Russian Chemical Reviews **76** (7) 599 – 615 (2007) © 2007 Russian Academy of Sciences and Turpion Ltd

DOI 10.1070/RC2007v076n07ABEH003714

Spin chemistry of enzymatic processes

M S Afanasyeva, P A Purtov, M B Taraban, T V Leshina

Contents

I.	Introduction	599
II.	Phenomenological description of the magnetic field and spin effects in enzymatic systems	600
III.	Theory of the magnetic field and spin effects in enzymatic multispin systems	602
IV.	Magnetic field effects in enzymatic reactions catalysed by horseradish peroxidase	604
V.	Conclusion	612

Abstract. The results of spin chemistry studies of the single electron transfer step and the role of spin states of paramagnetic intermediates in the enzymatic oxidation of organic substrates induced by horseradish peroxidase are generalised. Detailed theoretical description of the approach used to analyse the magnetic field effects in enzymatic multispin systems is presented. Prospects for the use of spin chemistry techniques for elucidation of the mechanisms of radical steps of enzymatic processes are considered. The bibliography includes 80 references.

I. Introduction

Spin chemistry methods are used in studies of radical reactions the rates and, often, directions of which depend on the orientation of the electron and nuclear spins of transient radical species and on the strength of external and local magnetic fields. Spin chemistry became an individual avenue of chemical research nearly twenty-five years ago and currently there is no need to introduce it to the reader. The term 'spin chemistry' refers to the chemistry of the radical processes mentioned above and to the methods employed. The latter comprise the methods based on the phenomena of chemically induced dynamic electron and nuclear polarisation (CIDEP and CIDNP, respectively), magnetic field effect (MFE), magnetic isotope effect (MIE) and related techniques that have been comprehensively described in a number of reviews and monographs (see, *e.g.*, Refs 1 and 2).

After completion of theoretical description of the magnetic field effects detected in model systems, the last decade was marked by the trend to practical application of this knowledge to the studies of complex chemical and biochemical processes by spin chemistry. This avenue of research seems to be quite important because elucidation of the structure and properties of short-lived intermediates in chemical reactions belongs to the key problems in modern chemistry. Spin chemistry methods make it possible to study paramagnetic intermediates with lifetimes from a few nanoseconds to a few microseconds, *viz.*, radical ions and neutral free radicals, biradicals, carbenes and carbene analogues and triplet-excited molecules.

The involvement of radical ions, radicals and biradicals in some reactions was only postulated; remarkable examples of successful use of the CIDNP and MFE techniques for elucidation of the mechanisms of these processes have been reported.[3–5] Of particular interest are recent spin chemistry studies in biology[6] including investigations of oscillatory enzymatic reactions,[7] structural features of reversible protein folding,[8] type of the intermediates in the reactions of B_{12}-dependent enzymes[9] and simulation of protein – nucleic acid interactions.[10]

Recently, the experimental data suggesting that free-radical states initiate processes that damage cell structures, certain proteins and nucleic acids have been accumulated increasingly.[11] It is believed that free radicals are responsible for the development of cardiovascular diseases,[12] compromised immune response,[13] cataract development,[14] development of Parkinson's disease,[15] schizophrenia,[16] and Alzheimer's disease,[15,17] as well as accelerated ageing and higher risk of cancer.[18] According to modern concepts, destructive changes in living organisms are most probably due to the processes involving reactive oxygen species and oxygen-containing radicals ($O_2^{\bullet-}$, $HO_2^{\bullet}$, $HO^{\bullet}$ and $^1O_2$) that induce oxidative stress, diseases and senilism.[19] In this connection, a demand arose for spin chemistry studies of reactive paramagnetic intermediates in biological systems.

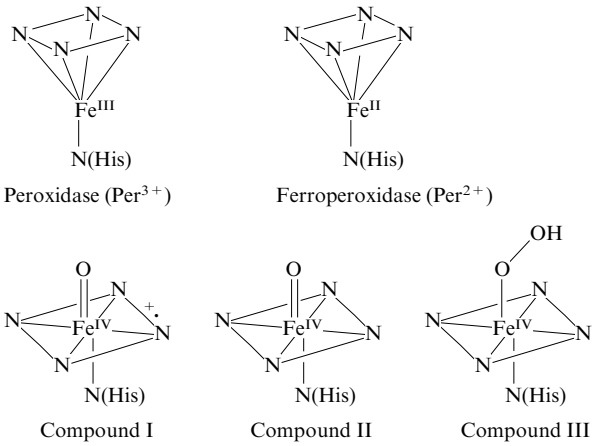
Paramagnetic species play an important role in some processes that are responsible for normal activity of mammalian cells.[20] The involvement of radical species in many biological processes was reviewed.[21,22] Enzymatic processes in living systems are sources of various free radicals, in particular, $HO_2^{\bullet}$ (Ref. 23). The present review is devoted to reaction mechanisms of horseradish peroxidase (HRP), which is the source of intermediates that react with many free radicals; of note is the appearance of the expression 'the free-radical chemistry of peroxidase'.[24] Peroxidase is present both in mammalian organisms and plants, where it decomposes toxic hydrogen peroxide and can serve as a scavenger of free radicals. It should be noted that at present researchers are far from

M S Afanasyeva, P A Purtov, M B Taraban, T V Leshina Institute of Chemical Kinetics and Combustion, Siberian Branch of the Russian Academy of Sciences, ul. Institutskaya 3, 630090 Novosibirsk, Russian Federation. Fax (7-383) 330 73 50, tel. (7-383) 333 14 05, e-mail: afanasieva@ns.kinetics.nsc.ru (M S Afanasyeva), taraban@ns.kinetics.nsc.ru (M B Taraban), leshina@ns.kinetics.nsc.ru (T V Leshina), tel. (7-383) 333 11 02, e-mail: purtov@ns.kinetics.nsc.ru (P A Purtov)

Received 5 April 2007
Uspekhi Khimii **76** (7) 651 – 668 (2007); translated by A M Raevskiy

knowing all sources of free radicals in the living systems in health and disease.

Taking into account the aforesaid, studies on the elementary processes resulting in emergence of free radicals, in particular, enzymatic reactions, are of considerable interest. The mechanisms of enzymatic reactions are studied by various modern physicochemical methods. Identification of paramagnetic species often faces severe difficulties.[25] Problems concerned with detection of free radicals by EPR spectroscopy (low steady-state concentrations of radicals in biological systems) are well known.[26] Using the spin trap technique in EPR spectroscopy, one can solve the 'concentration' problem, but some new problems often arise, *e.g.*, the need of suppression of EPR signals corresponding to further transformations of spin traps and stable radicals that formed.[26] In addition, when observing the EPR signal of a free radical, it is unclear whether the radical is involved in the process under study or it is the side reaction product. Spin chemistry studies of radical intermediates in biological processes could answer this question. Clearly, enzymatic processes are also promising subjects for spin chemistry studies, because chemical transformations in the active sites of many metal-containing enzymes involve spin-change reactions and interconversions of the intermediates existing in different spin states; it is the oxidation states of such metals as Fe, Cu, Zn, *etc*. that are dealt with. For instance, the catalytic cycle of HRP involves reliably characterised reactive intermediates, the so-called HRP-Compounds the active sites of which contain iron atoms in different oxidation and spin states (high-spin state in native HRP and ferroperoxidase and low-spin state in Compounds I – III).[24]



N(His) is the nitrogen atom of histidine.

However, until very recently the lack of comprehensive theoretical description of multispin systems precluded the extension of spin chemistry studies of enzymatic processes. Even a few years ago a relevant theoretical description (radical pair theory) was only developed for the cases where reaction products were formed from radical pairs with half-integer spins ($S = 1/2$). Meanwhile, iron-containing intermediates in the high-spin states are paramagnetic intermediates in many biological processes (*e.g.*, in reactions involving iron ions or haem-containing enzymes including HRP and cytochromes). Usually, in such systems a multi-electron atom having several unpaired electrons serves as the radical centre.[27] Here, analysis of the MFE requires knowledge of the specific features of the evolution of spin states in multispin systems. At present, intensive experimental research of multispin systems has been carried out taking the processes in radical pairs (RP) induced by the so-called third or fourth spin (usually, the spins of stable radicals or biradicals) as examples.[28, 29] Progress in this field of research, which is in the early stage of development (accumulation of experimental results), was stimulated by the studies [30, 31] in which a novel phenomenon called 'spin catalysis' was discovered.

The magnetic field effects treated in a broad sense, *i.e.*, the effects of external magnetic fields on various processes in living organisms, from migratory orientation of birds to the effect of the so-called magnetic storms on human health, belong to popular subjects of research.[32] Since such effects undoubtedly have a complex nature, detection of the changes occurring in the presence of a magnetic field usually provides no information on the mechanisms of generation of the MFE signals and on the magnetic field-sensitive steps of the processes under study.

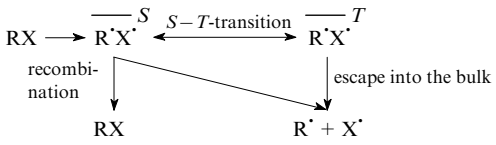
Mechanistic studies of the magnetic field effect on particular stages of *in vitro* processes using spin chemistry techniques are much more informative. These methods were used for comprehensive treatment of enzymatic processes involving magnetic field sensitive steps with participation of the pairs of paramagnetic species generated in single-electron transfer reactions.[33 – 35] Conclusions about the involvement of radical pairs in an enzymatic process are based on a comparison of the experimental and calculated dependences of the rate constants for transformations of paramagnetic intermediates on the magnetic field strength. Such studies were carried out for transformations of reactive paramagnetic intermediates in the catalytic cycle of the oxidation of some HRP substrates in the steady-state [33 – 35] and oscillatory regimes.[7] An important result of these investigations consists of the demonstration of the role of spin states of the paramagnetic intermediates in the catalytic cycle of the enzymatic process.

The magnetic field effects in biological systems were considered in a review,[6] which also summarised the data on possible biological effects under the action of static and alternating magnetic fields. In the present review, we make an attempt at systematisation and interpretation of the available material concerning theoretical description of the spin and magnetic field effects in multispin enzymatic systems and the results obtained in experimental studies of the dependences of the rates for enzymatic oxidation of organic substrates by horseradish peroxidase on the strength of the external magnetic field.

II. Phenomenological description of the magnetic field and spin effects in enzymatic systems

Reactions the rates and, sometimes, directions of which depend on the orientation of the electron and nuclear spins of the paramagnetic precursors of reaction products are the major subject of spin chemistry studies. An example of such a reaction is shown in Scheme 1.

Scheme 1



A radical pair emerged in, *e.g.*, photochemical or thermal decomposition of a molecule exists in one of the two correlated spin states, namely, in a singlet (S) or a triplet (T) state. The total electron spin of the radicals is equal to zero in the former state and unity in the latter. Usually, the triplet state of the radical pair is non-reactive (or low reactive),[1] and the RP recombination mainly occurs from the singlet state. Magnetic and spin interactions induce transitions between the non-reactive T-state and reactive S-state (the so-called $T-S$-transitions responsible for the change in the fraction of the reactive species). These singlet – triplet transitions represent the

physical essence of the spin and magnetic field effects in chemical processes. The most important magnetic interactions that induce the singlet–triplet transitions and affect their efficiency include the Zeeman interaction of the unpaired electrons in the RP with the external magnetic field, hyperfine coupling (HFC) between the electron spins and the magnetic nuclei (nuclei with a non-zero nuclear spin I) of the radicals and electron exchange interaction between the unpaired electrons in the RP. Figure 1 schematically presents two limiting cases of singlet–triplet mixing. For instance, in weak magnetic fields (Fig. 1 *a*) the hyperfine interaction induces $S-T$-transitions between all the three triplet Zeeman sublevels (T_0, T_{+1} and T_{-1}) and the recombining singlet state (HFC-mechanism of singlet–triplet transitions). In strong external magnetic fields that exceed substantially the local magnetic fields due to HFC, a large splitting between the T_{+1} and T_{-1} sublevels leads to the situation where the hyperfine interaction only affects the transitions between the singlet state and one triplet state (T_0). Isotropic hyperfine interaction does not involve the other two triplet states (T_{+1} and T_{-1}) in the $S-T$-transition (Fig. 1 *b*). Mixing of the S and T_0 states of a radical pair can also be due to the g-factor difference between the radicals (Δg-mechanism of singlet–triplet transitions). Thus, even weak (*e.g.*, Zeeman and hyperfine) magnetic interactions the constants of which are 5–6 orders of magnitude lower than kT can increase the fraction of the reactive state of the radical pair owing to the $S-T$-transition.

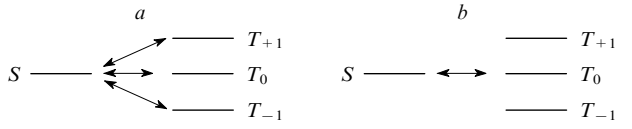


Figure 1. Energy levels of a radical pair and allowed transitions between the S- and T-states in weak (*a*) and strong (*b*) magnetic fields.

The manifestation of singlet–triplet transitions requires that the RP lifetime be rather long. In a condensed medium, this can be attained due to the cage effect. This effect determines two key features of the reactions under study, namely, the relatively long time of contact between partners and the possibility of repeated contacts of the same pair of reactants in the course of diffusion motion. The characteristic cage size (R) and the residence time (τ) of the reactants in the cage are governed by the kinematics of the relative motion, interaction between the reactants and some other properties. For instance, the hyperfine coupling constant between the unpaired electrons and magnetic nuclei (ω, in frequency units) in organic free radicals is of the order of about 10^8-10^9 rad s^{-1} and the probability of singlet–triplet transitions on the time scale $\tau \sim 10^{-9}-10^{-10}$ s is

$$\omega\tau \sim 0.01-0.1.$$

The probability of $S-T$-transitions increases with an increase in the viscosity of the solution (as τ increases) or in the reactions involving radical ion pairs (due to the Coulomb interaction between the partner radicals).

The singlet–triplet conversion depends on the orientation of electron and nuclear spins of the partners in the RP and the singlet–triplet conversion rate is affected by the HFC, Δg and external magnetic field. An important consequence is the possibility of manifestation of the magnetic field and spin effects (MIE and CIDNP) in chemical and biochemical reactions.

The MIE is due to the $T-S$ conversion rate difference between the radical pairs containing magnetic nuclei and the RP devoid of such nuclei. The MIE provides the isotopic selectivity in different reaction products. Chemically induced dynamic nuclear polarisation is a manifestation of the α- and β-spin selection in different reaction products. When carrying out the reaction in the probe of an NMR spectrometer, the lines observed in the NMR spectra at the very beginning of the process correspond to the distribution of the spin sublevel population that differs from the Boltzmann (equilibrium) distribution. A detailed description of the spin and magnetic field effects can be found in a monograph.[1]

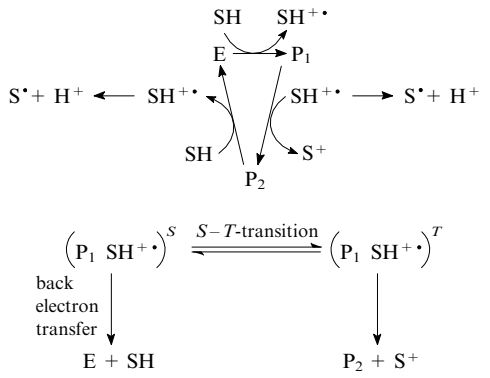
At present, the effect of the external magnetic field and CIDNP were detected for a number of chemical processes, such as single-electron transfer steps in the reactions previously treated as nucleophilic substitution;[3] *cis–trans*-isomerisation of alkenes including biologically valuable polyenes (retinal and carotenoids);[36] donor-acceptor interactions of synthetic analogues of nicotinamide adenine dinucleotide (NADH), namely, 1,4-dihydropyridines containing electron-withdrawing groups[37–40] and many other processes.

Now we will consider the specific features of manifestation of the magnetic field and spin effects in biochemical systems. In homogeneous solutions, a strong cage effect is most probably due to a high viscosity of the solvent and to the electrostatic interaction between partners in radical ion pairs. Pronounced cage effects are also characteristic of the reactions occurring in molecularly organised media, *e.g.*, liposomes, micelles, 'guest–host' inclusion complexes, *etc.* (here, the time spent by radical pairs in the contact zone due to the so-called weak interactions is longer than in non-viscous systems). Weak interactions also include the enzyme–substrate interactions (enzyme–substrate binding) in the active site of enzyme, which is usually considered as the main factor responsible for the high selectivity of enzymatic processes.[41] By analogy with well-studied processes in complexes or micelles,[42] one can expect that the radical intermediates formed in an enzymatic process will also form quite a long-lived enzyme–substrate complex. Binding of the partners in the active site of enzyme gives grounds to expect manifestations of rather strong electron exchange interaction between paramagnetic species in the enzyme–substrate complexes, which can be detected by spin chemistry techniques.

Now we have to dwell on basic differences between manifestations of the magnetic field and spin effects in homogeneous radical and enzymatic processes. In a radical reaction, the magnetic field effects consist of a change in the ratio of the recombination probability of free radicals to the probability of their escape into the bulk.[1] In enzymatic reactions, where the paramagnetic intermediates are involved in the catalytic cycle, two different spin states of a pair of paramagnetic species (corresponding to recombination and escape to the bulk, see Scheme 1) can characterise different steps in the catalytic cycle. Thus, the magnetic field will affect the ratio of recombination probabilities (or back electron transfer in the case of single-electron transfer) and transition to the next step in the catalytic cycle. As an example, we will consider a model catalytic cycle shown in Scheme 2.

The cycle begins with the formation of an enzyme–substrate complex [E···SH] in which an electron is transferred from the donor (substrate) to acceptor (enzyme) to form the (P_1 $SH^{+\bullet}$) radical pair in, *e.g.*, the singlet state. Magnetic interactions induce the singlet–triplet conversion; when the RP is in the singlet state, back electron transfer (recombination) and regeneration of the starting enzyme (E) and substrate (SH) are possible. The triplet RP is a precursor of the next stage of the catalytic cycle, which involves the formation of the intermediate P_2. Thus, this example clearly demonstrated the possibility of the effect of the spin states of paramagnetic

Scheme 2



intermediates on the rates of the processes occurring in the catalytic cycle of the enzyme.

The effect of the magnetic field on an enzymatic process will manifest itself as the dependence of particular steps of the catalytic cycle on the strength of external magnetic field. In this case, the magnetic field a effect can be defined as the ratio of the rate constants for the reactions carried out in a magnetic field of specified strength to the corresponding rate constants measured in zero (geomagnetic) field. Analysis of the magnetic field effects detected in enzymatic processes involves measurements of the dependences of the rates of particular steps of the catalytic cycle or the overall process on the strength of the external magnetic field (magnetic field dependence) and a comparison of the experimental and calculated dependences on the magnetic field. In the case of enzymatic processes, the recombination (back electron transfer) probabilities are calculated for a pair of paramagnetic species with specified magnetic resonance parameters (HFC constants, *g*-factors of radicals, effective electron exchange interaction constant J) with allowance for the lifetimes of the paramagnetic pair and the multiplicity of the initial and recombining states. Now we will consider an approach to the calculation of a recombination probability in multispin systems including enzymes.

III. Theory of the magnetic field and spin effects in enzymatic multispin systems

A model of a multispin system differs from that model used in the radical pair theory in the number of unpaired electrons (>2). For instance, the spin catalysis [30] deal with systems comprising three radicals (triads) or pairs of species with spins $I > 1/2$. Thus, the description of multispin systems requires taking account of possible correlated doublet, quartet, quintet and higher spin states. Recombination probability calculations for such multispin pairs should be carried out with allowance for a large number of subensembles of radical pairs, each corresponding to particular configurations of electron and nuclear spins. The interaction of RP spins with external magnetic fields and the spin–spin interactions induce transitions of a given RP from one subensemble to another. These interactions are described by systems of a large number of coupled kinetic equations. In addition, description of the dynamics of the RP spin state requires the use of quantum mechanics. The density matrix formalism is the most appropriate for simultaneous consideration of the spatial motion and spin dynamics of a radical pair. Now we will calculate the recombination probability in the framework of this method.

If the density matrix, $\rho(\vec{r}, t)$, of a pair of paramagnetic species separated by the distance r depends on r, changes in $\rho(\vec{r}, t)$ with time (t) are governed by three factors, *viz*., the spin evolution of the unpaired electrons due to the spin–spin interactions and spin interactions with external and local magnetic fields, the relative motion of reactants and recombination of radicals [1]

$$\frac{\partial\rho(\vec{r},t)}{\partial t} = \left(\frac{\partial\rho}{\partial t}\right)_{\text{spin}} + \left(\frac{\partial\rho}{\partial t}\right)_{\text{motion}} + \left(\frac{\partial\rho}{\partial t}\right)_{\text{recomb}}. \quad (1)$$

This equation is usually written as follows:[43]

$$\frac{\partial\rho(\vec{r},t)}{\partial t} = \mathrm{i}\hat{L}(\vec{r})\rho(\vec{r},t) + \hat{R}(\vec{r})\rho(\vec{r},t) - \hat{U}(\vec{r})\rho(\vec{r},t), \quad (2)$$

where $\hat{L}(\vec{r}) = \hat{\Omega} + \mathrm{i}\hat{R} + \hat{J}(\vec{r})$ is the Liouville operator (Liouvillian), which allows for the coordinate-independent spin evolution due to isotropic HFC and the Zeeman interaction with external magnetic fields ($\hat{\Omega}$), the spin relaxation processes ($\hat{R}$), and the coordinate-dependent spin evolution induced by the exchange interaction [$\hat{J}(\vec{r})$]. The operator $\hat{U}(\vec{r})$ describes the spin-dependent RP recombination, and its matrix elements characterise the rate of transformation of different components of the density matrix to the reaction product (or products). The functional operator $\hat{R}(\vec{r})$ specifies the motion of reactants, which is usually treated in the framework of classical mechanics.

The probability of RP recombination from a certain spin state at the instant t is given by the corresponding components of the vector $\hat{w}(t)$

$$\hat{w}(t) = \int_0^t\int \hat{U}(\vec{r})\rho(\vec{r},\tau)\mathrm{d}\tau\mathrm{d}\vec{r}. \quad (3)$$

Among a large number of methods for solving Eqn (2), the Green function formalism is most often used. The Green functions specify the character of the relative motion of other paramagnetic species in the pair.[44] This method makes use of a physically substantiated admission that the reaction zone is always a rather narrow region in the space of motion of reactants. In this case, the general matrix expression for the probability of recombination of a pair of paramagnetic species from a certain spin state has the form:[43]

$$\hat{w} = \hat{U}_0\hat{g}\left(1 + \hat{U}_0\hat{g}\right)^{-1}\rho_0, \quad (4)$$

where $\hat{U}_0$ is the matrix of recombination constants for different spin states (generally, recombination from any electron spin state is possible, but at different rate); $\hat{g}$ is the operator (averaged over the reaction zone) characterising the relative motion and the spin dynamics in the pair. The matrix of this operator is the matrix of characteristic 'times'. For instance, the matrix element $g_{nn,kk}$ is the mean lifetime of the RP in the spin state $|nn\rangle$ provided that the system initially was in the electron spin state $|kk\rangle$. All elements of this matrix are expressed through the one and only function $g(s)$ characterising the relative motion (changes in the mutual position) in the pair of recombining paramagnetic species.

Carrying out particular calculations often requires knowledge of only certain elements of the matrix $\hat{g}$, *i.e*., one or several of characteristic 'times'. Consider a typical case where the RP can recombine only in a particular electron spin state, *e.g*., a doublet or singlet state. Let the spin evolution in the RP occur in such a way that no transitions between different recombining states of the radicals occur. Then, the whole ensemble of radical pairs can be divided into subensembles with a particular configuration, $\{m\}$, of nuclear spins in the doublet (or singlet) state. Calculations for each subensemble are performed independently; the recombination probability for a specified subensemble is given by

$$w_m = \frac{U_0\tau_m}{1 + U_0\tau_m}, \tag{5}$$

where the quantity τ_m is the mean residence time of the radical pair in the reaction zone in the doublet state with the specified spin configuration $\{m\}$ provided that the pair was born in the reaction zone in the doublet state with the same spin configuration $\{m\}$.

In some cases, transitions between doublet states can play an important role. Suppose that a transition from the doublet state with the nuclear spin configuration $\{m\}$ to the doublet state with the spin configuration $\{m'\}$ is possible. We thus obtain a more general expression compared to Eqn (5)

$$w_m = U_0 \frac{\tau_m + \tau_{mm'} + U_0(\tau_m\tau_{m'} - \tau_{mm'}\tau_{m'm})}{1 + U_0(\tau_m + \tau_{m'}) + U_0^2(\tau_m\tau_{m'} - \tau_{mm'}\tau_{m'm})}. \tag{6}$$

Here $\tau_{mm'}$ is the mean residence time of the RP in the reaction zone in the doublet state with the preset spin configuration $\{m\}$ provided the start is from the doublet state with the preset spin configuration $\{m'\}$. The parameter $\tau_{m'm}$ has a similar meaning.

Further calculations require specifying of the spin Hamiltonian of the RP to describe the spin evolution and choosing an appropriate model for the relative motion to describe the spatial motion of the species. Changes in the mutual position of the species in the enzyme-substrate complex can be described using a rather simple two-position model,[45] according to which two states of an RP in the cage are possible: (i) radicals contact each other and can recombine and (ii) radicals do not contact each other but can return to the contact state. The state (i) is characterised by the time τ_1 necessary for transition of RP from this state to the state (ii). The state (ii) is characterised by two parameters, namely, the time τ_2 of back transition from the state (ii) to the state (i) and the time τ_c of irreversible decomposition of the RP. The singlet–triplet transitions occur in the state (ii) of the RP.

In the framework of the two-position model, the function $g(s)$ characterising the changes in the mutual position in a pair of recombining paramagnetic species is given by:[45]

$$g(s) = \frac{\tau_p}{n+1}\left(1 + \frac{n}{1 + s\tau_c}\right), \tag{7}$$

where n is the number of repeated contacts and $\tau_p = n\tau_1$.

The spin evolution is described using appropriate basis spin states φ_i. For instance, the singlet–triplet basis is suitable for the description of a pair of species with $I = 1/2$. If a pair comprises two species with $I = 1/2$ and 1, the resultant spin state can be doublet or quartet. The spin functions of these states have the form

$$|D\rangle = \begin{cases} \sqrt{\frac{2}{3}}\beta T_{+1} - \sqrt{\frac{1}{3}}\alpha T_0 \\ \sqrt{\frac{2}{3}}\alpha T_{-1} - \sqrt{\frac{1}{3}},\beta T_0 \end{cases}, \tag{8}$$

$$|Q\rangle = \begin{cases} \alpha T_{+1} \\ \sqrt{\frac{1}{3}}\beta T_{+1} + \sqrt{\frac{2}{3}}\alpha T_0 \\ \sqrt{\frac{1}{3}}\alpha T_{-} + \sqrt{\frac{2}{3}}\beta T_0 \\ \beta T_{-1} \end{cases}.$$

By specifying the spin Hamiltonian of the system it is possible to determine the stationary energy levels (E_i) and the eigenfunctions (wavefunctions) of the system (ψ_i). The stationary wavefunctions in the φ_i basis set are represented by

$$\psi_i = \sum_j (\hat{Q})_{ij}\varphi_j,$$

where $(\hat{Q})_{ij}$ is the matrix that describes transition from the initial spin basis set to the basis of eigenfunctions. The dependence of the wavefunctions on time is as follows:

$$|\psi(t)\rangle = \exp(-\mathrm{i}\hat{H}t)|\psi(0)\rangle.$$

Now we will write the density matrix in the basis of the φ_i functions. The action of the operator $\hat{Q}$ on the functions φ_i leads to

$$|\varphi(t)\rangle = \hat{Q}^{-1}|\psi(t)\rangle = \hat{Q}^{-1}\exp(-\mathrm{i}\hat{H}t)|\psi(0)\rangle = \tag{9}$$

$$= \hat{Q}^{-1}\exp(-\mathrm{i}\hat{H}t)\hat{Q}|\varphi(0)\rangle;$$

since

$$\hat{\rho}(t) = |\varphi(t)\rangle\langle\varphi(t)|,$$

one gets

$$\hat{\rho}(t) = \hat{Q}^{-1}\exp(-\mathrm{i}\hat{H}t)\hat{Q}|\varphi(0)\rangle\langle\varphi(0)|\hat{Q}^{-1}\exp(\mathrm{i}\hat{H}t)\hat{Q}. \tag{10}$$

For instance, the probability for the RP to exist in a doublet state at any instant is given by:

$$\rho_{DD}(t) = \left\langle D\middle|\hat{Q}^{-1}\exp(-\mathrm{i}\hat{H}t)\hat{Q}\middle|D\right\rangle\left\langle D\middle|\hat{Q}^{-1}\exp(\mathrm{i}\hat{H}t)\hat{Q}\middle|D\right\rangle. \tag{11}$$

Here $\hat{Q}$ represents the matrix coefficients; therefore, the expression for $\rho_{DD}(t)$ can be transformed as follows:

$$\rho_{DD}(t) = \sum_i Q_{i1}^4 + \sum_{i,j} Q_{i1}^2 Q_{j1}^2 \exp\left[-\mathrm{i}(E_i - E_j)\right]t + \tag{12}$$

$$+ \sum_{i,j} Q_{i1}^2 Q_{j1}^2 \exp\left[\mathrm{i}(E_i - E_j)\right]t.$$

The average lifetime of an RP in a doublet state provided that the RP was born in the same state can be determined from the following expression

$$\tau = (\hat{g})_{DD,\,DD} = \sum_i Q_{i1}^4 g(0) + \sum_{i,j} Q_{i1}^2 Q_{j1}^2 g\left[-\mathrm{i}(E_i - E_j)\right] + \tag{13}$$

$$+ \sum_{i,j} Q_{i1}^2 Q_{j1}^2 g\left[\mathrm{i}(E_i - E_j)\right].$$

For the two-position model, the equation for τ takes the form

$$\tau = \tau_p\left[\sum_i Q_{1i}^4 + 2\sum_{i>j}\frac{Q_{1i}^2 Q_{1j}^2}{n+1}\left(\frac{1+n}{1 + [(E_i - E_j)\tau_c]^2}\right)\right]. \tag{14}$$

The probability of recombination from the doublet state provided that the RP was formed in the doublet and quartet state, respectively, can be calculated using the following relationships:

$$w^D = \frac{1}{2}\,\frac{U_0\tau}{1 + U_0\tau}, \tag{15}$$

$$w^Q = \frac{1}{4}\,\frac{U_0(\tau_p - \tau)}{1 + U_0\tau}. \tag{16}$$

Recombination probability calculations for such systems are exemplified by the reactions catalysed by horseradish

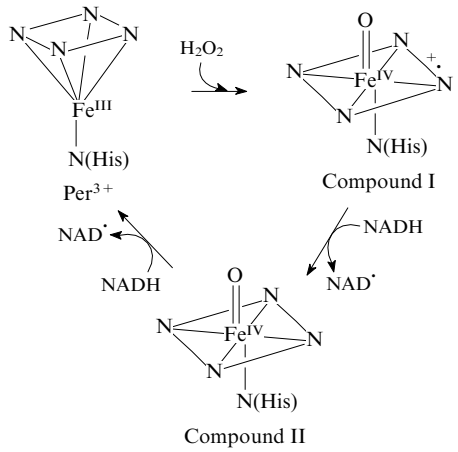
peroxidase containing an iron atom in the active site (see below).

IV. Magnetic field effects in enzymatic reactions catalysed by horseradish peroxidase

1. Modern concepts of the mechanisms of horseradish peroxidase-catalysed oxidation of substrates

Two models for the mechanism of the HRP catalytic cycle are available in the literature, a 'simplified' model [27] and a 'chemically realistic' one.[24] The former model (Scheme 3) was proposed as a possible mechanism of the oxidation of the natural peroxidase substrate (NADH) in the presence of an initiator (hydrogen peroxide) and involves successive transformation of native HRP (Per^{3+}, Fe^{III}) into reactive paramagnetic HRP intermediates, namely, Compound I containing a haem oxoform π-radical cation ($Fe^{IV}{=}O^{+\bullet}$) in the active site and Compound II ($Fe^{IV}{=}O$).

Scheme 3

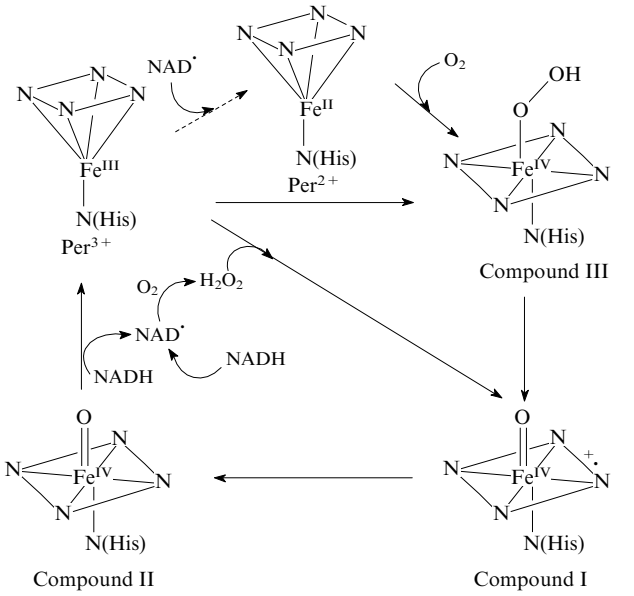


The second model (Scheme 4) was used to describe the NADH oxidation in the absence of H_2O_2. It is based on the assumption that slow oxidation of NADH with oxygen present in the buffer solution in an acid medium results in the formation of H_2O_2 and that the amount of H_2O_2 formed is sufficient for initiation of the catalytic cycle,[46] which includes interconversions of native HRP (Per^{3+}, Fe^{III}), Compound I ($Fe^{IV}{=}O^{+\bullet}$), Compound II ($Fe^{IV}{=}O$), Compound III ($Fe^{IV}{-}O{-}OH$) and ferroperoxidase (Per^{2+}, Fe^{II}).

However, the efficiency of the hypothetical step of H_2O_2 generation casts some doubts. The authors of the present review believe that single-electron transfer between the native HRP and substrate (NADH) is much more probable, because this is a commonly accepted starting point of oxidation in the reactions catalysed by other haem-containing enzymes.[47, 48] Indeed, according to published data on HRP-catalysed oxidation of substrates,[49, 50] the binding of a substrate in the active site of enzyme occurs near the haem plane, which permits direct electron transfer from the substrate to the haem. Here, the rate of single-electron transfer from the substrate to the haem in the enzyme–substrate complex should be much higher than the rate of the substrate oxidation by dissolved oxygen (for NADH, $k = 3.0 \times 10^{-6}$ s^{-1}).[24] It should be noted that high probability of single-electron transfer is also due to the donor properties of NADH ($E^0 = -320$ mV) and acceptor properties of HRP ($E^0 = -306$ mV).[51]

However, in spite of these arguments, the concept considering the formation of H_2O_2 as the starting point of the peroxidase-catalysed substrate oxidation still dominates in the literature.[24, 47] Nevertheless, the aforesaid suggests[34, 35] that the single-electron transfer step can occur in the [$Per^{3+}\cdots$ NADH] enzyme–substrate complex. It should be noted that the possibility of electron transfer between HRP and NADH to

Scheme 4



give ferroperoxidase (Per^{2+}) was discussed in a study of HRP-catalysed oxidation of photoexcited NADH.[52]

Thus, the possibility of electron transfer between the native HRP and NADH requires experimental proof. Of particular interest are the experimental proofs of the involvement of the $NADH^{+\bullet}$ radical cation in the enzymatic oxidation processes. Discussion of whether the multistep mechanism involving electron transfer is possible in biological systems

$$NADH \longrightarrow NADH^{+\bullet} \longrightarrow NAD^{\bullet} \longrightarrow NAD^{+},$$

or the oxidation occurs as the hydride transfer remains topical.[53] It should be emphasised that the radical steps were proposed for almost all processes involving HRP, that is, hydrogen peroxide utilisation, initiation of polymerisation in the synthesis of plant cell membranes, *etc*.[47] Meanwhile, only reactive paramagnetic intermediates of the native HRP, *i.e*., Compounds I–III, were experimentally detected to date.[46] Since the involvement of paramagnetic intermediates (Per^{3+}, Per^{2+}, Compounds I–III) in the catalytic cycle of HRP can be considered proven (see Schemes 3 and 4), it was quite natural to expect that the use of spin chemistry methods will provide reliable information on hypothetical reactive paramagnetic substrate species generated in these processes.

Further, we briefly outline the methodology of experiments and analysis of the magnetic field effects, which permits reliable establishment of manifestations of the radical steps in the HRP-catalysed oxidation of organic substrates.

2. Methodology of studies on the magnetic field effects in the substrate oxidation catalysed by horseradish peroxidase

To date, detailed magnetic-field-effect-based spin chemistry studies were carried out of the HRP-catalysed oxidation of organic substrates, namely, 2-methyl-1-(trimethylsilyloxy)-prop-1-ene ($Me_2C{=}CHOSiMe_3$) in the presence of H_2O_2,[33] NADH [34] and its synthetic analogue, 3,5-di(methoxycarbonyl)-2,3-dimethyl-4-nitrophenyl-1,4-dihydropyridine (nifedipine, NF), in the absence of H_2O_2.[35] In all the three cases, the effective rate constants for transformations of particular intermediates of the catalytic cycle of HRP (Per^{3+}, Per^{2+}, Compounds I–III) were measured using a rapid-scanning stopped-flow spectrophotometer. Since the intermediates listed above show different absorption spectra [46] (Fig. 2), it is possible to monitor the kinetics of corresponding interconversions. For instance, in the study of $Me_2C{=}CHOSiMe_3$

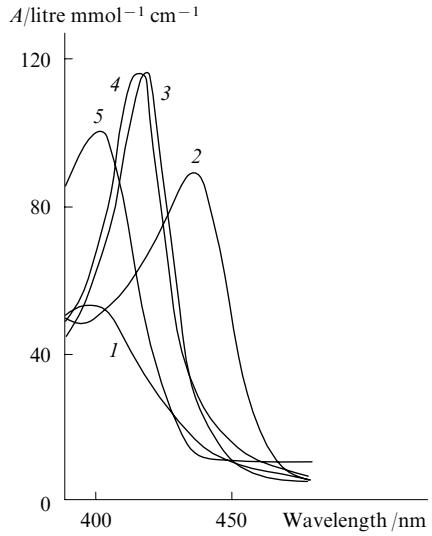


Figure 2. Absorption spectra of horseradish peroxidase and peroxidase reactive intermediates.[47]
Compound I (*1*), Per^{2+} (*2*), Compound III (*3*), Compound II (*4*) and Per^{3+} (*5*).

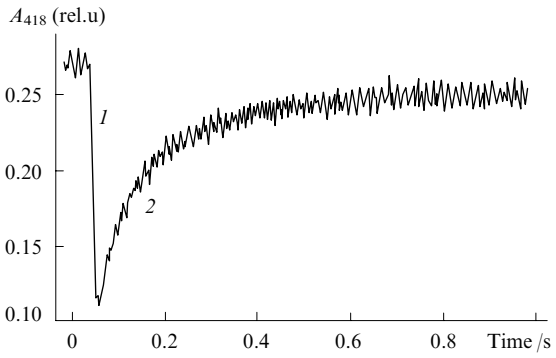


Figure 3. Kinetic curve of chemical transformations of reactive paramagnetic peroxidase intermediates observed at 418 nm.
Reaction conditions (all concentrations after mixing): 100 mM phosphate buffer (pH 7.4), $[Per^{3+}] = 4$ μmol litre^{-1}, $[H_2O_2] = 0.1$ mmol litre^{-1}, [2-methyl-1-(trimethylsilyloxy)prop-1-ene] = 16 mmol litre^{-1}; 25 °C.

oxidation, the changes in the absorption (A) of the reactive HRP intermediates were detected at 418 nm (maximum absorption of Compounds II and III, see Fig. 2) over a period of 1 s with a time resolution of 1 ms.[33] The time resolution was 16 ms in the NADH oxidation[34] and 32 ms in the NF oxidation,[35] because the reactions with these substrates took much longer time to complete (up to 500 s). To study the effect of external magnetic field, the mixing cell in which the reaction of the substrate with HRP occurred, was placed between the poles of an electromagnet and the magnetic field strength was varied from 0 to 4500 G. The kinetic traces recorded in each case were compared with the corresponding traces obtained in zero magnetic field (to this end, the residual magnetisation of the electromagnet was compensated up to a value of 0.3 to 0.5 G). The effective rate constants for interconverions of the HRP intermediates were determined using the Levenberg–Marquardt non-linear regression algorithm. The analytical solution to the system of differential equations describing successive transformations of intermediates of the catalytic cycle of HRP with allowance for their molar extinction coefficients at 418 nm[24,47] was used as the fitting function. The magnetic field effects were detected as the dependence of the ratio of the effective rate constant in a particular magnetic field to the correspondig rate constant determined in terrestrial magnetic field on the strength of the external magnetic field.

3. Analysis of magnetic field effects in the oxidation of 2-methyl-1-(trimethylsilyloxy)prop-1-ene by horseradish peroxidase

The oxidation of $Me_2C{=}CHOSiMe_3$ by horseradish peroxidase in the presence of hydrogen peroxide[33] was the first example of the substrate oxidation by a haem-containing enzyme, in which the effect of magnetic field on transformations of paramagnetic intermediates was detected.

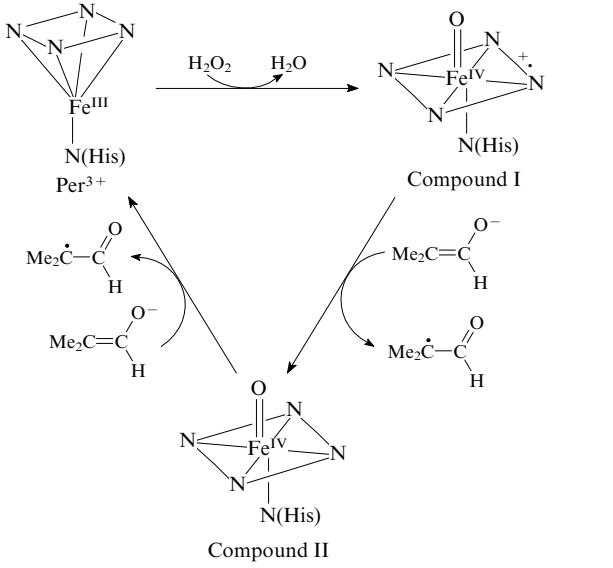
The kinetic curves (Fig. 3) with allowance for absorption of reactive peroxidase intermediates at 418 nm (see Fig. 2) can be explained by a sequence of conversions of the paramagnetic species. The initial decrease in the absorbance (see Fig. 3, region *1*) can be explained by the formation of Compound I (extinction coefficient $\varepsilon_{418} = 35$ litre mmol cm^{-1}) in the reaction of the native HRP with H_2O_2. The subsequent reaction of Compound I with the first substrate molecule results in Compound II ($\varepsilon_{418} = 115$ litre mmol cm^{-1}) and, consequently, in an increase in the absorbance (see Fig. 3, region *2*). Then Compound II reacts with the second substrate molecule to regenerate the native HRP.

The reaction of HRP with hydrogen peroxide is too fast to be treated correctly under the experimental conditions and at the time resolution mentioned above. In this connection the initial region of the fall-off of the kinetic curve was ignored in the design of the kinetic model for theoretical description of the process. Therefore, changes in the optical absorption can be described, in the framework of the sequence of conversions shown in Scheme 5, using the kinetic model for two successive unimolecular reactions

$$\text{Compound I} \xrightarrow{k_1} \text{Compound II} \xrightarrow{k_2} \text{Compound III}. \qquad (17)$$

Since the extinction coefficient of HRP at 418 nm is much lower than that of Compound II (see Fig. 2), the overall function for the description of the experimentally observed kinetics of changes in the optical absorption (A_{418}) is given by:

Scheme 5



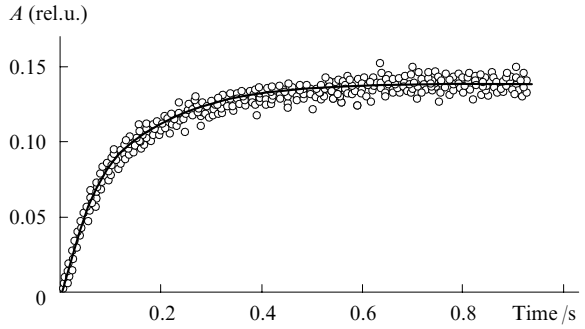


Figure 4. Fitting of the experimental kinetic curve using the Levenberg – Marquardt non-linear regression algorithm and analytical solution (18).
Experimental data are shown by open circles and the fitting results are shown by the solid line.

$$A_{418} = \left[\text{compound II} + \text{Per}^{3+}\right] \approx \tag{18}$$

$$\approx \frac{\left[\text{compound I}\right]_0}{2}\left[1 - \frac{(k_2 - 2k_1)\exp(-k_1 t)}{k_2 - k_1} - \frac{k_1 \exp(-k_2 t)}{k_2 - k_1}\right].$$

To determine the effective rate constants k_1 and k_2, the analytical solution (18) is used for constructing a corresponding kinetic plot with allowance for the sequence of conversions of reactive paramagnetic intermediates of the peroxidase catalytic cycle (see Scheme 5) using the Levenberg – Marquardt non-linear regression algorithm. Figure 4 illustrates excellent agreement between the experimental kinetic curve and calculated function (18). The magnetic field effects were calculated as the ratio of the rate constants k_1 and k_2 measured in non-zero external magnetic fields to the corresponding rate constants determined in the zero magnetic field. The magnetic field dependences of the magnetic field effects determined for k_1 and k_2 are shown in Fig. 5. In Section III, we showed that the shape of the magnetic field dependence of the magnetic field effect and positions of extrema are determined by the magnetic resonance parameters of two paramagnetic species.[1] By comparing the experimental magnetic field dependence (see Fig. 5) and the results of calculations using the known semiclassical approximation,[54] one can determine the magnetic field-sensitive step of the process, namely, the second stage of the catalytic cycle, which involves the interaction of Compound II with the substrate. In this stage, the event of single-electron transfer is followed by the formation of the radical form of Compound II and the radical anion of the enol form of 1-isobutyraldehyde ($Me_2C{=}CHO^{-\bullet}$). In this case the magnetic field effect is due to the probability difference between back electron transfer in the radical pair in weak and strong magnetic fields. Here, the reversible electron transfer event in the collective triplet state of the RP is accompanied by regeneration of Compound II, whereas the singlet state eventually results in the starting HRP (Per^{3+}) (Scheme 6).

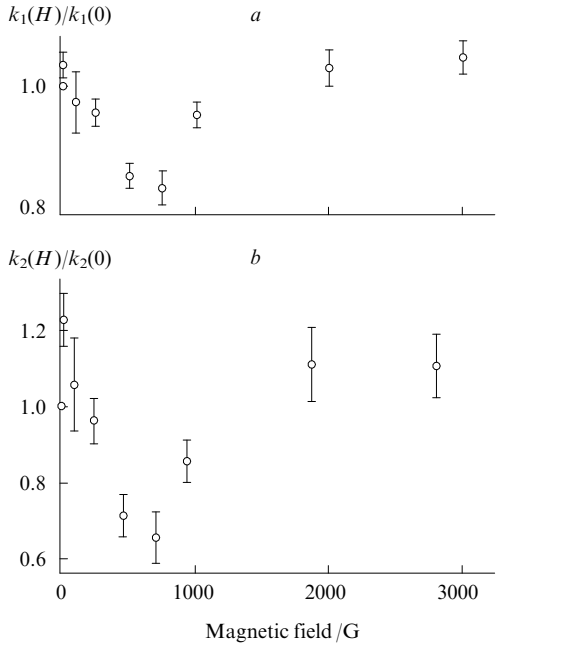
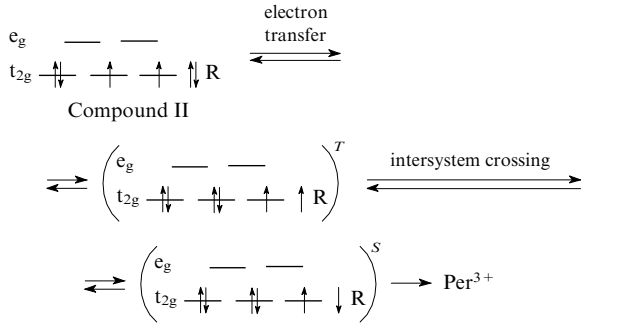


Figure 5. Magnetic field effect on the rate constants k_1 (*a*) and k_2 (*b*) of the oxidation of 2-methyl-1-(trimethylsilyloxy)prop-1-ene catalysed by horseradish peroxidase. Shown are the standard deviations (averaging over three experimental series including 5 or 6 measurements each).
Reaction conditions (all concentrations are reported after mixing): 100 mM phosphate buffer (pH 7.4), $[Per^{3+}] = 4$ μmol litre^{-1}, $[H_2O_2] = 0.1$ mmol litre^{-1}, [2-methyl-1-(trimethylsilyloxy)prop-1-ene] = 16 mmol litre^{-1}; 25 °C.

Scheme 6



Thus, manifestation of the magnetic field effect confirms the assumption of involvement of the paramagnetic intermediates in the catalytic cycle of HRP. This also indicates the possibility for a new, non-studied, short-lived radical form of Compound II to exist.[24, 27, 47]

4. Magnetic field effects in the oxidation of NADH and its synthetic analogues by horseradish peroxidase

a. Enzymatic oxidation of NADH catalysed by horseradish peroxidase

Yet another example of manifestation of the magnetic field effect in the reactions catalysed by HRP is provided by the oxidation of NADH, a natural HRP substrate. In the above discussion of modern concepts of the mechanisms of oxidation of organic substrates catalysed by HRP, mention was made that the initiation step of the catalytic cycle in the absence of hydrogen peroxide requires additional studies. Electron transfer from the donor molecule (NADH) to acceptor (*e.g.*, haem of the active site of HRP) can be a highly probable starting point of the catalytic cycle. The use of spin chemistry methods to prove this assumption seems to be correct.

The oxidation of NADH catalysed by HRP in the absence of H_2O_2 was studied by the stopped-flow spectrophotometric technique (see above). The reactive HRP intermediates (Compounds I, II and III) were also detected at the wavelength of maximum absorption of Compounds II and III (418 nm). Based on the known sequences of interconverions of HRP intermediates [46] and their molar extinction coefficients [24] (see

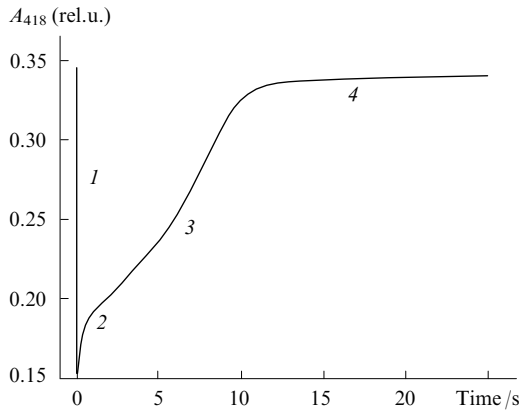
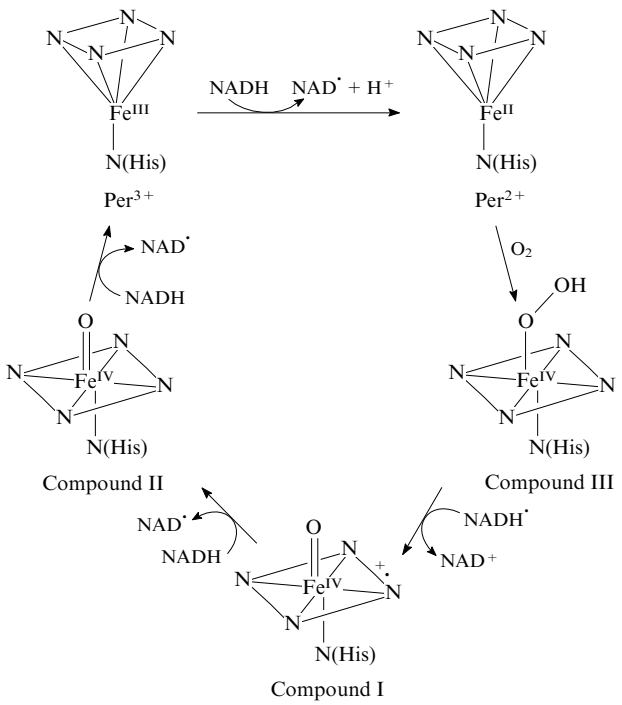


Figure 6. Kinetic curve of chemical transformations of horseradish peroxidase reactive paramagnetic intermediates observed at 418 nm in the NADH oxidation catalysed by peroxidase.
Reaction conditions (all concentrations after mixing): 100 mM MES buffer, pH 5.6; $[Per^{3+}] = 1$ μmol litre^{-1}, [NADH] = 100 μmol litre^{-1}; 25 °C.

Fig. 2), the characteristic kinetic curve of this process (Fig. 6) was explained as follows:[34] a decrease in absorption in the first step (see Fig. 6, region *1*) is associated with the transformation of native HRP ($\varepsilon_{418} = 62$ litre mmol^{-1} cm^{-1}) to ferroperoxidase (Per^{2+}) ($\varepsilon_{418} = 62$ litre mmol^{-1} cm^{-1}) upon electron transfer event from the substrate (NADH) to the starting HRP, which results in the formation of the reduced form (Per^{2+}). This is followed by the reactions of ferroperoxidase with oxygen dissolved in the buffer solution (Scheme 7), which leads to rapid conversion of Per^{2+} ($k = 5.8 \times 10^4$ litre mol^{-1} s^{-1}) to Compound III ($\varepsilon_{418} = 115$ litre mmol^{-1} cm^{-1})[24] and subsequent increase in absorption (see Fig. 6, region *2*). Fast deprotonation of the $NADH^{+\bullet}$ radical cation ($k = 3.6 \times 10^6$ s^{-1}) (Ref. 55) produces the $NAD^{\bullet}$ radical, which reacts with Compound III to give Compound I ($\varepsilon = 35$ litre mmol^{-1} cm^{-1}) (see Scheme 7). This process

Scheme 7



corresponds to the 'deflection' region of the kinetic curve (see Fig. 6, region *3*). Then, the single-electron reduction of Compound I produces Compound II ($\varepsilon_{418} = 115$ litre mmol^{-1} cm^{-1}), which leads to an increase in the absorption at 418 nm (Fig. 6, region *4*).

The initial portion of the kinetic curve of ferroperoxidase formation was too fast for quantitative treatment; therefore, the corresponding rate constant was excluded from the theoretical kinetic model. Therefore, in accordance with the proposed sequence of reactions (see Scheme 7), an increase in the absorption (see Fig. 6, regions *2–4*) was described using a kinetic model that assumes three unimolecular reactions

$$Per^{2+} \xrightarrow{k_1} \text{Compound III} \xrightarrow{k_2} \text{Compound I} \xrightarrow{k_3} \text{Compound II}. \quad (19)$$

Compounds II and III are characterised by equal extinction coefficients that are about three times higher than the molar extinction of Compound I and twice as high as that of Per^{2+}; therefore, the overall fitting function for the description of the experimental changes in absorbance with time can be written as follows:

$$A_{418} = [\text{compound III}] + [\text{compound II}] + \frac{[\text{compound I}]}{3} + \frac{[Per^{2+}]}{2} = [Per^{2+}]_0 - \frac{2[\text{compound I}]}{3} - \frac{[Per^{2+}]}{2}. \quad (20)$$

The curve calculated using experimental data and the Levenberg–Marquardt non-linear regression algorithm for the overall analytical solution (20) to the system of equations that describe the sequence of reactions in the catalytic cycle of HRP (19) virtually coincides with the experimental kinetic curve shown in Fig. 7. It should be noted that kinetic simulation with allowance for ferroperoxidase formation allowed one to obtain the best fit of the experimental and theoretical curves. This made it possible to determine the rate constants k_1, k_2 and k_3 for the reactions (19).

Yet another proof of occurrence of single-electron transfer between HRP and NADH in the enzymatic oxidation was obtained by analysing the magnetic field dependences of the magnetic field effect detected in this process. The magnetic field effect calculated as the ratio of the effective rate constants in a magnetic field to the corresponding rate constants in the zero magnetic field was found only for the constant k_1 (Fig. 8). The most probable magnetic field sensitive step is electron transfer

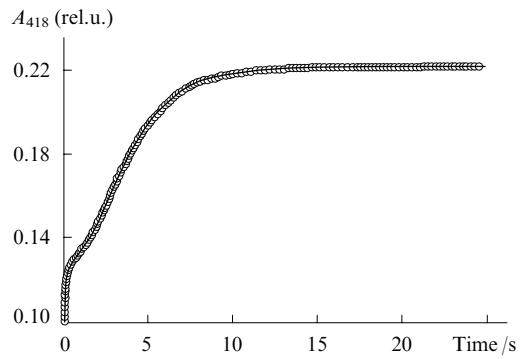


Figure 7. Fitting of the experimental kinetic curve for the reaction of peroxidase with NADH using the Levenberg–Marquardt non-linear regression algorithm and analytical solution (20).

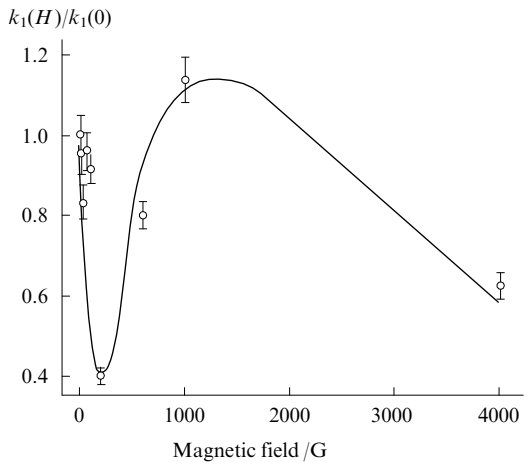


Figure 8. Magnetic field effect on the rate constant k_1 of the oxidation of NADH catalysed by horseradish peroxidase.
The error bars show the sum of the instrumental error (3.5%) and the standard error of averaging over three experimental series including 5 or 6 measurements each. Reaction conditions (all concentrations after mixing): 100 mM MES buffer (pH 5.6); $[Per^{3+}] = 1$ μmol litre^{-1}, [NADH] = 100 μmol litre^{-1}; 25 °C.

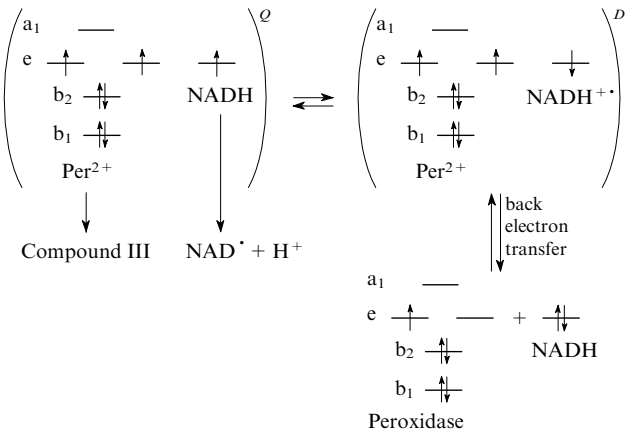
between the native HRP and NADH. Subsequent conversion of ferroperoxidase in the reaction with oxygen occurs under kinetic control ($k = 6 \times 10^4$ litre mol^{-1} s^{-1});[24, 47] therefore, the rate of formation of Compound III will be much lower than the rate of spin conversion in the pair (Per^{2+} O_2) and the process will most probably be spin-independent. The interaction of Compound III with the radical $NAD^{\bullet}$ also cannot be the source of a strong magnetic field effect, if a collision of this radical with the radical $NAD^{\bullet}$ in the bulk produces the so-called F-pair (strong magnetic field effects are not characteristic of this species).[56] However, is it possible to consider enzymatic reactions as bulk processes? The issue has not been answered so far. As to the subsequent steps of the catalytic cycle, the interaction of Compound I with NADH produces no RP and the formation of peroxidase as a result of the reaction between Compound II and NADH was not included in the analytical function at all, because this step was not observed on the time scale studied. Thus, we have formulated a hypothesis that the dependence of the rate constant k_1 on the strength of the external magnetic field is due to the effect of the magnetic field on the rate of ferroperoxidase formation in the event of single-electron transfer between HRP and NADH.

To check the assumption that the magnetic field effect is formed in the first step of the catalytic cycle, the magnetic field dependence of the magnetic field effect for the paramagnetic pair (Per^{2+} $NADH^{+\bullet}$) was calculated. The RP multiplicity was established in the framework of the theory of crystal field by analysing [34] the spin state of the central iron atom in the ferroperoxidase haem.[57]

According to modern concepts, the inorganic complex (ferroperoxidase, Per^{2+}) can be treated as a stable polyatomic system in which the effect of ligands [porphyrin (N) and histidine (N_{His})] on the central ion Fe(II) is electrostatic in nature. It was established that in a free iron atom five d-states (d_{xy}, d_{xz}, d_{yz}, d_{z^2} and $d_{x^2-y^2}$) with the same energy are split into four groups with different energies in the pyramidal field of ferroperoxidase ligands (N and N_{His}), *i.e.*, the quintuply degenerate ^{5}D-term of free ion is split in the ligand field of the pyramidal complex into four terms, namely, b_1 ($d_{x^2-y^2}$), b_2 (d_{xy}), a doubly degenerate e (d_{xz}, d_{yz}) and a (d_{z^2}).

The electron states of the RP are shown in Scheme 8. Donation of a single electron from NADH to the native HRP causes the formation of the (Per^{2+} $NADH^{+\bullet}$) RP in the quartet and doublet states. Owing to intersystem crossing, the non-reactive quartet spin state becomes a reactive doublet state. Recombination of the latter (back electron transfer) maintains the possibility of regeneration of the starting HRP and NADH.

Scheme 8



The recombination probability for the RP under study was calculated in the framework of the density matrix formalism for the two-position model. The basics of this approach have been considered above (see Section III). It was assumed [34] that the observed quartet–doublet spin evolution of the RP is governed by the electron spin interaction with the constant external magnetic field, isotropic hyperfine interaction between the unpaired electrons and magnetic nuclei in the system and by the exchange interaction between the unpaired electrons, which causes splitting of the RP terms into a doublet and a quartet.

The Hamiltonian of this system of interactions has the form:

$$\hat{H} = \omega_1\hat{S}_1 + \omega_2\hat{S}_2 + a\hat{S}_2\hat{I} + J\hat{S}_1\hat{S}_2, \qquad (21)$$

where $\omega_1 = g_1\beta\hbar^{-1}H_0$ and $\omega_2 = g_2\beta\hbar^{-1}H_0$ are the spin precession frequencies of the radicals in the pair of species with the electron spins 1 and 1/2, respectively; the *g*-factors are $g_1 = 3.25$ (for ferroperoxidase) [58] and $g_2 = 2.003$ (for the substrate radical cation);[55] $a = 32.9$ G (Ref. 55) is the constant of isotropic hyperfine interaction between the unpaired electron of the radical cation $NADH^{+\bullet}$ and the magnetic nucleus in this radical cation ($I = 1/2$); and J is the exchange integral between the doublet and quartet terms of the radical pair. It should be noted that in the general case the exchange interaction constant depends on the distance between the radicals (r) and the orientation angles (θ and φ), *i.e.*, $J = f(r,\theta,\varphi)$. For simplicity, only two possible conformational states of the complex [$Per^{2+}\cdots NADH^{+\bullet}$] characterised by the quartet–doublet (Q–D) evolution with the corresponding exchange integrals J_1 and J_2 varied from 200×10^7 to 300×10^7 rad s^{-1} were analysed.[34] The best agreement between the calculated and experimental dependences on the magnetic field applied was attained at $J_1 = 200 \times 10^7$ and $J_2 = 265 \times 10^7$ rad s^{-1}.

The spin Hamiltonian (21) commutates with the projection of the total spin of the electrons and nucleus on the direction of the external magnetic field H_0, the total projection being equal to

$$\hat{\Sigma}_Z = \hat{S}_{1Z} + \hat{S}_{2Z} + \hat{I}_Z\,. \qquad (22)$$

Because only the states with the same total projection participate in quantum transitions, all spin states of the radical pair

can be divided into the following subensembles: $\Sigma_Z = \pm 2$, $\Sigma_Z = \pm 1$ and $\Sigma_Z = 0$, only the subensembles with the total spin projections equal to $+1$, -1 and 0 contributing to the RP recombination. The behaviour of the stationary energy levels obtained by solving the Schrödinger equation with the Hamiltonian given by expression (21) for the subensembles mentioned above is illustrated in Fig. 9. Recombination probability calculations were carried out for each of the three subensembles. The final recombination probability for the RP (Per^{2+} $NADH^{+\bullet}$) was calculated as the sum of the probabilities for each subensemble with allowance for the statistical weights. The recombination probability $W(Q)$ thus calculated for the radical pair (Per^{2+} $NADH^{+\bullet}$) born in the quartet state is plotted *vs*. magnetic field in Fig. 10.

The magnetic field dependence of the recombination probability first calculated for the multispin system was interpreted as follows.[34] According to the classical radical pair theory, a radical pair born in the non-reactive quartet state is characterised by a minimum recombination probability in zero magnetic field, which is confirmed by calculations. Further increase in the recombination probability $W(Q)$ in weak magnetic fields corresponds to removal of degeneracy of the Zeeman energy levels on going from the zero to a non-zero magnetic field. Interpretation of the remaining regions of the magnetic field dependence requires consideration of the behaviour of the stationary energy levels of the subensembles with the spin projections equal to $+1$, 0 and -1 (see Fig. 9) in different magnetic fields. A decrease in separation between the energy levels in the range 200–250 G indicates that here one should expect manifestation of specific features of the recombination probability behaviour. Indeed, the theoretical curve (see Fig. 10) shows an extremum at 250 G the position of which unambiguously indicates manifestation of electron exchange interaction (J). The role of J is clearly illustrated in

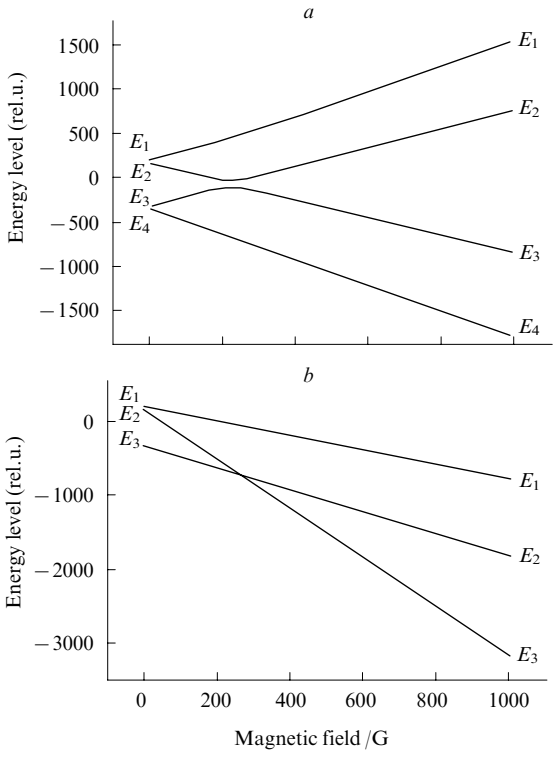


Figure 9. $Q-D$ transitions in the paramagnetic pair (Per^{2+} $NADH^{+\bullet}$)^Q.
Splitting of stationary energy levels with the total spin projection equal to 0 (*a*) and -1 (*b*) in an external magnetic field.

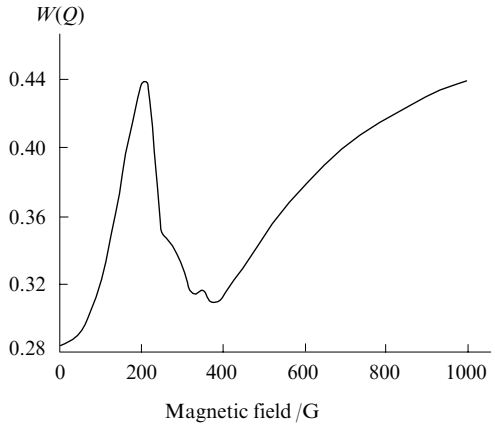


Figure 10. Calculated recombination probability $W(Q)$ for the paramagnetic pair (Per^{2+} $NADH^{+\bullet}$)^Q plotted *vs*. the magnetic field induction.

the scheme of the RP terms in the magnetic field (Scheme 9). Effective quartet–doublet mixing is possible in the external magnetic field equal to

$$H_0 = \frac{(3/2)J}{(g_1 + g_2)\beta}.$$

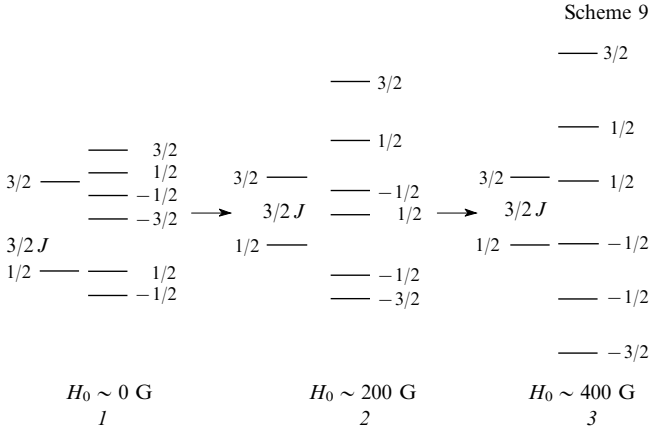
Then, after passage of the local maximum of the magnetic field dependence, the splitting between the energy levels (see Scheme 9, lines *2*, *3*) increases again due to the Zeeman interaction. As a result, transitions from the quartet state to the doublet state occur at lower rates and the recombination probability decreases (see Fig. 10). Since the frequency of transitions between states is determined by the Larmor frequency difference between the radicals in the pair

$$\omega_{QD} = \omega_1 - \omega_2 = (g_1 - g_2)\beta\hbar^{-1}H_0\,,$$

the efficiency of transitions between the non-reactive quartet state and reactive doublet state will increase again with an increase in the magnetic field. Therefore, the recombination probability increases in strong magnetic fields (400–1000 G).

The calculated recombination probability characterises the magnetic field effect on the $Q-D$ transition in the radical pair (Per^{2+} $NADH^{+\bullet}$), resulting in regeneration of the starting HRP. Therefore, the experimentally detected formation of Compound III in the bimolecular reaction between ferroperoxidase and oxygen molecule with the rate constant k_1 is described by the dependence of $[1 - W(Q)]$ on the magnetic field applied (Fig. 11). A comparison of the calculated (see

Scheme 9



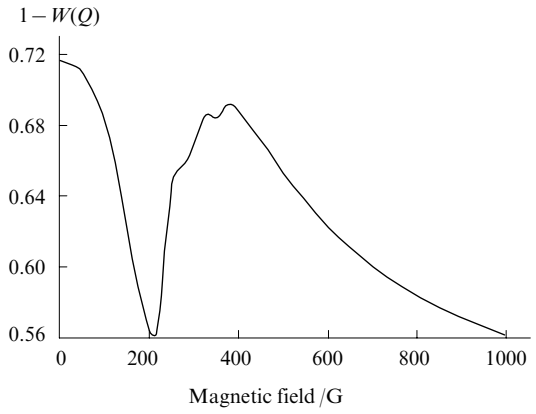


Figure 11. Escape probability $[1 - W(Q)]$ calculated for the paramagnetic pair (Per^{2+} $NADH^{\bullet+}$)^{Q} plotted *vs.* the external magnetic field (simulation of experimental dependence of the rate constant k_1 in the NADH oxidation catalysed by horseradish peroxidase on the external magnetic field).

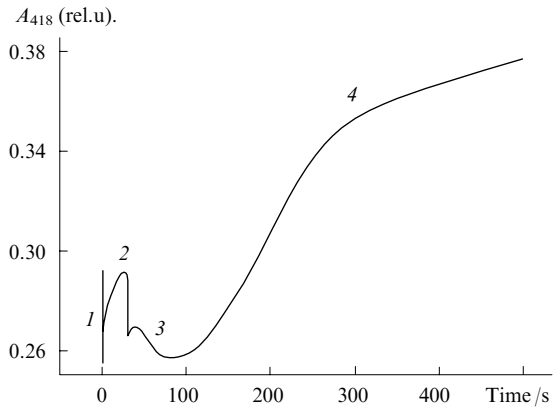


Figure 12. Kinetic curve of chemical transformations of the peroxidase reactive intermediates observed in the oxidation of nifedipine. Reaction conditions (all concentrations after mixing): 100 mM phosphate buffer (pH 7.0); $[Per^{3+}] = 1$ μmol litre^{-1}; [NF] = 15 μmol litre^{-1}; 15 vol.% EtOH; 25 °C.

Fig. 11) and experimental (see Fig. 8) magnetic field dependences of the magnetic field effect revealed qualitative agreement between them.

Thus, manifestation of the magnetic field effect, the magnetic field dependence of which is described in the framework of the radical pairs theory, indicates involvement of two paramagnetic species in the NADH oxidation catalysed by HRP. Agreement between the experimental and calculated magnetic field dependences is attained assuming formation of the magnetic field effect in the pair (Per^{2+} $NADH^{+\bullet}$). It should be emphasised that the conclusion about the presence of single-electron transfer step in the catalytic cycle of HRP also follows from the analysis of the experimental kinetic curves (see, *e.g.*, Fig. 6).

b. Magnetic field effect in the enzymatic oxidation of nifedipine, a synthetic analogue of NADH

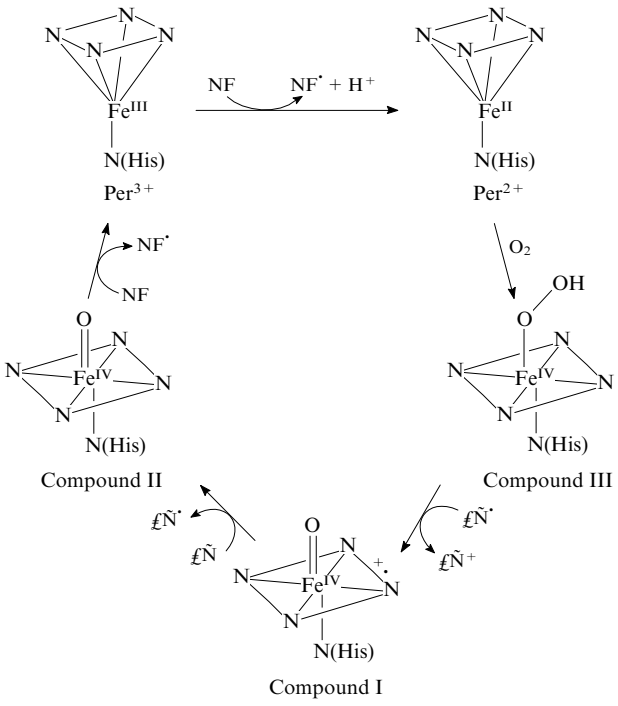
The presence of the single-electron transfer step in the catalytic cycle of HRP was additionally substantiated in the studies of the enzymatic oxidation of nifedipine [3,5-di(methoxycarbonyl)-2,3-dimethyl-4-nitrophenyl-1,4-dihydropyridine], a synthetic analogue of NADH, which were also carried out using the stopped-flow technique.[35]

The characteristic kinetic curve of the formation and decay of the peroxidase reactive intermediates in the oxidation of NF observed at 418 nm (maximum absorption wavelength of Compounds II and III) is shown in Fig. 12. The kinetic curve was analysed using the approach similar to that described above for the enzymatic oxidation of NADH. It was found that the NF oxidation kinetics can be explained by a sequence of elementary steps[35] identical to that proposed [34] for the description of the enzymatic NADH oxidation (Scheme 10).

A decrease in the absorbance in the initial portion of the kinetic curve (see Fig. 12, region *1*) is associated [35] with the formation of ferroperoxidase ($\varepsilon_{418} = 62$ litre mmol^{-1} cm^{-1}) upon electron transfer from NF to the haem iron atom of the enzyme, which also leads to formation of the radical cation $NF^{+\bullet}$. Further increase in absorbance (see Fig. 12, region *2*) occurs during the reaction of ferroperoxidase with the dissolved oxygen to give Compound III ($\varepsilon_{418} = 115$ litre mmol^{-1} cm^{-1}). In the next stage, a neutral nifedipine radical ($NF^{\bullet}$) formed as a result of deprotonation of the $NF^{+\bullet}$ radical cation is involved in the reaction. A CIDNP study [35] of the transformations of $NF^{+\bullet}$ in a model process showed that the deprotonation takes a few nanoseconds. The radical thus formed reacts with Compound III, which causes a dramatic decrease in absorbance (see Fig. 12, region *3*) due to the formation of Compound I ($\varepsilon_{418} = 35$ litre mmol^{-1} cm^{-1}). Single-electron reduction of Compound I by the second NF molecule results in the formation of Compound II ($\varepsilon_{418} = 115$ litre mmol^{-1} cm^{-1}); as a result, absorbance increases (see Fig. 12, region *4*). No rapid regeneration of the native HRP occurs in the course of the reaction between NF and Compound II; this seems to be due to inactivation of the enzyme in the reactions with radicals.[35]

At the same time, a comparison of the kinetic curves of the HRP-catalysed oxidation of NADH and NF (*cf.* Figs 6 and 12) clearly demonstrates significant differences between these processes. For instance, it should be noted that each step of the NF oxidation is much slower than the corresponding step of the reaction of HRP with NADH. This can be explained by several factors, in particular, a lower concentration of NF compared to the NADH concentration (15 and 100 μmol

Scheme 10



litre^{-1}, respectively) and by the use of the phosphate buffer (pH 7.0) instead of the MES buffer (pH 5.6) in which HRP is more reactive.[24] The activity of the enzyme is also affected by the addition of EtOH (15%) necessary for solubilisation of NF, because ethanol acts as aninhibitor of HRP.

The effective rate constants for the formation of Compounds III and I were determined by treating the kinetic curves measured at different strengths of external constant magnetic field using the Levenberg–Marquardt non-linear regression algorithm. In this case, the kinetic model for two successive unimolecular stages was considered

$$\mathrm{Per}^{2+} \xrightarrow{k_1} \text{Compound III} \xrightarrow{k_2} \text{Compound I}, \qquad (23)$$

and fitting was performed only for the portion of the kinetic curve that describes the kinetics of formation and consumption of Compound III (see Fig. 12,*2*). It turned out that, similarly to the NADH oxidation, only the rate constant k_1 is magnetosensitive. The experimental magnetic field dependence of the magnetic field effect (Fig. 13) agrees with the theoretical curve (see Fig. 11) assuming that the magnetic field effect is formed in the pair (Per^{2+} $\mathrm{NF}^{+\bullet}$) (Scheme 11).

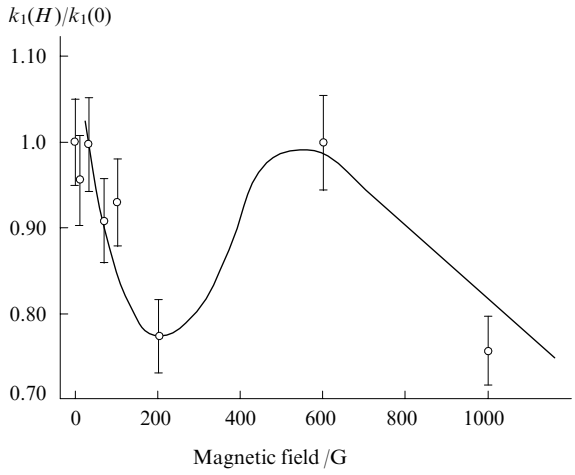
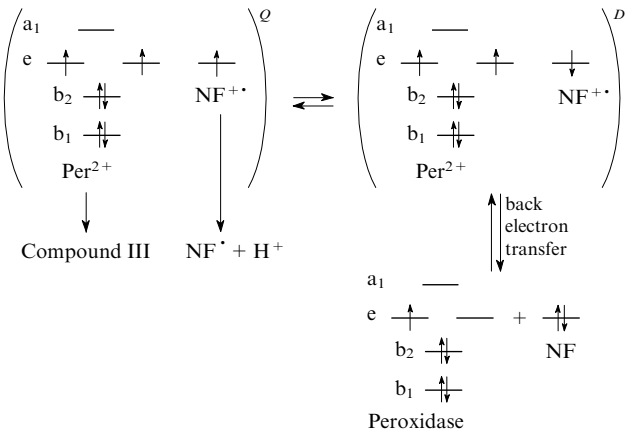


Figure 13. Magnetic field effect on the rate constant k_1 for oxidation of nifedipine catalysed by horseradish peroxidase.
The error bars show the sum of the instrumental error (3.5%) and the standard error of measurements (averaged over three experimental series including 5–6 measurements each). Reaction conditions (all concentrations after mixing): 100 mM phosphate buffer (pH 7.0); [Per^{3+}] = 1 μmol litre^{-1}, [NF] = 15 μmol litre^{-1}; 15 vol.% EtOH; 25 °C.

Thus, we can conclude that, similarly to the case of NADH, back electron transfer in the pair (Per^{2+} $\mathrm{NF}^{+\bullet}$) occurs in the doublet state of the RP, whereas the quartet spin state is the source of Compound III (see Schemes 8 and 11).

An important conclusion that can be drawn based on the analysis of the magnetic field effects detected in the enzymatic oxidation of the natural substrate of horseradish peroxidase, NADH and its synthetic analogue nifedipine, is as follows. In the absence of hydrogen peroxide, the enzymatic oxidation begins with electron transfer between the native HRP and substrate rather than the reaction with trace amounts of hydrogen peroxide formed as a result of NADH (NF) oxidation by dissolved oxygen, which was earlier considered as the initiation step.[24,47]

Scheme 11



5. Studies of the magnetic field effect on the NADH oxidation by horseradish peroxidase in an oscillatory reaction

Of particular interest is the oscillatory reaction of NADH with O_2 catalysed by HRP, which has been a subject of intensive studies. The reaction dynamics shows a number of specific features dependent on the reaction conditions.[59] The peroxidase–oxidase system exhibits bistability (*i.e.*, two different dynamic states can exist under identical experimental conditions).[24] Examples are provided by two coexisting stable states[60] and a stable state that coexists with periodic oscillations.[61]

It should be noted that oscillations in an enzymatic system are complex biochemical processes that often involve up to 10–20 coupled elementary reactions associated with either generation or consumption of free radicals. To study the oscillatory oxidation reaction, NADH and oxygen are continuously added to a stirred aqueous buffer solution (pH 5.0–6.5) containing HRP and an initiator (usually, an aromatic compound, such as 4-hydroxybenzoic acid, melatonin or Methylene Blue). Under such conditions one can observe oscillatory processes.[62,63] Reaction products include hydrogen peroxide and a number of oxygen-containing free radicals.[24] The formation of reactive oxygen species is undesired for cell metabolism due to their destructive properties; additionally, too high concentrations of H_2O_2 cause inactivation of HRP in the reaction of peroxide with Compound I.[64,65] A mechanism of inactivation of the enzyme by free-radical species is described[66] and the role of aromatic initiators in this process was discussed. It was assumed that inactivation is a result of the interaction between reactive oxygen species and the side chains of amino acid molecules and peroxidase sugar residues.[67] It was shown that reduction of inactivation of the enzyme in the oscillatory system is followed by an increase in the concentration of melatonin, which rapidly reacts with the oxygen radical species formed, *e.g.*, $OH^{\bullet}$.

Studies of the effect of external magnetic field on the rate of oscillatory processes are of particular interest. It is quite important to answer the question whether or not does the enzymatic oxidation retain its magnetic field sensitivity in the oscillatory reaction and what is the role of its initiator?

The effect of external magnetic field on the consumption of oxygen in the oscillatory reaction of NADH with O_2 catalysed by horseradish peroxidase was first observed by Olsen *et al.*[7] The oxygen concentrations in the solution during the reaction in a magnetic field of strength varied from 0 to 4000 G were measured with a Clark-type oxygen electrode. The frequency and amplitude of [O_2]-oscillations are plotted *vs.* magnetic field in Fig. 14. The plots exhibit characteristic extrema at about

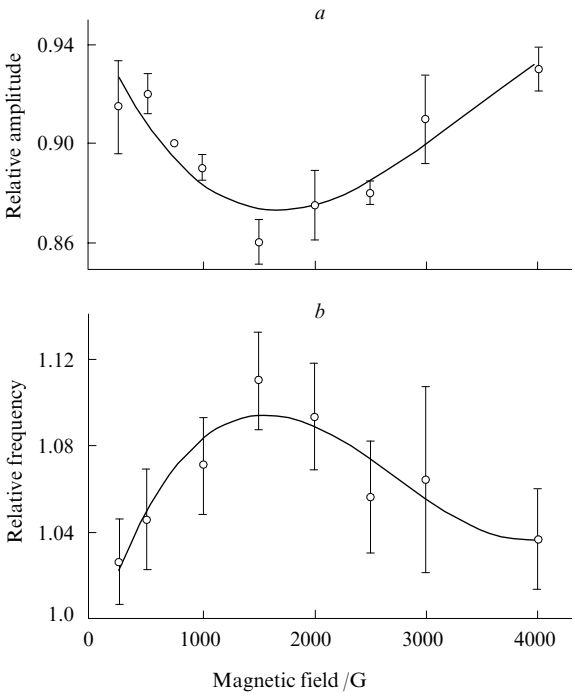


Figure 14. Effect of the magnetic field on the [O_2]-oscillation amplitude (*a*) and frequency (*b*).

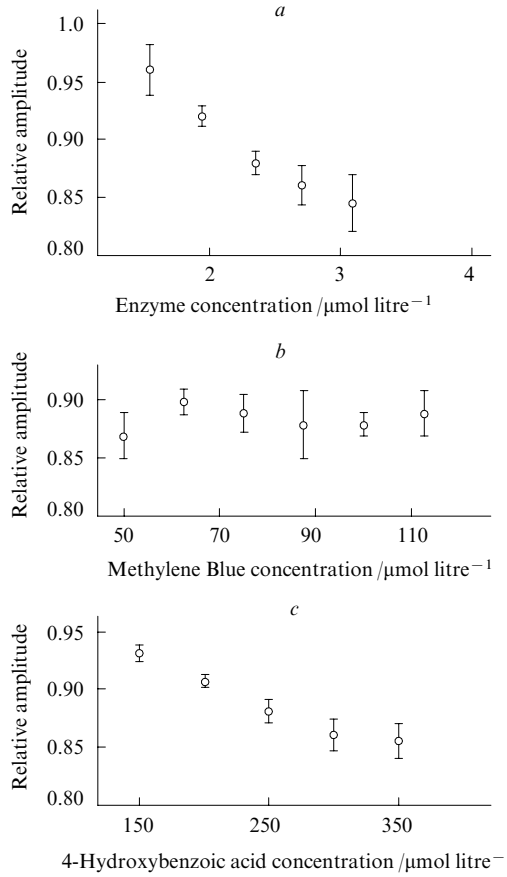


Figure 15. Effect of the concentrations of peroxidase (*a*), Methylene Blue (*b*) and 4-hydroxybenzoic acid (*c*) on the [O_2]-oscillation amplitude.

1500 G. The magnetic field effect is independent of the pH value, NADH and methylene blue concentrations, being sensitive to the change in the concentrations of HRP and initiator, 4-hydroxybenzoic acid (Fig. 15).[7] It was assumed that the magnetic field sensitive steps of the oxidase oscillatory reaction can involve radical pairs including Compounds I and II.

$$\text{Compound I} + \text{NADH} \longrightarrow \text{Compound II} + \text{NAD}^{\bullet}, \quad (24)$$

$$\text{Compound II} + \text{NADH} \longrightarrow \text{Per}^{3+} + \text{NAD}^{\bullet} + \text{H}_2\text{O}. \quad (25)$$

The dependence of the magnetic field effects observed on the concentration of phenolic compounds can be explained [7] by participation of the latter in electron transfer between Compounds I and II in the course of the reactions

$$\text{Compound I} + \text{PhOH} \longrightarrow \text{Compound II} + \text{PhO}^{\bullet}, \quad (26)$$

$$\text{Compound II} + \text{PhOH} \longrightarrow \text{Per}^{3+} + \text{PhO}^{\bullet} + \text{H}_2\text{O}, \quad (27)$$

$$\text{PhO}^{\bullet} + \text{NADH} \longrightarrow \text{PhOH} + \text{NAD}^{\bullet}. \quad (28)$$

Note that the lack of a theoretical magnetic field dependence [7] precludes drawing unambiguous conclusions about the magnetic field sensitive step of the process. We can only point to significant differences between the magnetic field dependences obtained for the steady-state HRP-catalysed oxidation of organic substrates [$Me_2C{=}CH(OSiMe_3)$, NADH and NF] (see above) and measured in the course of oscillatory NADH oxidation by peroxidase (*cf.* Figs 5, 8, 13 and 14). These differences can be explained taking into account the fact that in the course of the steady-state substrate oxidation catalysed by HRP the magnetic field effects were detected in particular stages of the catalytic cycle, whereas in the case of oscillatory oxidase reaction [7] the effect of the magnetic field on the rate of the overall process was studied. The observed dependence of the magnetic field effect on the concentration of phenol [7] gives grounds to assume the presence of a number of magnetic field sensitive steps in the reaction under study, which can be a possible reason for the differences between the magnetic field dependences (see above).

V. Conclusion

The material presented in this review demonstrates the applicability of spin chemistry methods to studies of paramagnetic species involved in the enzymatic oxidation. Manifestation of the magnetic field effects in enzymatic reactions points to a relationship between the reactivity and spin states of paramagnetic intermediates of the catalytic cycle of HRP. For instance, it was shown that in the absence of hydrogen peroxide the enzymatic oxidation is initiated by electron transfer between the native enzyme and substrate. In addition, observation of the CIDNP effect [68] in the interaction of photoexcited NADH with horseradish peroxidase not only substantiates the conclusion about the possibility of electron transfer between the peroxidase haem and NADH, but also proves the formation of the radical cation $NADH^{+\bullet}$, which was postulated in the analysis of the magnetic field effect.[34]

Agreement between the experimental magnetic field dependences of the magnetic field effects detected in studies of the oxidation catalysed by horseradish peroxidase and the results of calculations in the approximations used in the radical pair theory makes it possible to draw some conclusions on the subtle mechanisms of the processes occurring in the catalytic cycle.

For instance, the molecular dynamics of partners in the enzyme–substrate complex should meet key requirements of the radical pair theory, namely, the rates of electron transfer in

enzymatic processes should be similar to the diffusion rates (otherwise, manifestation of the magnetic field effect is impossible), and the lifetimes of reactive intermediates should not exceed a few microseconds. The calculated magnetic field dependence [34] showed the best agreement with experimental data at submicrosecond lifetimes of the pair of paramagnetic species. Since the theoretical model is based on the concept of diffusion of partners, one should assume that reactants to some extent retain their mobilities in the enzymatic processes. In addition, extrema present in the magnetic field dependences at a magnetic fields strength of the order of hundreds of Gauss attest unambiguously to electron exchange interaction between partners in the catalytic cycle of horseradish peroxidase. This is most probably due to the substrate binding in the active site of the enzyme. One can hope that analysis of positions of the extrema in the plots of the magnetic field dependences of the magnetic field effects in the enzymatic oxidation of different substrates in combination with the data obtained by other methods for detection of enzyme–substrate interactions will in the future allow one to formulate criteria for evaluation of binding.

An important result is also the observation of the magnetic field effect in the oscillatory reaction of NADH oxidation by peroxidase. To draw unambiguous conclusions about changes in the processes occurring in the catalytic cycle of peroxidase in the course of oscillatory oxidation in the presence of additional sources of free radicals (Methylene Blue, phenol, *etc.*), one should study the effect of magnetic field on interconversions of HRP-Compounds, as was done for the steady-state oxidation. Differences in the character of the magnetic field dependences of the magnetic field effects detected in particular steps of the steady-state oxidation and in the oscillatory reaction (overall reaction) suggest the presence of non-identified magnetic field-sensitive steps of the oscillatory process.

Summing up, it seems useful to list other enzymatic processes that are most promising for spin chemistry studies in order to establish the mechanisms of elementary reactions. These are first of all the processes involving cytochrome P450 and cytochrome *c* oxidase,[69] which have well-established paramagnetic intermediates and are structurally similar to the peroxidase Compounds.

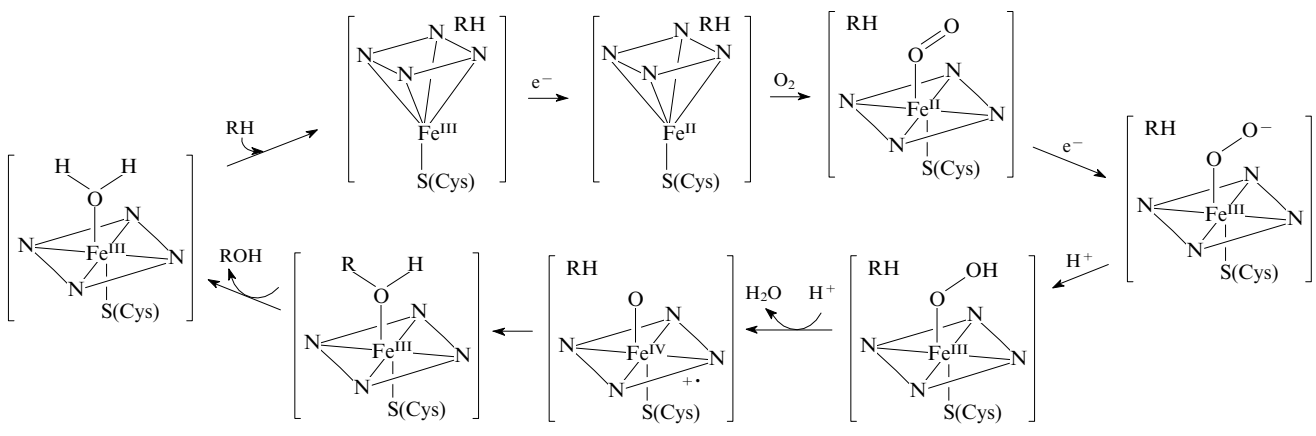
Cytochrome P450 plays an important role in enzymatic catalysis by metal-containing enzymes:[47] it participates in hydroxylation of xenobiotics, such as saturated and unsaturated fatty acids,[70] steroid hormones,[71] cholesterol,[72] bile acids [73] and prostaglandins.[74] Despite the fact that the mechanism of substrate oxidation catalysed by cytochrome P450 has been studied for two decades, it is still to be clarified. The catalytic cycle of hydroxylation by cytochrome P450 involves radical steps (Scheme 12).[47] For instance, it was assumed that the substrate oxidation starting with the binding by the $Fe^{IV}=O$ group can proceed by either a radical ('radical clock' of norcarane and spiro-2,5-octane) or cationic mechanism. This results in different reaction products.[75]

In addition, the state (high-spin or low-spin) of the active site of enzyme in the enzyme–substrate complex and the changes in this spin state upon reduction as a result of single-electron transfer are still unclear.[48]

Spin chemistry methods can be useful in studies of the mechanism of hydroxylation by cytochrome P450. Indeed, by analysing the magnetic field dependences of the effective rates of the interconversions of reactive intermediates in the catalytic cycle of cytochrome and the dependences of the product ratio of substrate oxidation on the strength of external magnetic field it is possible to determine the spin state of iron in the enzyme active site and the spin configurations of the cytochrome reactive intermediates. Analysis of the magnetic field effects also permits monitoring of the effect of electron spins of the cytochrome P450 intermediates on the ratio of the radical and cationic oxidation pathways.

Yet another enzymatic system that can (potentially) be studied by spin chemistry methods is the cytochrome *c*–cytochrome *c* oxidase system. The role of electron-transfer steps in this system has been a subject of considerable discussion.[76] The case in point is the generation of the chemical potential within a mitochondrion in the respiratory chain, which produces most of the free energy necessary for life processes in aerobic organisms by combining electron transfer and the synthesis of adenosine triphosphate (ATP).[77] The electron transfer steps involving proteins and highly reactive paramagnetic cytochrome intermediates in the mitochondrial chain is the key to this process. At present, we know that the cytochrome *c*–cytochrome *c* oxidase system contains four cofactors, namely, two copper atoms, a low-spin haem group and a haem group that is a constituent of the protein containing haem iron and copper.[78] During the reaction, the system adds four electrons in four successive single-electron steps, thus reducing oxygen to water, and at the same time creates a proton gradient across the inner mitochondrial membrane (see Scheme 12). Recently,[76] the reactive paramagnetic intermediates of cytochrome *c* oxidase were determined spectroscopically. These are peroxide linkers (Compounds P_M and P_R), ferryl intermediate (Compound F), ground state (Compound O), a singly reduced form (Compound E) and a doubly reduced form (Compound R). Interconversions of these species involve single-electron transfer between the paramagnetic intermediates of the enzyme.[69] Based on the results of structural studies of cytochrome *c*,[79,80] possible pathways of electron transfer in the enzyme and a mechanism involving interconversion of cytochrome *c* intermediates were proposed; however, all these assumptions

Scheme 12



require additional proofs. It is desired to prove them in studies of the dynamics of the processes occurring in the cytochrome *c*–cytochrome *c* oxidase system. One can expect that spin chemistry methods, such as the magnetic field effect and chemically induced dynamic nuclear polarisation, will provide the possibility of establishing the role of the electron spin states in different paramagnetic intermediates and obtaining information on the subtle mechanism of functioning of the system of mitochondrial breath, namely, the cytochrome *c*–cytochrome *c* oxidase system.

References

1. K M Salikhov, Yu N Molin, R Z Sagdeev, A L Buchachenko *Spin Polarization and Magnetic Effects in Radical Reactions* (Amsterdam: Elsevier, 1984)
2. U Steiner, T Ulrich *Chem. Rev.* **89** 51 (1989)
3. I P Gragerov, L A Kiprianova, A F Levit *Khimicheskaya Polyarizatsiya Yader v Issledovaniyakh Mekhanizma Reaktsii Organicheskikh Soedinenii* (Chemically Induced Nuclear Polarisation in the Mechanistic Studies of Organic Reactions) (Kiev: Naukova Dumka, 1985)
4. M B Taraban, V I Rakhlin, T V Leshina *Ross. Khim. Zh.* **43** 80 (1999) [a]
5. T V Leshina, O S Volkova, M B Taraban *Izv. Akad. Nauk, Ser. Khim.* 1830 (2001) [b]
6. C B Grissom *Chem. Rev.* **95** 3 (1995)
7. A C Møller, A Lunding, L F Olsen *Phys. Chem. Chem. Phys.* **2** 3443 (2000)
8. P J Hore, S L Winder, C H Roberts, C M Dobson *J. Am. Chem. Soc.* **119** 5049 (1997)
9. T T Harkins, C B Grissom *Science* **263** 958 (1994)
10. O A Snytnikova, Yu P Tsentalovich, R Z Sagdeev *Appl. Magn. Reson.* **26** 183 (2004)
11. C E Cross, B Halliwell, E T Borish *Ann. Intern. Med.* **107** 526 (1987)
12. S Matsuzaki, L I Szweda *Biochemistry* **46** 1350 (2007)
13. G R Castro, B Panilaitis, E Bora, D L Kaplan *Mol. Pharm.* **4** 33 (2007)
14. C Brady, S E J Bell, C Parsons, S P Gorman, D S Jones, C P McCoy *J. Phys. Chem. B* **111** 527 (2007)
15. E Gaggelli, H Kozlowski, D Valensin, G Valensin *Chem. Rev.* **106** 1995 (2006)
16. A Zhang, J L Neumeyer, R J Baldessarini *Chem. Rev.* **107** 274 (2007)
17. D P Smith, G D Ciccotosto, D L Tew, M T Fodero-Tavolotti, T Johanssen, C L Masters, K J Barnham, R Cappai *Biochemistry* **46** 2881 (2007)
18. M Lukin, C de los Santos *Chem. Rev.* **106** 607 (2006)
19. C J Burrows, J G Muller *Chem. Rev.* **98** 1109 (1998)
20. B Halliwell, J M C Gutteridge *Methods of Enzymology* Vol. 186 (Eds L Parker, A N Glazer) (San Diego, CA: Academic Press, 1990) p. 1
21. A R Cross *The Molecular Basis of Oxidative Damage by Leukocytes* (Eds A J Jesaitis, E A Dratz) (Boca Raton, FL: CRC Press, 1992. P. 215
22. *The Respiratory Burst and Its Physiological Significance* (Eds A J Sbarra, R R Strauss) (New York: Plenum Press, 1988)
23. T M Penning, S T Ohnishi, T Ohnishi, R G Harvey *Chem. Res. Toxicol.* **9** 84 (1996)
24. A Scheeline, D L Olson, E P Williksen, G A Horras, M L Klein, R Larter *Chem. Rev.* **97** 739 (1997)
25. R P Mason, M B Kadiiska *Biological Magnetic Resonance. Biomedical EPR. Part A: Free Radicals, Metals, Medicine and Physiology* Vol. 23 (Eds S S Eaton, G R Eaton, L J Berliner) (New York: Kluwer Academic, Plenum, 2005) p. 93
26. H Hirata, H Fujii *Curr. Org. Chem.* **10** 521 (2006)
27. J H Dawson *Science* **240** 433 (1988)
28. O S Volkova, M B Taraban, V F Plyusnin, T V Leshina, M P Egorov, O M Nefedov *J. Phys. Chem. A* **107** 4001 (2003)
29. I M Magin, P A Purtov, A I Kruppa, T V Leshina *J. Phys. Chem. A* **109** 7395 (2005)
30. A L Buchachenko, V L Berdinsky *J. Phys. Chem.* **100** 18292 (1996)
31. A L Buchachenko, V L Berdinsky *Chem. Rev.* **102** 603 (2002)
32. *Biological Effects of Electromagnetic Fields* (Ed. P Stavroulakis) (Berlin: Springer, 2003)
33. M B Taraban, T V Leshina, M A Anderson, C B Grissom *J. Am. Chem. Soc.* **119** 5768 (1997)
34. M S Afanasyeva, M B Taraban, P A Purtov, T V Leshina, C B Grissom *J. Am. Chem. Soc.* **128** 8651 (2006)
35. M S Afanasyeva, M B Taraban, N E Polyakov, P A Purtov, T V Leshina, C B Grissom *J. Phys. Chem. B* **110** 21232 (2006)
36. N E Polyakov, T V Leshina, L Kispert *RIKEN Rev.* **44** 140 (2002)
37. M B Taraban, A I Kruppa, N E Polyakov, T V Leshina, V Lūsis, D Muceniece, G Duburs *J. Photochem. Photobiol., A* **73** 151 (1993)
38. A I Kruppa, M B Taraban, N E Polyakov, T V Leshina, V Lūsis, D Muceniece, G Duburs *J. Photochem. Photobiol., A* **73** 159 (1993)
39. N E Polyakov, M B Taraban, A I Kruppa, N I Avdievich, V V Mokrushin, P V Schastnev, T V Leshina, V Lūsis, D Muceniece, G Duburs *J. Photochem. Photobiol., A* **74** 75 (1993)
40. N E Polyakov, A I Kruppa, T V Leshina, V Lūsis, D Muceniece, G Duburs *J. Photochem. Photobiol., A* **111** 61 (1997)
41. V P Komov, V N Shvedova *Biokhimiya* (Moscow: Drofa, 2004)
42. S S Petrova, A I Kruppa, T V Leshina *Chem. Phys. Lett.* **385** 40 (2004)
43. P A Purtov, A B Doktorov *Chem. Phys.* **178** 47 (1993)
44. P A Purtov, Doctoral Thesis in Physical and Mathematical Sciences, Institute of Chemical Kinetics and Combustion, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, 2000
45. S A Mikhailov, P A Purtov, A B Doktorov *Chem. Phys.* **166** 35 (1992)
46. K Yokota, I Yamazaki *Biochemistry* **16** 1913 (1977)
47. I Schlichting, J Berendzen, K Chu, A M Stock, S A Maves, D E Benson, R M Sweet, D Ringe, G A Petsko, S G Sligar *Science* **287** 1615 (2000)
48. H A Harbury *J. Biol. Chem.* **225** 1009 (1957)
49. S Colonna, N Gaggero, C Richelmi, P Pasta *Trends Biotechnol.* **17** 163 (1999)
50. L Casella, M Gullotti, R Ghezzi, S Poli, T Beringhelli, S Colonna, G Carrea *Biochemistry* **31** 9451 (1992)
51. G Battistuzzi, M Borsari, A Ranieri, M Sola *J. Am. Chem. Soc.* **124** 26 (2002)
52. F I Ataullakhanov, A M Zhabotinskii *Biofizika* **20** 596 (1975) [c]
53. J Gębicki, A Marcinek, J Zielonka *Acc. Chem. Res.* **37** 379 (2004)
54. K Schulten, P G Wolynes *J. Chem. Phys.* **68** 3293 (1978)
55. S Fukuzumi, T Tanaka *Photoinduced Electron Transfer Reactions. Part C. Photoinduced Electron Transfer Reactions: Organic Substrates* (Eds M A Fox, M Chanon) (Amsterdam: Elsevier, 1988) Ch. 4.10
56. A L Buchachenko, R Z Sagdeev, K M Salikhov *Magnitnye i Spinovye Effekty v Khimicheskikh Reaktsiyakh* (Magnetic and Spin Effects in Chemical Reactions) (Novosibirsk: Nauka, 1978)
57. I B Bersuker *Elektronnoe Stroenie i Svoistva Koordinatsionnykh Soedinenii* (Electronic Structure and Properties of Coordination Compounds) (Leningrad: Khimiya, 1986)
58. E G Pavel, N Kitajima, E I Solomon *J. Am. Chem. Soc.* **120** 3949 (1998)
59. M J B Hauser, L F Olsen *Transport and Structure — Their Competitive Role in Biophysics and Chemistry* (Eds S C Müller, J Parisi, W Zimmermann) (Berlin: Springer, 1999)
60. H Degn *Nature (London)* **217** 1047 (1968)
61. B D Aguda, L-L H Frisch, L F Olsen *J. Am. Chem. Soc.* **112** 6652 (1990)
62. S Nakamura, K Yokota, I Yamazaki *Nature (London)* **222** 794 (1969)
63. L F Olsen, H Degn *Biochim. Biophys. Acta* **523** 321 (1978)
64. J N Rodriguez-Lopez, J Hernández-Ruiz, F Garcia-Cárnovas, R N F Thorneley, M Acosta, M B Arnao *J. Biol. Chem.* **272** 5469 (1997)
65. J Hernández-Ruiz, M B Arnao, A N P Hiner, F Garcia-Cvrnovas, M Acosta *Biochem. J.* **354** 107 (2001)
66. L F Olsen, M J B Hauser, U Kummer *Eur. J. Biochem.* **270** 2796 (2003)
67. C L Hawkins, M J Davies *Biochim. Biophys. Acta* **1504** 196 (2001)
68. M S Afanas'eva, P A Purtov, M B Taraban, T V Leshina, Ch B Grissom *Izv. Akad. Nauk, Ser. Khim.* 1090 (2006) [b]

69. P R Rich, S E J Rigby, P Heathcote *Biochim. Biophys. Acta* **1554** 137 (2002)
70. M L Das, S Orrenius, L Ernster *Eur. J. Biochem.* **4** 519 (1968)
71. R I Salganik, S V Nedel'kina, S V Argutinskaya, L P Kusmartseva *Voprosy Med. Khim.* **20** 135 (1974)
72. G S Boyd, A C Brownie, C R Jefcoate, E R Simpson *Biochem. J.* **125** 1 (1971)
73. K Einarason, G Johansson *FEBS Lett.* **4** 177 (1969)
74. U Israelsson, H Hamberg, B Samuelsson *Eur. J. Biochem.* **11** 390 (1969)
75. M Newcomb, R Shen, Y Lu, M J Coon, P F Hollenberg, D A Koop, S J Lippard *J. Am. Chem. Soc.* **124** 6879 (2002)
76. B G Malmstrom *Chem. Rev.* **90** 1247 (1990)
77. H S Carr, D R Winge *Acc. Chem. Res.* **36** 309 (2003)
78. M Hartmut *Proc. Natl. Acad. Sci. USA* **95** 12819 (1998)
79. T Tsukihara, H Aoyama, E Yamashita, T Tomazaki, H Yamaguchi, K Shinzawa-Itoh, R Nakashima, R Yaono, S Yoshikawa *Science* **272** 1136 (1996)
80. S Iwata, C Ostermeier, B Ludwig, H Michel *Nature (London)* **376** 660 (1995)

[a] — *Mendeleev Chem. J. (Engl. Transl.)*
[b] — *Russ. Chem. Bull., Int. Ed. (Engl. Transl.)*
[c] — *Biophysics (Engl. Transl.)*