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Activation of oxygen by metalloenzymes and their models

N B Kozlova, Yu I Skurlatov
Institute of Chemical Physics, USSR Academy of Sciences, Moscow

ABSTRACT. Studies on the activation of molecular oxygen by metalloenzymes and their models are surveyed.
The bibliography includes 105 references.

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I. Introduction

The description of biological oxidation-reduction processes at the elementary step level is virtually impossible owing to the complexity of the protein objects studied, but the various kinds of model systems which reproduce in a simplified form particular functional features of biological systems can help.

In the present review, we shall confine ourselves to the examination of problems of the modelling of the elementary mechanisms of the action of metalloenzymes catalysing processes with participation of O_2.[1] Attention has been traditionally concentrated on these processes as a consequence of the great importance of O_2 for the vital activity of organisms, in connection with the prospect of using O_2 as an ecologically pure oxidant, and also because of the abundance of processes with participation of O_2 in the environment.

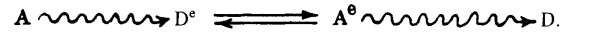
In examining metalloenzyme systems and their models, one must take into account the chemical and biological characteristics of each elementary step. The chemical characteristics are of a general character for many enzyme systems, while the biological characteristics define the specificity of the given system, determined by the structural features of the active centre and of the entire metalloenzyme molecule, and the dynamic properties of macromolecules with their multipoint intermolecular interactions. It would be premature to make any kind of generalisations for complex systems of this kind. On the other hand, the chemical characteristics of the elementary step make it possible to consider from general standpoints the mechanisms of oxidation-reduction reactions in both enzyme and model systems. This postulate constitutes the basis of the review. Its aim is an attempt to represent biological oxidation-reduction processes in terms of the electron transfer step.

The theory of outer-sphere electron transfer has been developed[2] and its quantitative aspect has been applied.[3]

Outer-sphere electron transfer takes place in a collision (unoriented and unstabilised) complex as a consequence of the fluctuations in the dipoles of the medium which equalise the energy levels of the initial and final states:[2,3]

$$A + D^e \rightarrow (A...D^e \longleftrightarrow A^e...D)^{\neq} \rightarrow A + D^e,$$

where A is the electron acceptor and D^e the one-electron donor. The energy of the reorganisation of the solvent in the outer-sphere electron transfer depends quadratically on the number of electrons transferred. By virtue of this, the transfer of two electrons via the outer sphere mechanism is relatively ineffective although it has been detected in many instances. On the other hand, when one speaks of reactions with participation of O_2, in enzyme systems its reduction takes place as a two-electron process with formation of $O_2^{2-}(H_2O_2)$ or effectively as a four-electron process with formation of $O^{2-}(H_2O)$. These reactions occur mostly as a result of the formation of complexes between O_2 and reduced variable valence metal ions—usually copper and iron ions. In general, one may call such reactions inner-sphere electron transfer reactions. Intramolecular electron transfer is sometimes also implied by the inner-sphere transfer:



Such transfer usually takes place either when the molecule contains bridging electron-conducting groups (with a "rigid" molecular structure) or as a result of intramolecular collisions of the donor–acceptor functional groups (flexible dynamic structure).

Consideration of complexes between electron-donating and electron-accepting species led Mulliken to introduce the concept of charge transfer complexes (CTC).[4,5] The studies by one of the authors of the present review have been devoted to the role of such complexes in oxidation-reduction reactions.[6,7] Subsequently oxidation-reduction reactions with participation of O_2 in model and enzyme systems were considered from the standpoint of ideas postulating intramolecular electron transfer as a chemical step in which the products of the final electron transfer are formed on decomposition of the intermediate donor–acceptor complex with *partial* charge transfer (PCTC):

$$A + D^e \rightleftarrows (AD)^e \rightarrow A^e + D,$$

where $(AD)^e$ is a PCTC with one-electron inner-sphere transfer.

The final two-electron process can be represented analogously:

$$A + D^{2e} \rightleftarrows (AD)^{2e} \rightarrow A^{2e} + D.$$

The fundamental difference between the "activated" collision complex and the PCTC consists in the mutual orientation of the donor and acceptor and the distance between them or, more precisely, in the extent of the electron-exchange interaction (V) of the donor–acceptor orbitals. In the outer-sphere case, the reactants approach one another to a distance $\gtrsim 4$ Å and $V < kT$; in the inner-sphere case, the distance between the reacting species is less than the sum of the van der Waals or ionic radii but is greater than the sum of the covalent radii of the atoms in contact, while the energy of the exchange donor–acceptor interaction exceeds the energy of the thermal motion: $kT < V \lesssim 10\,kT$. Evidently, depending on the values of V, the intermolecular transfer can take place either via the "outer-sphere" mechanism or via the "inner-sphere" mechanism [a partial charge transfer (PCT) state is produced]. Intramolecular electron transfer over long distances is the most typical property of biological electron-transport systems.

II. Characteristic features of inner-sphere electron transfer reactions and of intermediate complexes with partial charge transfer

We shall consider the distinctive features of the donor–acceptor PCTC by virtue of which they play an important role in metal complex and especially metalloenzyme catalysis. Their main feature is the existence of a rapidly established (thermal) equilibrium between the ground (N) and electronically excited (E) states. At the present time, complexes of this kind are referred to as examples of redox isomerism. In the ground state, the complex decomposes into the initial reactants, while in the electroncially excited state it decomposes into the electron transfer products:

$$A + D^{e} \rightleftarrows (AD)^{e}_{N} \overset{kT}{\rightleftarrows} (AD)^{e}_{E} \rightarrow A^{e} + D,$$
$$A + D^{2e} \rightleftarrows (AD)^{2e}_{N} \overset{kT}{\rightleftarrows} (AD)^{2e}_{E} \rightarrow A^{2e} + D.$$

As a result, the PCTC can interact with any "third" species and, existing simultaneously in two (ground and electronically excited) states, it can manifest the properties of both the initial reacting species and of the electron transfer products. Such "duality" in the properties of the PCTC has been clearly interpreted in terms of the ideas of Mulliken's resonance theory,[4,5] according to which the PCTC can be represented by a "superposition" of states without charge transfer and with complete charge transfer:

$$(AD)^{e} \equiv (AD^{e} \longleftrightarrow A^{e}D),$$
$$(AD)^{2e} \equiv (AD^{2e} \longleftrightarrow A^{2e}D).$$

Identical contributions of the limiting structures obtain when the energies of the donor–acceptor pair without (i) and with (f) complete charge transfer in the absence of electron-exchange interaction are equal.[6,8] The difference between the energies of these states (ΔH_e) can be estimated from thermodynamic considerations:[5]

$$\Delta H_e \simeq \Delta\sigma^0_{el} + RT \ln (K_{c,i}/K_{c,f}),$$

where $\Delta\sigma_{el}$ is the difference between the redox potentials of the donor and the acceptor and the constants K_c are the stability constants of the hypothetical complexes of the initial and final states. When the values of $K_{c,i}$ and $K_{c,f}$ are similar, the condition $\Delta H_e \simeq \Delta\sigma_{el} \simeq 0$ implies the approximate equality of the redox potentials of the partners involved in the bond:

$$\varphi_0(A/A^{e,2e}) \simeq \varphi_0(D^{e,2e}/D).$$

When the values of $K_{c,i}$ and $K_{c,f}$ or methods for their formation are available, account can be taken also of the contribution by the complex formation effect to ΔH_e.

The thermodynamic factor determines not only the properties of the PCTC (the degree of charge transfer in the ground and electronically excited states depends on ΔH_e) but also the relative probability of the decomposition into the electron transfer products.

The rate of the final electron transfer via the inner-sphere mechanism depends on the nature of the intermediate PCTC and the process thermodynamics. It is possible to differentiate two extreme cases:[8] the rate of the electron transfer is determined either by the rate of formation of the PCTC (as a rule when $\Delta\sigma^0_{el} < 0$) or by the rate of its decomposition into the electron transfer products (usually when $\Delta\sigma^0_{el} > 0$).

Since the properties of the PCTC and the probability of their decomposition into the electron transfer products depend on parameters such as ΔH_e and $\Delta\sigma^0_{el}$, there is a possibility of the operation of several "external" mechanisms whereby these parameters are influenced.

When ionic and highly polar reactants participate in the reactions, the values of $\Delta\sigma^0_{el}$ depend on the polarity of the medium. Solvation effects diminish ΔH_e and correspondingly increase the degree of charge transfer in the donor – acceptor complex. Appreciable changes in the polarity of the medium usually occur at the water–non-polar phase interface, as a result of which effective charge transfer, induced by relatively small movements of the reacting species in a medium with a polarity gradient, are to be expected in precisely such boundary regions.

Another pathway leading to the final electron transfer in PCTC is associated with the effects of the coupling of oxidation-reduction and acid–base reactions. The mechanism whereby a proton is supplied to the acceptor can be various: a proton can be drawn from the aqueous medium or from other proton donors in close proximity. The transition from an ionic to an electrically neutral reaction entails a sharp alteration of $\Delta\sigma^0_{el}$ and ΔH_e. In fact such energy coupling of electron and proton transfer appears as the transfer of a hydrogen atom in a one-electron process or of a hydride ion in a two-electron process. In protein macromolecules, there is an inter-relation between small local changes in the relative disposition of the atoms in the active centre and the change in the structure in the entire macromolecule.[9] As a result of this, there is a possibility of the energy coupling of the structural and electronic-structural characteristics accompanied by a change in V and $\Delta\sigma^0_{el}$. The dynamic conformational properties of the macromolecule thereby determine also its functional possibilities in effecting electron transfer. Any coupling merely "manifests" some property characteristic of the PCTC, while the essential feature of the inner-sphere electron transfer remains unaltered.

The intrinsic concept of an "inner-sphere" process refers usually to electron transfer reactions in the inner coordination spheres of transition metal ions.

In contrast to the intermolecular donor–acceptor (valence) bond, in the case of metal ions the coordinate bond is collective in character. By virtue of this feature, appreciable changes in the geometrical and electronic structures of the complex can occur without appreciable energy expenditure.[11] Two consequences of such collective binding are very important for the occurrence of "true" inner-sphere electron transfer reactions.

The first concerns the dynamic character of the metal–ligand bond: despite the high strength of each bond considered separately, in the absence of steric limitations or chelation the complex ion exists permanently in the state of ligand exchange. This occurs either as a result of conformational instability (the dynamic Jahn–Teller effect)[12] or as a result of transitions between close conformationally excited states of the complex differing in the symmetry of the ligand environment and hence in the electronic-structural characteristics of the central ion.[9] In transition metal ions, there may be three types of conformationally excited states:

(1) For an unchanged coordination number (CN), only the symmetry of the ligand environment changes, which can entail a change in the spin state and population of the *d* orbitals of the central ion.

(2) In conformational excitation, the CN increases by 1 or 2—vacant sites appear in the coordination sphere and are available for the introduction of one or two ligands.

(3) On conformational excitation, the CN diminishes by 1 or 2—the dissociation of the ligands (one or two) takes place.

The second consequence is associated with the possibility of identifying the donor–acceptor bond between the metal ion and the redox ligand as a "valence" bond. Bearing in mind the limitations to the strength of such a bond ($V \lesssim 10\ kT$) and the types of conformationally-excited states of the metal complex ion quoted above, one can infer *a priori* the presence or absence of sites in the coordination sphere of the metal ion potentially vacant and available for the binding of a redox ligand with formation of a coordination PCTC.[9]

Variable-valence metal ions in the oxidised state (M^{2+}) are usually coordinatively unsaturated to the maximum extent. In this case the incorporation of redox ligands—two-electron donors (D^{2-})—in the inner coordination sphere is possible only via the ligand substitution mechanism:

$$LM^{2+} + D^{2+} \overset{-L}{\rightleftarrows} MD \rightleftarrows M^{+} + D^{\cdot-}.$$

On the other hand, in the reduced form, metals frequently have potentially vacant sites for the binding of a redox ligand. The incorporation of the ligand in the inner coordination sphere takes place in this case via a mechanism of the oxidative addition type:

$$LM^{+} + A \rightleftarrows L(MA)^{+} \rightarrow LM^{2+} + A^{\cdot-}.$$

If A is a two-electron acceptor, there is a possibility of an inner-sphere two-electron transfer with formation of the metal ion in the superoxidised state M^{3+}:

$$M^{+} + A \rightleftarrows MA^{+} \rightleftarrows M^{3+} + A^{2-}.$$

An inner-sphere two-electron reaction can take place analogously also in the interaction of the M^{3+} ion with a two-electron donor D^{2-}:

$$M^{3+} + D^{2-} \rightleftarrows MD \rightarrow M^{+} + D.$$

In each of the examples of the one- and two-electron inner-sphere transfer, the intermediate complex exists simultaneously in the ground and electronically excited states, decomposing respectively into the initial reactants or the electron transfer products. In order to have a clear idea about the nature of the PCTC, the initial reactants from which it is formed, and the products into which it decomposes, in describing the PCTC it is convenient to use Mulliken's terminology,[5] defining "limiting structures" as respectively the initial reactants and the products of the final electron transfer. In this case, the intermediate binary PCTC with inner-sphere one- or two-electron transfer can be represented as a "superposition" of structures without and with complete charge transfer:

$$(MA^{+})_{I} = (M^{+}A \leftrightarrow M^{3+}A^{2-}),$$
$$(MA^{+})_{II} = (M^{+}A \leftrightarrow M^{2+}A^{\cdot-}),$$
$$MD = (M^{2+}D^{2-} \leftrightarrow M^{+}D^{\cdot-},$$
$$MD^{+} = (M^{3+}D^{2-} \leftrightarrow M^{+}D).$$

The double arrow between states without charge transfer (initial reactants) and those with full charge transfer (products) reflects here the mixing of these two stages on formation of the PCTC.

Ternary PCTC can include either one or two metal ions. In the former case, inner-sphere electron transfer takes place in the coordination sphere of one metal ion. Such complexes are formed in the interaction of binary PCTC with a redox ligand. Accordingly, four types of ternary complexes can be formed:

$$(DMA^{-})_{I} = (D^{2-}M^{+}A \leftrightarrow D^{\cdot-}M^{2+}A^{2-} \leftrightarrow DM^{+}A^{2-}),$$
$$(DMA^{-})_{II} = (D^{2-}M^{+}A \leftrightarrow D^{\cdot-}M^{+}A^{\cdot-} \leftrightarrow D^{\cdot-}M^{2+}A^{2-} \leftrightarrow DM + A^{2-}),$$
$$(AMD) = (AM^{2+}D^{2-} \leftrightarrow A^{\cdot-}M^{2+}D^{\cdot-} \leftrightarrow A^{\cdot-}MD \leftrightarrow A^{2-}M^{2+}D),$$
$$(AMD^{+}) = (AM^{3+}D^{2-} \leftrightarrow A^{\cdot-}M^{2+}D \leftrightarrow A^{\cdot-}M^{3+}D^{\cdot-} \leftrightarrow A^{2-}M^{3+}D).$$

Their decomposition into particular electron transfer products is determined by the corresponding values of $\Delta\sigma^{0}_{el}$ and can be controlled by the energy coupling effect manifesting particular "limiting" structures.

A second metal ion can also play the role of a redox partner in the binary PCTC with the resulting formation of dinuclear bridged PCTC:

$$M^{+} + (MA^{+})_{II} \rightleftarrows (MAM)^{2+} \rightarrow 2M^{2+} + A^{2-},$$
$$MD + M^{2+} \rightleftarrows (MDM)^{2+} \rightarrow 2M^{+} + D,$$
$$M^{+} + M(A{-}A)^{+}_{I} \rightleftarrows (MA{-}AM)^{2\cdot} \rightarrow 2(MA^{+})_{I} \rightarrow 2M^{3+} + 2A^{2-},$$
$$MD^{+} + MD^{+} \rightleftarrows (MD-DM)^{2+} \rightarrow 2M^{+} + D-D.$$

Dinuclear bridged PCTC can be formed also in the interaction of the initial dinuclear metal complex ion (ion pair) with the corresponding redox ligand:

$$\underset{\text{|——|}}{M^{+}\ldots M^{+}} + A \rightleftarrows \underset{\text{|——|}}{(MAM)^{2+}} \rightarrow \underset{\text{|——|}}{M^{2+}\ldots M^{2+}} + A^{2-},$$
$$\underset{\text{|——|}}{M^{2+}\ldots M^{2+}} + D^{2-} \rightleftarrows \underset{\text{|——|}}{(MDM)^{2+}} \rightarrow \underset{\text{|——|}}{M^{+}\ldots M^{+}} + D.$$

Polynuclear cluster complexes with a strong metal–metal bond can also participate in energy coupling effects:

$$-M^{+}-M^{+} + A \rightleftarrows (-MMA)^{2+} \rightarrow -M^{2+}-M^{2+} + A^{2-},$$
$$-M^{2+}-M^{2+} + D^{2-} \rightleftarrows -M^{+}-M^{+} + D.$$
$$\uparrow$$
$$\rightleftarrows (-MMD)^{2+} \rightleftarrows$$

The above schemes describe in a general form virtually all cases of the formation of molecular and coordination PCTC in oxidation-reduction reactions.

The proposed classification of the PCTC is useful when the nature, properties, and reactivities of the intermediate complexes formed in catalytic redox reactions are considered. In fact, knowing the structural and energy characteristics of the metal ion and the redox ligand, it is possible to predict the possibility of the formation of the PCTC, its reactivity in relation to "third" species, the preferred decomposition channels, and the rate constant for the final electron transfer.[7,10] Ideas about PCTC have proved extremely productive in the analysis of both the general questions and specific examples of the activation of molecular oxygen by variable valence metal ions.[7]

III. The ligand properties of molecular oxygen and the reactivity of metal—oxygen complexes

Molecular oxygen is a redox ligand without unshared electron pairs for the formation of the usual (σ-donor) coordinate bond. By virtue of this factor, the incorporation of O_2 in the coordination sphere of a metal ion is possible only at a potentially vacant site in the conformationally excited complex. A donor-acceptor metal—oxygen bond is formed on partial transfer of one or two electrons from the t_{2g} orbital of a metal ion, which existed initially in the reduced form, to the antibonding orbitals of the O_2 molecule.

This question has been investigated in detail[9,13,14] and we shall consider only the geometrical structure of the metal—oxygen complexes. It follows from symmetry considerations that the combination of the $\pi^*(O_2)$ and $t_{2g}(M^+)$ orbitals can be achieved in two ways—with retention or breakdown of the equivalence of the O atoms. In the former case, the product is only PCTC of the peroxy-type (oxygen acting as a bidentate ligand) with inner-sphere two-electron transfer:

$$(MO_2^+)_I = \left(M^+ : \overset{\cdot\cdot O}{\underset{\cdot\cdot O}{\|}} \longleftrightarrow M^{3+} \begin{matrix} O^- \\ | \\ O^- \end{matrix} \right)$$

The latter case involves a one-electron transfer with formation of a complex of the superoxide type (oxygen acting as a monodentate ligand) with a "linear" structure:

$$(MO_2^+)_{II} = \left(M^+ \ldots \overset{O}{\overset{/\!/}{O}} \ (^1\Delta) \longleftrightarrow M^{2+} - \overset{O^-}{\overset{/}{O}} \right).$$

The establishment of singlet oxygen (sp^2-hybridisation of the atomic orbitals) in a complex of the "superoxide" type has been confirmed by the results of quantum-chemical calculations[15] and by direct measurements using IR spectroscopic methods.[16] Together with the singlet oxygen structure, the presence of the superoxide radical has been detected in the complexes with the "linear" structure.[17,18]

Complexes of the "superoxide" type have been isolated in the solid state for Co^{2+} (d^7) and Fe^{2+} (d^6) ions[16] in a planar ligand environment: salicylideneamines, porphyrins, and other macrocyclic ligands. Many such complexes have been characterised in solution by EPR. These are $(OH^-)Ti^{4+}O_2^-$,[19] Mn^{3+}(porphyrin)O_2^-,[20] Co^{3+}(corrin)O_2^{2-}, vitamin B_{12},[21] $Sn^{4+}O_2^-$,[22] etc. Analogous metal—oxygen complexes are formed in the interaction of HO_2 with Ti^{4+} [23] and other d^0 elements (V^{5+}, Zr^{4+}, U^{6+}, Th^{4+}, Mo^{6+}) [23-25] and also in the interaction of vitamin B_{12} in the oxidised form with the O_2^- radical-ion.[26]

The equivalence of the O atoms in the complex with the "linear" structure is attained only on formation of dinuclear μ-peroxo-complexes:

$$(MOM)^{2+} = \left(M^+ \ldots \overset{O \ldots M^+}{\overset{/}{O}} \longleftrightarrow M^{3+} - \overset{O^- - M^{3+}}{\overset{/}{O^-}} \right).$$

Complexes of this type are most characterstic of ions with the d^7 electronic configuration (Co^{2+}) [27,28] and also with the d^5 electronic configuration: Mn^{2+}(phthalocyanine) in the presence of pyridine[29] and Ru^{2+} + EDTA in an alkaline medium.[30,31] Analogous complexes are formed in the interaction of Ru^{3+} EDTA with H_2O_2.[31] The formation of dinuclear μ-peroxo-complexes is possible also for M^+ ions with the d^9, d^{10} electronic configuration.

The preferential formation of a particular type of oxygen complex depends on the presence of one or two potentially vacant sites in the coordination sphere of the metal ion and on the value of $\Delta\sigma_{el}^0$ for the corresponding electron transfer reaction.

Although the binding of O_2 in complexes with variable valence metals is of intrinsic interest as regards the study of natural O_2 transferring agents,[14] the question of the activation of O_2 as an oxidant is more significant. The complete or partial reduction of O_2 to reactive products such as HO_2 and OH leads to the activation of O_2. Attention should be drawn to the fact that, the higher the reactivity of the intermediate species, the smaller the chance of its being detected or isolated in the solid state. The "unobserved" oxygen complexes can therefore play a role in the activation of O_2.

From the standpoint of the PCT concept, the reactivity of coordinated oxygen is determined by the nature of the oxygen complex and the possibility of the incorporation of the oxidised substrate in the inner coordination sphere of the metal ion.

In the case of complexes of the "superoxide" type, reactions of three kinds are most characteristic.

1. Outer-sphere electron transfer reactions similar to the reactions of the superoxide radical-ion[32] but occurring much more effectively as a consequence of the increase in the redox potential of the oxygen coordinated to the highly charged central ion:

$$(MO_2^+)_{II} + D^{2-} \rightarrow MO_2 + D^-.$$

(2) Inner-sphere redox reactions for the *cis*-coordination of the oxidised substrate: in this case the incorporation of the substrate in the inner coordination sphere is possible either at a potentially vacant site or via the ligand substitution mechanism in the *cis*-position relative to the coordinated oxygen:

$$(MO_2^+)_{II} + RH \rightleftarrows (MO_2RH)^+ \begin{cases} \longrightarrow M^{3+} + HO_2^- + R' \\ \longrightarrow M^+ + HO_2 + R' \\ \longrightarrow M^+ + H_2O_2 + \text{product}. \end{cases}$$

The latter reaction is of greatest interest, since it entails the two-electron oxidation of the substrate in the inner coordination sphere of the metal ion with formation of hydrogen peroxide as the O_2 reduction product and without the final change in the (reduced) oxidation state of the metal ion. Such a reaction has been discovered in the oxidation of ascorbic acid by copper ions[7,33-36] and in the oxidation of methanol by the complexes $L_2Cu^+O_2$ (L = 2,2'-bipyridyl).[37]

When the substrate has reducing (DH^-) and ligand properties, there is a possibility of the formation of a ternary PCTC and the substrate can be bound to the metal also before the interaction with O_2.

(3) Inner-sphere redox reactions with a second metal ion. These are common in autoxidation processes:

$$(MO_2^+)_{II} + M^+ \xrightarrow{2H^+} 2M^{2+} + H_2O_2.$$

Two M^+ ions may exist also in a "pair":

$$M^+ \ldots M^+ + O_2 \rightleftarrows (M{-}O_2{-}M)^{2+} \begin{cases} \xrightarrow{2H^+} M^{2+}M^{2+} + H_2O_2 \\ \xrightarrow{2H^+} M^{2+}M^{2+} + 2H_2O \end{cases}$$

The reactivity of mononuclear oxygen complexes of the peroxy-type is due to two factors.

(1) The manifestation by such complexes of the reactive properties of metal ions in the superoxidised state as one- and two-electron acceptors. The formation of superoxidised states of the metal ion can be accompanied by the complete or partial oxidation of the coordinated ligands. For example, the autoxidation of the complexes of Fe^{2+} with 1-hydrazinophthalazine,[38] phosphines,[39] and EDTA is accompanied by inner-sphere oxidation of these ligands. The incorporation of a redox ligand in the coordination sphere is possible both in the interaction of M^+ with O_2 and after the binding of O_2 in a complex with the metal. A high reactivity in relation to H^+ and OH^- with the "appearance" of coordinated hydrogen peroxide or the superoxidised metal ion respectively is characteristic of complexes of the peroxy-type.

(2) In complexes with strong two-electron charge transfer, the dissociation of the O—O bond as a result of the stabilisation of O^{2-} with "partial" formation of atomic oxygen may prove to be more favourable than the hydrogen peroxide structure by virtue of the increase in the effective charge on the central metal ion, especially in weakly polar media:[40]

$$(MO_2^+)_I = \left(M^+ \ldots \overset{O}{\underset{O}{\|}} \longleftrightarrow M^{3+}\!\!<\!\!\begin{matrix} O^- \\ | \\ O^- \end{matrix} \longleftrightarrow M^{3+}\!\!:\!\!\begin{matrix} O^{2-} \\ | \\ O \end{matrix} \right).$$

Accordingly, complexes of the peroxy-type with strong charge transfer can exhibit the reactivity characteristic of the superoxidised metal ion and can participate, like atomic oxygen, in substrate hydroxylation and epoxidation reactions.

Among the model hydroxylating systems based on O_2, the Fe^{3+}/Fe^{2+}—EDTA—ascorbic acid (DH_2) system, discovered in 1954 by Udenfriend[41] and catalysing reactions of the type

$$RH + DH_2 + O_2 \rightarrow ROH + D + H_2O$$

has been most widely investigated. The role of the electron donor in this reaction reduces to regenerating the reduced form of the iron ethylenediaminetetra-acetate complex participating in the activation of O_2 with formation of an oxygen complex of the peroxy-type.

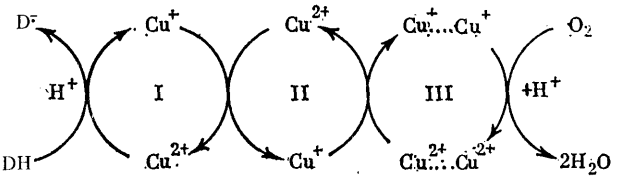
IV. Activation of oxygen by metal-containing enzymes

The biological catalysts and processes involving oxidation by molecular oxygen are widely distributed in nature. Resorting to the ideas and characteristic mechanisms of the activation of O_2 described above, we shall consider metal-containing enzymes—oxidases and oxygenases. The former use oxygen as an electron acceptor and the latter use it as a donor of an O atom.[42]

Oxidases—the catalysts of the four-electron reduction of O_2 to water—have been most thoroughly investigated: laccase, ascorbate oxidase, and cytochrome *c* oxidase. A characteristic feature of all these enzymes is the separation in space of the centres for the transformation of the substrate and reduction of O_2.

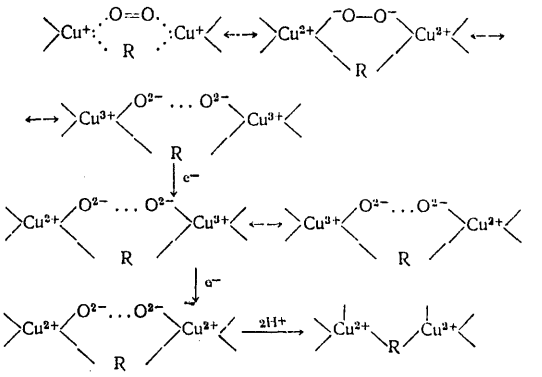
Thue "blue oxidases" (laccase, ascorbate oxidase) contain copper ions of three types: EPR-detectable "blue" [type (I)] and "non-blue" [type (II)] Cu^{2+} ions as well as a pair of Cu^{2+} ions [type (III)] which are not detectable by EPR.[43] In these oxidases, there is direct electron transfer between copper ions of types (I) and (II) and between types (II) and (III).[44] In fact, O_2 is not activated in the substrate oxidation process for this group of enzymes. The role of O_2 consists in the regeneration of the oxidised form of the enzyme. The rate of the enzyme reaction is limited by the interaction of the substrate with the "blue" Cu^{2+} ion, while the rate of regeneration of the oxidised form of the oxidase is limited by the interaction of O_2 with the reduced type (III) ions.

The mechanism of the action of laccase and ascorbate oxidase is of the same type and includes the usual cyclic scheme involving the alternate oxidation of the substrate and reduction of O_2:



The reduced enzyme is oxidised via the intermediate formation of a dinuclear μ-peroxo-complex analogous to the complexes of O_2 with the copper-containing O_2 transferring agent (haemocyanine) and ceruloplasmin.[42] The reduction of the bridging hydrogen peroxide in such a complex to water requires the transfer of two additional electrons from the substrate binding centre. Such electron transport takes place via a "non-blue" copper ion in the case of laccase and ascorbate oxidase.

Within the framework of the concept of the PCTC, the mechanism of the four-electron reduction of O_2 in "blue" oxidases can be represented by a sequence of the following inner-sphere reactions of type (III) ions:



R = bridging ligand.

The inner-sphere two-electron oxidation of each copper ion in the dinuclear complex is called upon to play a stabilising role by virtue of the inertness of Cu^{2+} [Cu^{3+}? (Ed. of Translation)] in ligand substitution reactions. In the binding of O_2, the rate of the intermolecular electron transfer from the centre (I) to the centre (III) increases by two orders of magnitude,[42] which apparently in fact ensures the possibility of the four-electron reduction of O_2 before the complex decomposes to H_2O_2 via the μ-peroxo-complex state.

A copper–polymer complex (Cu^{2+}–polyvinylpyridine) is a functional model of ascorbate oxidase:[44,45] in its presence, ascorbate anions are oxidised via a one-electron process and O_2^- radical-ions do not migrate outside the limits of the macromolecular coil because of the high local concentration of copper ions in the metal–polymer complex.

Cytochrome *c* oxidase is an enzyme which plays an important role in respiratory processes, catalysing the oxidation of cytochrome *c*:

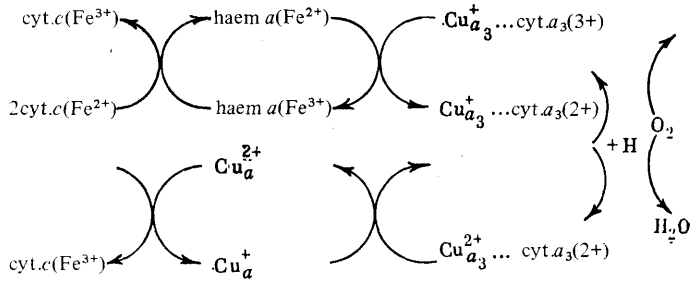
$$4\text{cytochrome } c(2+) + 4H^+ \rightarrow 4\text{cytochrome } c(3+) + H_2O.$$

It has been established by EPR that two magnetically isolated centres enter into the composition of the enzyme—the low-spin cytochrome *a* and Cu_a ($S = 1/2$; $\varphi_0 = 0.25$ V) as well as the $S = 2$ centre due to the antiferromagnetic coupling of cytochrome a_3 ($S = 5/2$) and Cu_{a_3} ($S = 1/2$), separated by a distance of 5 Å ($\varphi_0 = 0.35$ V).[46] These two centres are apparently linked via a bridge comprising a common ligand-oxygen (cytochrome a_3-O-Cu^{2+}), imidazole, or sulphur.[47–52] However, in the study of various synthetic imidazole-bridged dimers, it was demonstrated by measurements of the magnetic susceptibility between 4 and 300 K that the antiferromagnetic coupling does not correspond to that observed in cytochrome *c* oxidase ($-J > 200$ cm^{-1}).[52] It is most likely that in the oxidised enzyme the cytochrome a_3 and Cu_{a_3} centres are linked via oxygen or sulphur atoms.[49] The ligands in Cu_a are cysteine and histidine residues.[53] There is a haem–haem interaction between cytochromes *a* and a_3 accompanied by a change from the high-spin to the low-spin state of iron in cytochrome a_3 as the molecular environment in haem *a* is altered.[54] The study of the partly reduced form of cytochrome *c* oxidase demonstrated the presence of reduced low-spin haem iron, type (I) copper ions in the reduced form, and the high-spin ferrihaem a_3. Both the oxidised and the one-electron reduced enzymes oxidise cytochrome *c* also in the absence of O_2. The two-electron reduction of the oxidised form of cytochrome *c* oxidase converts it into the active form. The subsequent reduction is not significant for the manifestation of catalytic activity.

The activation of the enzyme is apparently associated with the reduction of a dinuclear haem a_3–Cu centre, which results in the possibility of the incorporation of O_2 in the coordination spheres of copper and iron ions as a bridging ligand.

In the functioning of the enzyme, haem *a* and Cu_a are independent electron acceptors on contact with the substrate, while haem a_3 and Cu_{a_3} form jointly a dinuclear O_2 binding centre.[53,55] Experiments with cross-link reactants established that the electron transfer from the haem of ferrocytochrome *c* to the haem *a* of cytochrome *c* oxidase takes place over a long distance and at a constant rate close to the rate of diffusion. The interaction of cytochrome *c* with the Cu_a centre takes place independently but much more slowly than the interaction with haem *a*.[53]

Structural studies have shown that the plane of haem *a* is at right angles to the plane of the phospholipid membrane near the water–non-polar phase interface, i.e. in the region of a dielectric constant gradient in the medium. The "cooperation" with electron transfer as a result of low changes in the polarity of the medium, mentioned previously, is thereby achieved for this enzyme. The "proton cooperation" in the stage involving the reduction of O_2 to water is also significant for the functioning of the enzyme.[55,56] The mechanism of the action of cytochrome *c* oxidase can be represented by a scheme analogous to that presented for ascorbate oxidase:

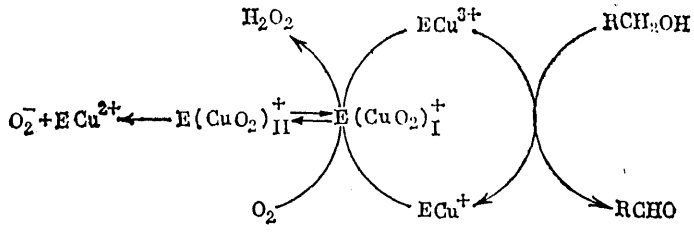


In the reduction of the bridging oxygen molecule to H_2O, three intermediate compounds are distinguished: $[Fe_{a_3}-O-O-Cu_{a_3}]^{3+}$, $[Fe_{a_3}-O-O-Cu_{a_3}]^{2+}$, and $[Fe_{a_3}-O-O-Cu_{a_3}]^{+}$.[57] These forms can be regarded as dinuclear PCTC.

It follows from the mechanisms of the action of oxidases presented that the metal ion in the oxidised form (Cu^{2+} or Fe^{3+}) participates in the oxidation of the substrate–electron donor (cytochrome *c*) or H atom (ascorbate), while a pair of metal ions in the reduced form, forming a relatively stable intermediate μ-peroxo-complex, participates in the reduction of O_2 to H_2O. The proton cooperation by the medium plays an important role in the subsequent reduction of this complex.

The metalloenzymes catalysing the reduction of O_2 to H_2O_2 contain in the active centre as a rule one "non-blue" copper ion [type (II)]. Certain amino-oxidases, galactose oxidase, and uricase are representatives of this group of "colourless" enzymes. The mechanism of the action of enzymes of this group can be various.

In the case of the galactose oxidase, the catalytic cycle includes the Cu(I) and Cu(III) enzyme forms, i.e. Cu(I) interacts with O_2 via the inner-sphere two-electron transfer mechanism. The formation of oxygen complexes of the peroxy-type is characteristic of copper(I) ions, but in the absence of the substrate they are readily transformed into complexes of the superoxide type.[58] The copper(II) formed on dissociation of this form of the enzyme is catalytically inactive. The inactive Cu(II) and the active Cu(III) forms of the enzyme can undergo interconversions under the influence of one-electron redox agents.[58] Overall, the mechanism of the action of galactose oxidase can be represented by the cyclic scheme



E = protein ligand

The copper ion in the superoxidised state functions as the oxidant species.

Reactions of this type have been modelled for the oxidation of ascorbic and dihydroxyfumaric acids and methanol using aquocopper ions and copper complexes of 2,2′-bipyridyl.[9]

In the case of the enzyme uricase, O_2 is activated as a result of the formation of a metal–oxygen complex of the superoxide type:

$$Cu\,(I) + O_2 \rightarrow CuO_2^{\cdot}(II),$$

$$CuO_2\,(II) + \text{substrate} \rightarrow Cu\,(I) + H_2O + \text{product}.$$

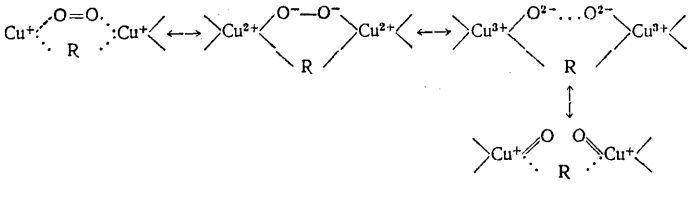
Here the oxidant species is a metal–oxygen complex of the "superoxide" type.[59]

Xanthine oxidase is the enzyme which catalyses the oxidation of hypoxanthine to xanthine and of xanthine to uric acid.[60] Xanthine oxidase is frequently used to generate O_2 radicals. The active centre incorporates a molybdenum ion, iron sulphur clusters (eight paired Fe atoms), and two FAD molecules. When FAD is removed, the enzyme loses its ability to reduce O_2 but retains its ability to oxidise xanthine. The function of molybdenum thus reduces to oxidising the substrate and the function of the iron–sulphur clusters is to transfer an electron from the reduced molybdenum to FAD, which in fact participates in the reduction of O_2. As for other FAD-dependent oxidases, O_2 is reduced without the formation of a metal–oxygen complex.

Oxygenases can be divided into copper- and iron-containing enzymes. The most typical representatives of the copper-containing oxygenases are tyrosinases, characterised by a multiplicity of forms. The coordination environment of the copper ions in the active centre is similar for enzymes of different origin and is made up of four histidine residues. The inhibition of the enzyme activity of tyrosinases by carbon monoxide indicates the involvement of the reduced form of copper in the process mechanism.[61] The mechanism of the activation of O_2 by tyrosinases has not been established.

Tyrosinase exhibits both hydroxylating and oxidase activities.[62] In the reduced state, the enzyme is capable of reversible binding of O_2, the spectrum of oxytyrosinase resembling that of oxyhaemocyanine. Like the latter, oxytyrosinase decomposes reversibly to a Cu^{2+}-enzyme and H_2O_2.[63] It has been suggested[64,65] that the structure of oxytyrosinase resembles that of oxyhaemocyanine, i.e. the active centre of this enzyme contains two copper ions. This makes it necessary to postulate distinct oxidative properties in the dinuclear copper–oxygen complex. The detection of electrophilic intermediates in the mechanism of the action of tyrosinase[66–68] indicates the possible dissociation of the O–O bond in this complex with formation of species of the type $ECuO^+$.

Within the framework of the concept of PCTC, one can postulate the coexistence of the following states of oxytyrosinase:



R = bridging ligand.

In the presence of the corresponding substrates (mono- and di-phenols), oxytyrosinase exhibits either the properties of Cu^{3+} as an electron acceptor, similarly to the dinuclear centre of the "blue" oxidases, or the properties of an O donor as a hydroxylating species.

Another representative of copper-containing monoxygenases is dopamine β-hydroxylase containing one or two Cu^{2+} ions in the active centre.[64] The activation of the apoenzyme requires two copper ions.[65] Whereas tyrosinase does not require an extraneous electron donor, dopamine β-hydroxylase functions only in the presence of a donor (ascorbate),[42] which reduces the Cu^{2+}-enzyme to the Cu^+-form.

Among iron-containing oxygenases, one may distinguish aromatic aminoacid monoxygenases (phenylalanine 4-monoxygenase, tyrosinase monoxygenase, tryptophan 5-monoxygenase) and hydroxylating enzymes containing cytochrome P-450.

Pterin-dependent aromatic acid monoxygenases contain one non-haem iron ion (Fe^{2+}) in the active centre.

As for the copper-containing oxygenases, the mechanism of the activation of O_2 has not been established. There is only evidence for the formation of a multicomponent charge transfer complex[70] (enzyme, substrate, and a coenzyme as the electron donor), which can decompose in different ways on interaction with O_2. In particular, the oxidation of the coenzyme can lead to the formation of hydrogen peroxide. The influence of the addition of peroxidase and catalase on the process stoichiometry[70] shows that the mechanism of the action of the iron-containing monoxygenases O_2 may be activated both as a result of the formation of the peroxy-type oxygen complex FeO_2 and as a result of the interaction of the reduced enzyme–substrate complex with H_2O_2.

During the last twenty years, the attention of investigators was concentrated on the study of non-specific hydroxylating enzyme systems containing cytochrome P-450.[69,71–77] These enzymes are widely distributed in nature and participate not only in processes involving the hydroxylation of endogenic substrates but also in detoxication reactions of various xenobiotics.[78,79] O_2 and two reduced equivalents, usually NADPH, participate in the substrate hydroxylation processes:

$$SH + NADP\cdot H + H^+ \rightarrow SOH + H_2O + NADP^+.$$

The haem of cytochrome P-450 participates in the activation of O_2, its reduction occurring with the aid of the electron-transport (reductase) enzyme system.[42]

Bacterial cytochrome P-450 has been isolated in a crystalline form and characterised by X-ray diffraction with a resolution of 5.5 Å.[75] The haem iron ion in the active centres of cytochrome P-450 is coordinated to cysteine residues in the 5-position and histidine residues or water in the 6-positions, undergoing comparatively readily a change in the valence spin states. In the reduced form, the enzyme forms a complex with CO, which has an intense absorption band at 447 nm and this has in fact given rise to the name of this group of haemoproteins. Oxidised cytochrome P-450 exists in a low-spin state and, on binding the substrate, it is fully or partly (depending on the temperature, pH, the polarity of the medium, and the nature of the substrate) converted into a high-spin (pentacoordinate) form. The structural aspects of cytochrome P-450 have been examined in reviews[80–83] and also in other communications.[84–90] In the reduced form, cytochrome P-450 exists predominantly in a high-spin state and readily interacts with O_2.[91]

The microsomal hydroxylating system in the liver contains, apart from cytochrome P-450, also NADPH-cytochrome P-450 reductase and phospholipids. The role of NADPH-cytochrome P-450 reductase consists in electron transport from NADPH to cytochrome P-450. The role of phospholipids in the hydroxylation process has not been ultimately elucidated. The phase transition in microsomal phospholipids at 16–25 °C alters sharply the activation characteristics of the oxidation substrates and in many instances leads to a change in the rate-limiting stage of the hydroxylation process. One cannot rule out the possibility that the effect of phospholipids on microsomal oxidation processes is associated with the manifestation of the influence of the local changes in the polarity of the medium, noted above, and the probability of electron transfer. The influence of certain lipids on the conformational states and the equilibrium involving the spin forms of cytochrome P-450 has been recorded.[92–97]

The mechanism of the enzyme reaction involves the formation of an enzyme-substrate complex with the oxidised form of cytochrome P-450 as a result of the binding of the substrate to a site adjoining haem. As a result of local structural changes, the cysteine ligand – iron ion distance changes and there is a corresponding alteration in the symmetry of the ligand environment, the spin state, and the redox characteristics of the iron ion.[98–100] After the one-electron reduction of the enzyme – substrate complex, Fe^{2+} – haem interacts with O_2 to form an oxygenated complex, which in many instances may be isolated and characterised spectroscopically.[101] The substrate is hydroxylated in the subsequent one-electron reduction of the oxygenated complex, which apparently takes place as a result of the formation of a peroxy-type iron – oxygen complex (with strong charge transfer) as the hydroxylating species.

In contrast to copper ions, whose oxidation state changes only within the limits $Cu^{+}-Cu^{3+}$, in the case of haem the range of the possible formal valence state extends from Fe^{+} to Fe^{6+}. Admittedly, the Fe^{3+} state is hardly realisable and in the $Fe^{5+,6+}$ state there is charge transfer from the porphyrin ligand to the central ion. The structural characteristics of iron-porphyrins (PFe) suggest the formation of a peroxy-type oxygen complex in the interaction of PFe^{2+} with O_2:[6]

$$(PFeO_2)^{2+}=P\left(Fe^{2+}\begin{matrix}O\\ \|\\ O\end{matrix}\longleftrightarrow Fe^{4+}\begin{matrix}O^{-}\\ |\\ O^{-}\end{matrix}\right)$$

The transfer of an additional charge to this complex leads formally either to the formation of the oxygen complex PFe^{+} or to the dissociation of the O – O bond in the limiting peroxy-structure:

$$P(FeO_2)^{+}=P\left(Fe^{+}\begin{matrix}O\\ \|\\ O\end{matrix}\longleftrightarrow Fe^{3+}\begin{matrix}O^{-}\\ |\\ O^{-}\end{matrix}\longleftrightarrow Fe^{3+}\begin{matrix}O^{2-}\\ \vdots\\ O\end{matrix}\longleftrightarrow Fe^{4+}\begin{matrix}O^{2-}\\ \vdots\\ O^{-}\end{matrix}\right)$$

On protonation of O^{2-}, the last two states are found to be stoichiometrically equivalent to the structure of compound (I) of peroxidase.[102]

The proportions of the "limiting" structures are determined by the structural-energetic characteristics of the enzyme – substrate complex and the polarity of the medium. The $PFe^{3+}O$ and $PFe^{4+}O^{-}$ states, which may be formed on protonation of a coordinated oxygen dianion, for example, via the transfer of H^{+} from an aminoacid in close proximity, are characterised by a high hydroxylating capacity. An oxenoid mechanism of the insertion of the O atom in a C – H bond is to be expected for the $PFe^{3+}O^{-}$ species and intramolecular oxidation of substrates by a radical mechanism is to be expected for species of the type $PFe^{4+}O^{-}$.[103] The ambiguity of the evidence concerning the radical or oxenoid mechanism of the hydroxylation of various substrates is apparently also associated with the "dual" reactivity of species of the type $PFeO^{3+}$.[42,104,105]

It is of interest to note that the oxidation of many substrates in the microsomal hydroxylating system takes place in the same way also in the presence of hydroperoxides instead of O_2. This indicates the presence of the same type of intermediate species in both systems. Thus the question of the activation of O_2 is indissolubly linked with the question of the activation of hydrogen peroxide in the case of cytochrome P-450 as well as other oxygenases.

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