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A Microheterogeneous Medium for Chemical (Enzymic) Reactions Based on a Colloidal Solution of Water in an Organic Solvent

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The structure and properties of inverted surfactant micelles in non-polar solvents are examined. The methods for the solubilisation of proteins (enzymes) in such systems and the molecular mechanisms of solubilisation are characterised. The kinetics of chemical enzymic reactions in inverted micelle systems are discussed. The factors influencing the catalytic activity and substrate specificity of the solubilised enzymes are analysed. The characteristic features of the shift of the chemical equilibrium in inverted micelle systems are considered and the prospects for the employment of such systems in fundamental research (particularly in the modelling of the structure and functions of biological membranes) and for the solution of applied problems in chemistry are discussed.
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I. INTRODUCTION

The most important elements in a chemical process are the selection of the reaction medium and the catalyst. Enzymes are the best catalysts of chemical reactions available at present. Their exceptionally high catalytic activity under mild conditions and the unsurpassed selectivity of their action[1] lead to extensive prospects for the employment of biocatalysis in technological processes[2]. The use of enzymes as catalysts in fine organic synthesis and in the preparation of medicinal drugs and valuable biologically active compounds is particularly promising. However, the wide scale employment of enzymes in practice is hindered by the fact they retain their unique catalytic properties usually only in

aqueous solution. On the other hand, it is known that many chemical reactions are driven by thermodynamic factors towards the required product only in specific organic solvents. This is associated both with specific solvation effects and with the solubilities of the individual reaction components or, in other cases, with the fact that water is formed together with the required product and the equilibrium is therefore displaced towards the starting materials when the reaction is carried out in aqueous solutions. Unfortunately the transition from water as the reaction medium to an organic solvent entails as a rule either complete denaturation of the enzyme or a sharp decrease of its catalytic activity and the disappearance of its substrate specificity[3].

One of the possible ways out of this situation is the creation of model catalytic systems, soluble in organic solvents, which make it possible to approach to some extent the efficiency of catalysis by enzymes in aqueous solutions. In particular, systems of inverted surfactant micelles in organic solvents can be used as such model systems. The inverted micelles consist of closed aggregates of associated surfactant molecules whose outer layer is made up of their hydrocarbon fragments and whose nucleus consists of their polar (ionic) groups. Such associated species are capable of solubilising in organic solvents considerable amounts of polar compounds, which are located within the inner cavity (nucleus) of the micelle.

The unique properties of the micromedium in the inner cavity of inverted micelles, namely a reduced polarity, a high microviscosity, and a high concentration of functional groups, justify the consideration of the inner cavity of inverted micelles as an extremely realistic model of the micromedium in the active centres of enzymes[4,5]. Indeed many reactions are greatly accelerated when they are carried out in systems of inverted micelles and the acceleration effects sometimes approach in order of magnitude those observed in enzyme catalysis[6]. The acceleration of reactions in inverted micelle systems is caused, apart from the trivial effect arising because the reactants are concentrated as a result of being bound to the micelles and the effects of the micromedium in the inner cavity, by the rigid fixation of the reactant molecules to the inner cavity of the micelles[6,7] as happens in enzyme catalysis[1]. Effects associated with specificity in relation to the size[8] and configuration[9] of the reactant molecules, characteristic of enzyme catalysis, can also be observed in catalysis by inverted micelles.

It is noteworthy that the acceleration and specificity effects, comparable in magnitude to those observed in enzyme catalysis, are manifested in an inverted micelle system only in individual instances and are not general. A procedure, which would permit a direct combination of the advantages of an enzyme as a catalyst with the advantages of an organic solvent as the reaction medium is therefore more promising. Its fundamental idea is to "force" enzymes to operate in an organic solvent with a low water content. Evidently, in employing this approach, it is necessary to solve the problem of the stabilisation of the enzyme in relation to the denaturing effect of the organic solvent[3].

This problem can be solved in principle by employing as the reaction medium a "water–water-immiscible organic solvent" system or an organic solvent which ensures the integrity of the native structure of the enzyme globule and hence its catalytic activity[10]. Another solution of the problem has been found recently. Namely we have shown[11], and this was subsequently confirmed in studies by other investigators (which are discussed below), that water-soluble enzymes can be solubilised in organic solvents with the aid of surfactants and that their catalytic activity can be simultaneously preserved. As a result of the solubilisation, the enzyme is incorporated in the inner cavity of an inverted surfactant micelle, as shown in Fig.1, and is thereby protected from the denaturing action of the organic solvent.

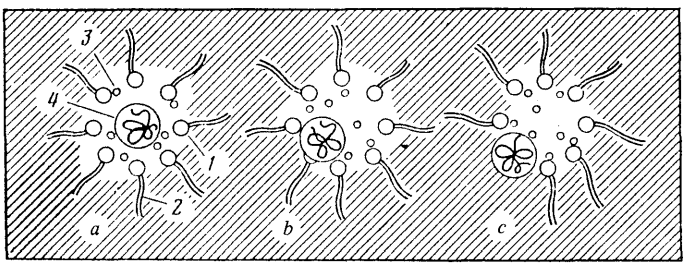


Figure 1. Schematic representation of an inverted micelle containing hydrophilic (*a*), surface-active (*b*), and hydrophobic (*c*) proteins: *1*) ionic (polar) group in the surfactant molecule; *2*) hydrocarbon group in the surfactant molecule; *3*) counterions and/or solubilised water molecules; *4*) enzyme molecule; the organic solvent is shown shaded.

We shall consider the principal aspects and achievements in this new field—micellar enzymology, which permits the employment of biocatalysts for the acceleration of chemical reactions in an organic solvent.

II. PHYSICOCHEMICAL PROPERTIES OF INVERTED MICELLES

The Structure of Inverted Micelles

In non-polar organic solvents inverted micelles consist of associated surfactant species whose inner component is made up of the polar "heads" and whose outer layer consists of the hydrocarbon "tails" of these molecules. The micelle-formation process in a non-polar medium can be described[12] in some cases within the framework of the mass action model, which presupposes an equilibrium between the monomeric surfactant molecules and the micelles of the "all or nothing" type and in other cases by a stepwise model, according to which a set of associated species with different aggregation numbers, which are in equilibrium with one another, exist in solution.

The size and form of the inverted micelles are closely related to the micelle formation mechanism: in the case of the mass action mechanism the micelles are nearly spherical, while in the case of the stepwise mechanism they assume extended, rod-like and lamellar structures[13]. The linear dimensions of the micelles do not usually exceed several tens of Angstroms. The micelles formed via the mass action mechanism are usually characterised by a narrow distribution with respect to size and shape, which is independent of the surfactant concentration[14–16]; this feature applies mainly to systems with anionic surfactants. In the case of surfactants undergoing association via the stepwise mechanism (these are as a rule cationic surfactants) the micelles are polydisperse and the distribution shifts towards larger associated species as the surfactant concentration rises[13,17,18].

On solubilisation of water in surfactant solutions in non-polar solvents, hydrated micelles are formed and differ significantly in their size and shape from the micelles which

exist in anhydrous systems. The hydration of the micelles reduces the degree of their asymmetry, since they acquire an almost spherical shape[19,20], and leads to a sharp increase of their size and molecular weight owing to the formation of a water-filled microcavity within the inverted micelle[16,19-23]. For example, in the system of inverted micelles formed by the sodium salt of the di-iso-octyl ester of sulphosuccinic acid (OT or AOT aerosol) in iso-octane, an increase of the degree of hydration, i.e. of the molar ratio $[H_2O]/[AOT]$, from 0 to 50 entails an increase of the hydrodynamic radius of the micelle from 17 to 120 Å.[22]

The Micromedium in the Inner Cavities of Inverted Micelles

The water filling the inner cavities of the hydrated inverted micelles differs in its physicochemical properties from the usual (bulk phase) water. The scale of the differences is determined by the degree of hydration by the inverted micelles, which is usually expressed as the ratio of the molar concentrations $[H_2O]/[\text{surfactant}] = w_0$. For low values of w_0 (of the order of several units), the properties of the micellar and bulk-phase water differ greatly but, as w_0 increases, the differences are gradually smoothed out. The cause of this is that the first portions of the water added to the system are bound firmly to the surfactant molecules as a result of the hydration of their polar components. The amount of strongly bound water is 4–10 molecules per surfactant molecule depending on the nature of the latter. The so-called "free" water, constituting an independent microphase and approaching in its properties the bulk-phase water, appears within the inverted micelles only after the completion of the hydration of the surfactant[20,24,25]. For example, for low values of w_0, the viscosity of the water solubilised by the inverted AOT micelles in an aliphatic hydrocarbon exceeds that of the bulk-phase water by a factor greater than 200 and its polarity corresponds to that of chloroform; with increase of w_0, these differences between the properties of the micellar and bulk-phase water decrease monotonically and disappear[25,26].

The aqueous cavities of the hydrated inverted micelles are inhomogeneous in their physicochemical properties and are characterised by different local polarities, viscosities, etc. at different points[20,26,27]. For example, the local viscosities and polarities of water within the cavities of the hydrated inverted AOT micelles in iso-octane respectively decrease and increase on moving away from the boundary of the aqueous cavity to its centre[26]. The existence of the inhomogeneity can be explained by the presence within the micelle of layers of water of hydration bound to different extents[20,26,27].

The pH Values in the Inner Cavities of the Inverted Micelles

The inner cavity of a hydrated inverted micelle is characterised by a high concentration of electric charges arising on ionisation of the polar groups in the surfactant molecules. As a result of this, the local pH of the aqueous cavity can differ significantly from the pH of the solubilised aqueous solution[28-32]. The question of the pH assumes a particular importance in the study of chemical (and particularly enzymic) reactions in the inner cavities of the inverted micelles.

In order to estimate the acid–base properties of the micromedium in the inner cavities of the inverted micelles, the method based on comparing the pK_a of different indicators dissolved in pure water and solubilised by the inverted micelles is most widely employed[29-32]. Analysis of the data obtained with the aid of the indicator method permits the conclusion that the micromedium in the inner cavity is in general more acidic than the initial aqueous solution in the case of an anionic surfactant[29,30] and more alkaline in the case of a cationic surfactant[32]. The pH shift is ~1–2 units. When a non-ionic surfactant is employed, the acidity in the inner cavity of the micelles hardly differs from that in the initial aqueous solution[31].

A new approach to the determination of the pH in the inner cavities of the inverted micelles, in which the dependence of the chemical shift of the ^{31}P NMR signal due to the ^{31}P nucleus, forming part of the composition of the phosphate ion, on the pH of the surrounding medium employed is used, has been proposed recently[28,33]. A relation between the pH of the aqueous cavity in an inverted AOT micelle in iso-octane and the initial aqueous solution (the "acidity scale") has been obtained with the aid of this method[28]. The pH shifts determined in this way agree with the values obtained by the indicator method[29,30].

Both approaches described suffer, however, from significant disadvantages. In particular the pK_a of the solubilised "reporter" molecule (indicator or phosphate anion) cannot be determined independently, which necessitates appropriate assumptions and hence leads to an uncertainty in the pH value obtained.

The Reactivity of the Water Solubilised by Inverted Micelles

The rates of chemical reactions involving water as a rule increase sharply on passing from an aqueous solution to a system of hydrated inverted micelles and the acceleration effect can reach a factor of 10^6 and more[5,34,35]. The acceleration effect is largely due to the high reactivity of the water solubilised in the inner cavities of the inverted micelles where the reaction takes place. For example, the nucleophilic activity of the water solubilised by inverted AOT micelles is at least 10^3 times higher than that of the bulk-phase water[5,35]. The water which is either involved in the hydration of the polar groups in the surfactant molecule[5,36] or appears directly after the completion of the hydration[35] shows the highest reactivity. Further addition of water leads to the formation of hydrogen bonds between the water molecules themselves which results in a decrease of their reactivity[36]. The high reactivity of the water molecules involved in the initial hydration of the surfactant can be caused by the high mobility and/or the change in their acid–base characteristics[5].

III. METHODS FOR THE INCORPORATION OF THE PROTEIN (ENZYMES) IN INVERTED MICELLES

Homogeneous (optically transparent) solutions of proteins (enzymes) in organic solvents can be obtained by employing one of the following three procedures.

The first procedure was proposed by ourselves[11] and is nowadays most widely used[37-44]. A small amount (of the order of several volume per cent) of the aqueous protein solution was introduced into a surfactant solution in an organic solvent (the actual ratio of the volumes of the aqueous and organic solutions is determined by the object of the experiment, the required degree of hydration of the surfactant, etc.). The resulting mixture is vigorously shaken (for several tens of seconds) until the formation of an optically transparent solution. Exposure of the

mixture to ultrasound is also used instead of mechanical shaking[45,46]. It must be emphasised that the choice of the method of stirring is important in the study of enzymes and particularly enzyme kinetics in inverted micelle systems, because the time taken to attain equilibrium in the colloidal system depends on its efficiency: in some cases the equilibration time in inverted micelle systems (in the absence of a solubilised protein) can be measured in hours and tens of hours (see, for example, Becher and Clifton[47] and Corkill et al.[48]).

The equilibration time in surfactant solutions apparently depends to a considerable extent on the nature of the surfactant and/or solvent. For solutions of one of the most widely used surfactants—the OT (OAT) aerosol—in aliphatic hydrocarbons, it has been shown[22,49] that equilibrium (the attainment of constant dimensions of the micelles) is established rapidly and there is no need for ultrasonic treatment. We arrived at an analogous conclusion using the same surfactant for the solubilisation of enzymes[50]. The question of the necessity for the ultrasonic treatment can, however, arise when other surfactants are used. One must then bear in mind that the action of ultrasound can inactivate the enzyme[51]. In the solubilisation of aqueous enzyme solutions without the application of ultrasound, there is virtually no inactivation of the enzyme[11,41,52,53].

The second procedure, proposed in Menger's laboratory[54], consists in the initial introduction of the required amount of water into the surfactant solution in an organic solvent (in order to attain the required degree of hydration of the surfactant), after which a dry (lyophilised) protein is dissolved in the resulting transparent solution with vigorous shaking. The time required for the dissolution of the dry protein is usually greater than in the procedure employing aqueous solutions but it diminishes with increase of the degree of hydration of the surfactant[50]. The complete dissolution of the dry protein sample takes place only at moderate protein concentrations, comparable to those obtained by the first method[50].

In order to obtain highly concentrated protein solutions, an excess of the dry protein is introduced into the surfactant solution in an organic solvent and its undissolved part (after several hours) is separated by centrifugation[50]. The repetition of the procedure for the dissolution of the protein in the resulting supernatant increases the protein content in the micellar solution. When this method is used to obtain concentrated protein solutions, it is necessary to monitor the surfactant and water contents in the supernatant after separating the soluble component, because the latter can entrain both the surfactant and water.

The third solubilisation method, proposed by Luisi and co-workers[55,56], involves the spontaneous transfer of the protein in a two-phase system consisting of approximately equal volumes of the aqueous protein solution and the organic solvent containing the surfactant. After the completion of the solubilisation, carried out with slight stirring or altogether without stirring, the organic phase containing the dissolved protein is separated. A disadvantage of this method is the long time required for the solubilisation process. In addition, the problem of determining the amount of water solubilised by the micelles (the degree of hydration of the surfactant) arises.

We draw attention to the fact that, for very low values of $w_0 < 1-2$, proteins are almost insoluble in inverted micelle systems. The solubilisation of the protein begins only for a certain value of w_0, beyond which an increase of the degree of hydration of the surfactant is accompanied by an almost linear increase of the solubility of the protein up to concentrations comparable to those attainable in an aqueous solution[50]. The specific solubilisation limits depend on the temperature, the pH of the aqueous solution, the nature of the surfactant, the concentrations of water and the surfactant in the system, and also the nature of the protein[41,50].

Molecular Mechanisms of the Incorporation of the Protein in Inverted Micelles

In organic solvents inverted micelles provide a methodologically unique possibility for the dissolution of proteins of different kinds under standard conditions where the dissolved protein molecule can itself "select" the microenvironment corresponding to its nature (optimal from the thermodynamic standpoint). In fact, as can be seen from the schematic illustration in Fig. 1*a*, the molecule of a hydrophilic protein can avoid direct contact both with the organic solvent and with the surface of the inner cavity of the inverted micelle formed by the polar "heads" of surfactants molecules, being localised in the aqueous nucleus of the hydrated inverted micelle. Surface-active enzymes, for example lipases, are able to interact with the surface layer of the inverted micelle or can be even partly immersed in the latter (Fig. 1*b*). Finally, typical membrane enzymes, can, in principle, come into contact even with an organic solvent, provided that this is favoured by thermodynamic factors (Fig. 1*c*).

Examples of the solubilisation by inverted micelles of enzymes of different kinds, corresponding to type *a*,[11,37-45,52-59] *b*,[60,61] and *c*,[46,62] have been described in the literature (Fig. 1). In all cases the solubilisation results in the formation of an optically transparent solution and the solubilised enzymes retain their catalytic activity. Only the molecular mechanism of the solubilisation of proteins (enzymes) of type *a* (Fig. 1), i.e. the water-soluble proteins, has been investigated quantitatively[38,63,64]. In particular, it has been shown by sedimentation analysis[63,64] that the molecules of typical water-soluble proteins (lysozyme, trypsin, α-chymotrypsin, ovalbumin, alcohol dehydrogenase, and γ-globulin) are capable of being incorporated into inverted AOT micelles in octane over a wide range of experimental conditions without a significant change in the initial dimensions of the micelle. This result agrees with the scheme in Fig. 1*a* and indicates the absence of a significant interaction of the protein with the surfactant.

IV. KINETIC THEORY OF CHEMICAL REACTIONS IN INVERTED MICELLE SYSTEMS

The rates of many chemical reactions increase sharply when they are transferred from an aqueous medium or an organic solvent to organic solvent–surfactant or organic solvent–surfactant–water systems (see the relevant reviews[6,24,65]). In discussing the reaction acceleration effects, the term "micellar catalysis" (which has come to be generally accepted for aqueous systems) must, however, be used with caution because the increase of the rates of reactions in the presence of surfactants in organic solvents is by no means always caused by micellar effects[66]. In certain cases inverted micelles are not formed at all and the acceleration can be achieved as a result of the homogeneous-catalytic effect of monomeric surfactant molecules[66].

Analysis of the numerous experimental data available at present permits the conclusion that in those cases where the acceleration is indeed micellar in nature, it can be caused by

the operation of factors analogous to those which ensure[67,68] the increase of the rates of reactions in aqueous micellar systems. Namely the acceleration can be due to the reactants becoming concentrated as a result of their binding to the micelles, the catalytic action of the polar groups in the surfactant molecules, the rigid fixation and/or orientation of the reactant molecules in the inner cavity of the micelles, and also the characteristic features of the micromedium in the inner cavity where the reaction takes place (reduced dielectric constant, increased reactivity of water, and a shift of the pK_a of the ionic groups of the interacting molecules).

In the kinetic analysis of chemical reactions in an inverted micelle system, it is necessary in the first place to take into account the distribution of the reactants in this microheterogeneous medium. The analogous problem in micellar catalysis in aqueous solutions has already been adequately solved[67,68] within the framework of the pseudophase model. The pseudophase approach can also be employed in the study of the kinetics of chemical reactions in inverted micelle systems.

According to the pseudophase model[69], a surfactant solution consists of the bulk phase of the organic solvent and the micellar "phase" and there is an equilibrium distribution of the reactants between these phases, for example:

$$A_{bulk} \overset{P_A}{\rightleftharpoons} A_{mic}, \qquad (1)$$

where P_A is the partition coefficient of the reactant A:

$$P_A = [A]_{mic}/[A]_{bulk}. \qquad (2)$$

Here and henceforth the subscripts "mic" and "bulk" refer respectively to the micellar and bulk phases. The concept of the set of micelles as an independent "continuous" phase agrees with experimental data, which indicate the free character of the intermicellar exchange of the solubilised molecules[45,70-74]. The applicability of the pseudophase model is limited by the assumption that the equilibrium distribution of the reactants between the phases [formula (1)] does not shift as a consequence of the chemical reaction, i.e. the exchange of matter between the phases is fairly rapid (takes place at diffusional rates). This assumption, which is valid for normal surfactant micelles in aqueous solutions[68], remains in force also on passing to inverted micelle systems[75-78]. We shall consider the application of the pseudophase approach to the analysis of chemical reactions of various types occurring in inverted micelle systems.

First-Order Reactions

Within the framework of the pseudophase model, first-order reactions can be described by the following scheme:

$$\begin{array}{ccc} A_{bulk} & \overset{P_A}{\rightleftharpoons} & A_{mic} \\ \downarrow k_{bulk} & & \downarrow k_{mic} \\ \text{products} & & \text{products} \end{array} \qquad (3)$$

where k_{bulk} and k_{mic} are the true rate constants for the transformation of A in the bulk phase and micellar phase respectively and the partition coefficient P_A is specified by Eqn.(2). Under conditions where [surfactant] $\gg$ [A], the expression for the effective rate constant is[67,68]

$$k_{obs} = (k_{bulk} + k_{mic}P_A cV)/(1 + P_A c) \qquad (4)$$

where c is the concentration obtained after subtracting the critical concentration for micelle formation (CCM), V the molar volume of the surfactant, and $cV \ll 1$ the volume fraction of the micellar phase. It follows from Eqn.(4) that the dependence of k_{obs} on c has the form of a curve with a plateau, which is indeed observed experimentally[4,5,79-81]. The limiting value of k_{obs} is equal to k_{mic} and characterises the true reactivity of the reactant A in the micellar medium.

Second-Order Reactions

Kinetic analysis of a second-order reaction A + B $\xrightarrow{k_{obs}}$ products, occurring in a micellar system under the conditions of strong binding of the reactants to the micelles, leads to the following expression for the dependence of the observed reaction rate constant on the surfactant concentration[67,68]:

$$k_{obs} = \frac{(k_{mic}/V)\,K_A K_B c + k_{bulk}}{(1 + K_A c)\,(1 + K_B c)} \qquad (5)$$

where $K_A = P_A - 1$, $K_B = P_B - 1$, and $P_A \gg 1$ and $P_B \gg 1$ are the partition coefficients of the reactants A and B between the phases determined in accordance with Eqn.(2). According to Eqn.(5), the dependence of k_{obs} on c should be represented by a curve with a maximum, which agrees with experimental data[82,83]. The proposed methods for the linearisation of the experimental k_{obs}–c curves[67,68] make it possible to find both the true rate constants k_{mic} in the micellar medium and the binding constants.

As a general comment on Eqns.(4) and (5), one must emphasise that, in the study of the dependence of k_{obs} on c within the framework of the model discussed, the surfactant concentration should be varied in such a way that the ratio $[H_2O]/[\text{surfactant}]$ is kept constant. In other words, the change in the surfactant concentration should be always accompanied by the corresponding change in the water concentration and conversely. Otherwise a change in c leads to an alteration of the properties of the micromedium within the inner cavities of the inverted micelles where the reaction proceeds (see Section II) and hence to a change in k_{mic} and a distortion of the type of dependence of k_{obs} on c predicted by Eqns.(4) and (5).

Enzymic Reactions in Inverted Micelle Systems

We shall consider the kinetics of the reaction between the enzyme E and the substrate S proceeding in accordance with the Michaelis mechanism

$$E + S \rightleftharpoons ES \rightarrow E + \text{products} \qquad (6)$$

in an organic solvent–water–surfactant system. We shall assume that an equilibrium distribution of the substrate between the phases has been established:

$$S_{bulk} \overset{P_S}{\rightleftharpoons} S_{mic}, \qquad (7)$$

where P_S is the partition coefficient of the substrate:

$$P_S = [S]_{mic}/[S]_{bulk}. \qquad (8)$$

The distribution of the enzyme need not be considered because proteins are almost insoluble in hydrophobic solvents[84]; in addition, enzymes are as a rule denatured in non-aqueous media (see the review of Martinek and Berezin[85]). We shall therefore assume that the catalytic activity is concentrated only in the micellar phase.

The rate of reaction (6) in the micelles, referred to the bulk phase of the entire system, can be expressed (in the initial instant when the concentration of the products is low compared with the initial concentration of the substrate,

in the presence of an excess of the substrate relative to the enzyme, and under stationary conditions) in the form of the Michaelis–Menten equation:

$$v = \frac{k_{cat,mic}[E]_{0,mic}[S]_{0,mic}}{K_{M,mic} + [S]_{0,mic}} \cdot \theta \qquad (9)$$

where θ is the volume fraction of the micellar phase and the subscript 0 indicates initial concentrations.

We shall assume that the exchange of substrate molecules between the phases is fairly rapid, i.e. the occurrence of the enzyme reaction (6) does not impair equilibrium (7). The concentrations of the reactants can then be found from Eqn.(8) and the material balance equations:

$$[S]_{0,mic} = [S]_{0,mic}\theta + [S]_{0,bulk}(1-\theta)$$
$$[E]_{0,tot} = [E]_{0,mic}\theta \,. \qquad (10)$$

However, one should remember that Eqn.(8) is valid only for fairly dilute solutions and the reactant concentrations must therefore be much lower than the surfactant concentration.

After substituting Eqns.(8) and (10) in Eqn.(9), we have

$$v = \frac{k_{cat,app}[E]_{0,tot}[S]_{0,tot}}{K_{M,app} + [S]_{0,tot}} \qquad (11)$$

where

$$k_{cat,app} = k_{cat,mic} \qquad (12)$$

$$K_{M,app} = K_{M,mic}[1+\theta(P_S-1)]/P_S \,. \qquad (13)$$

In the case of a reaction involving a charged substrate, Eqn.(13) can be simplified by assuming that the substrate molecules exist only in the aqueous micellar phase and hence not only $P_S \gg 1$ but also $P_S\theta \gg 1$. Then

$$K_{M,app} = K_{M,mic}\theta \qquad (14)$$

Here we have considered only the simplest pseudophase model[52,86,87], which presupposes a uniform distribution of the reactants throughout the bulk of the hydrated micelles. In the general case the model must naturally be more complex and must take into account the microheterogeneity of the inner cavities of the inverted micelles; in particular, one could take into account the distribution of the substrate between the inner surface layer and the aqueous nucleus of the inverted micelle. Nevertheless, even the simplest model and the equations obtained within its framework permit a successful kinetic analysis of enzymic reactions in inverted micelle systems.

In order to determine the "true" parameters of the Michaelis equation, characterising the enzymic reaction in the aqueous micellar phase, it is necessary to investigate the dependence of the observed rate of the enzymic reaction on the ratio of the bulk phase (organic solvent) and the aqueous micellar phase. The volume fraction of the aqueous micellar phase θ should then be varied in such a way that the ratio w_0 is kept constant, i.e. the properties of the aqueous micellar phase itself are not altered (see Section II).

The validity of Eqns.(11)–(14) has been confirmed[52,86,87] in relation to the trypsin-catalysed hydrolysis of the *p*-nitroanilide of *N*-benzoyl-D,L-arginine in the system of the inverted micelles of cetyltrimethylammonium bromide in a 1:1 chloroform–octane mixture. The dependence of the observed kinetic parameters of this reaction ($K_{M,app}$ and $k_{cat,app}$) on the volume fraction of water in the system is illustrated in Fig.2. Evidently conditions can be achieved (for low values of θ) where, according to Eqn.(14), the observed $K_{M,app}$ is superior to (smaller than) K_M in an aqueous solution (indicated by the dashed line in Fig.2). In principle, by virtue of this factor, it is possible to achieve higher observed reaction rates in an inverted micelle system than in an aqueous solution.

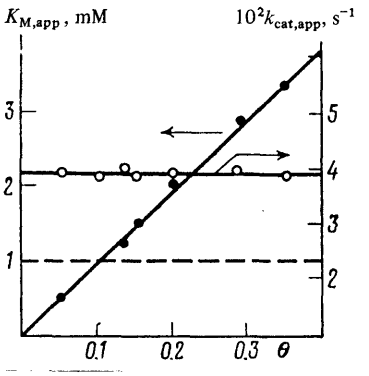


Figure 2. Hydrolysis of the *p*-nitroanilide of *N*-benzoyl-D,L-arginine catalysed by trypsin solubilised in the system cetyltrimethylammonium bromide–aqueous buffer (0.02 M phosphate–borate–acetate, pH 8.0)–1:1 chloroform–octane mixture[86]. The Michaelis parameters $K_{M,app}$ and $k_{cat,app}$ are plotted as a function of the volume fraction of water θ in the system with $[H_2O]/[surfactant] = 25$ and [surfactant] = 0.1–0.8 M. Experimental conditions: 26 °C, $[E]_{0,tot} = 5 \times 10^{-6}$ M. $K_{M,app}$ for the reaction in water is indicated by a dashed line.

This phenomenon, which has long been known for organic (non-enzymic) reactions in micellar solutions[67,68], is associated with the fact that the reactants become concentrated in the micelle. In principle, the concentration effect influences all the kinetic parameters whose dimensions include concentration. On the other hand, Fig.2 shows that the quantity K_{cat}, which has the dimensions [time^{-1}], is independent of θ and hence the observed value of k_{cat} is equal to the true value of this constant for the reaction in the aqueous micellar phase [see Eqn.(12)]. This is precisely why the *true* reactivity of the enzyme and hence its state in the micelle can be inferred from the observed value of $k_{cat,app}$.

V. CATALYTIC ACTIVITY OF SOLUBILISED ENZYMES

The Influence of the Degree of Hydration of the Inverted Micelles

One of the most striking effects observed in the study of enzymes in inverted micelle systems is the dependence of the catalytic activity of the solubilised enzymes on the degree of hydration of the inverted micelle w_0, i.e. on the parameter which determines the size and properties of the micromedium within the inner cavities of the micelles. The dependence of the catalytic activity on w_0 has been observed for enzymes such as α-chymotrypsin[40,52,58,87], trypsin[88], lysozyme[41], phospholipase A_2,[60] alcohol dehydrogenase[52], pyrophosphatase[52,57,87], and pancreatic lipase[61]. As a rule, this dependence is bell-shaped, i.e. there is an optimum value of w_0 for which the catalytic

activity of the solubilised enzyme is a maximum. The optimum values of w_0 differ for different enzymes.

As an example, we shall consider the dependence of the rate constant k_{cat} for the deacylation of *N*-trans-cinnamoyl-α-chymotrypsin, solubilised by inverted AOT micelles in octane, on the degree of hydration, which is illustrated in Fig.3. The possible causes of the appearance of the maximum in the relation between k_{cat} and w_0 have been analysed in detail[58]. Here we shall draw attention to the fact that, under the conditions of the maximum, k_{cat} exceeds the value observed in an aqueous solution (shown by a dashed line in Fig.3). This phenomenon of the "superactivity" of the α-chymotrypsin solubilised by inverted AOT micelles has also been observed by other workers[40,54]. The phenomenon of superactivity is apparently partly due to the effects of the microenvironment of the enzyme molecule in the inverted micelle.

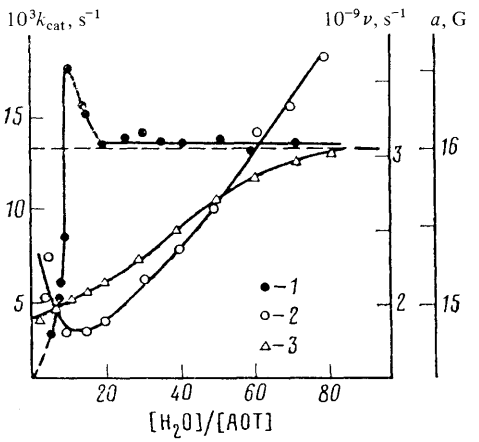


Figure 3. Dependence of the first-order rate constant k_{cat} for the deacylation of *N-trans*-cinnamoyl-α-chymotrypsin (curve *1*), the rotational frequency ν (curve *2*), and the hyperfine splitting constant *a* (curve *3*) for the spin-labelled α-chymotrypsin on the ratio $[H_2O]/[AOT]$ in the AOT (0.1 M)–octane–water (0.05 M tris buffer, pH 8.0) system at 25 °C.[89] The 4-iodoacetamido-2,2,5,5-tetramethylpyrrolidin-1-ylo y-radical was used as the spin label. The values of k_{cat} and *a* in aqueous solution are indicated by the dashed line.

Indeed, Fig.3 shows that the enzyme exhibits the maximum activity for moderate degrees of hydration of the surfactant, i.e. under conditions where the microenvironment of the enzyme molecule within the micelle differs significantly from that in aqueous solution. In particular, it follows from Fig.3 that the hyperfine splitting constant *a* of the EPR signal of a spin label introduced into the active centre of α-chymotrypsin is smaller under the conditions of the catalytic activity optimum than in an aqueous solution[89]. This implies that the polarity of the medium in which the active centre of the solubilised enzyme is located is significantly lower than the polarity of the aqueous solution[90].

On the other hand, the appearance of "superactivity" can also be caused by conformational changes in the enzyme molecule when it is incorporated in the inverted micelle. Thus, under the conditions of the catalytic activity optimum, a minimum is observed in the curve representing the dependence of the frequency of rotation ν of the spin label in the α-chymotrypsin active centre on w_0 (Fig.3), i.e. there is a minimum in the conformational mobility of the label. The circular dichroism spectra have led to the conclusion[40] that there is an increase of the rigidity (degree of helical configuration) of the α-chymotrypsin molecule solubilised by inverted AOT micelles in iso-octane (the region where $w_0 \cong 10$). The increased conformational rigidity of the enzyme molecule for $w_0 \cong 10$ can apparently be explained by the fact that under these conditions the diameter of the inner cavity of an empty inverted AOT micelle[21] is close to the dimensions of the α-chymotrypsin molecule (40 × 40 × 50 Å).[91]

Another example is provided by horse radish peroxidase solubilised by inverted AOT micelles in octane. Fig.4 shows that the optimum value of k_{cat} greatly exceeds the activity manifested by the enzyme in aqueous solution (dashed line). Furthermore, Fig.4 shows that the optimum value of k_{cat} increases with decrease of the surfactant concentration (with decrease of the area of the interface) and in the limit reaches values hundreds of times greater than the catalytic activity of the peroxidase in aqueous solution[59].

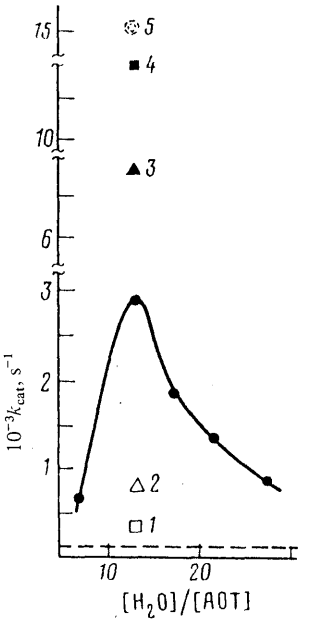


Figure 4. Dependence of the first-order rate constant k_{cat} for the peroxidase-catalysed oxidation of pyrogallol by hydrogen peroxide on the degree of hydration of the surfactant in the AOT (0.1 M)–octane–water (0.025 M phosphate buffer, pH 7.0) system at 26 °C.[59] The rate constants k_{cat} were measured for a fixed degree of hydration $[H_2O]/[AOT] = 13$ at different surfactant concentrations: *1*) 0.5 M AOT; *2*) 0.3 M AOT; *3*) 0.03 M AOT; *4*) 0.03 M AOT + 0.1 M imidazole; *5*) k_{cat} found by extrapolation to zero surfactant concentration. Dashed line—the value of k_{cat} in aqueous solution.

The "superactivity" of the enzyme solubilised by inverted micelles is so far a fairly rare phenomenon. In most cases the catalytic activity of enzymes under normal conditions is close to that observed in aqueous solution (for example in the

case of horse liver alcohol dehydrogenase[39,53], trypsin[45], and pyrophosphatase[57,87]). This fact is consistent with the results of spectroscopic studies[37,39,44–46,54–56,88], according to which the conformation of the protein molecules solubilised by inverted micelles is usually in the main identical with that observed in aqueous solution.

However, it must be emphasised that the relation between the conformational (according to spectroscopic data) and catalytic characteristics of enzymes is not always unambiguous. For example, the catalytic activity of lysozyme solubilised by inverted AOT micelles in iso-octane approaches under optimum conditions that observed in aqueous solution, although the conformation of this enzyme undergoes significant changes after its incorporation into the inverted micelle according to circular dichroism data[41]. On the other hand, spectroscopic characteristics of another enzyme, horse radish peroxidase, hardly change on solubilisation by inverted AOT micelles[46,59], while the optimum catalytic activity of the solubilised peroxidase greatly exceeds that manifested by the same enzyme in aqueous solution (Fig.4). The interpretation of spectroscopic data for solubilised enzymes must therefore be treated with caution.

The Influence of the pH

The rates of enzymic reactions and the kinetic parameters describing them in most cases depend on the pH (see, for example, Berezin and Martinek[1]). When an enzymic reaction was transferred from an aqueous solution to a system of inverted surfactant micelles, significant shifts of the pH profiles were observed[37,40,41,45,52,54,87]. This phenomenon is due to different causes. Firstly, the use of ionogenic surfactants, forming a charged layer (electrical double layer) around the enzyme molecule, can lead to a local shift of the pH (as a function of the sign of the charge of the ionic groups and the degree of hydration of the surfactant molecule). The apparent shift of the pH (and correspondingly of the effective pK) is in this case usually 1–2 units (see the section above dealing with the micromedium in the inner cavities of inverted micelles). Secondly, the acid–base properties of the ionogenic groups of the enzymes solubilised in the inverted micelles can vary with their microenvironment (for example, on partial dehydration). It is well known that the dehydration of ionic groups, which occurs when an aqueous solution is replaced by an aqueous organic solution or a purely organic solution, leads to changes in the pK of the ionic compounds by an amount up to several units[92]. Finally, it is necessary to take into account the possibility of a conformational change in the enzyme on solubilisation, which can also alter the observed pK of its ionogenic groups, including the functional groups involved in the enzymic reaction.

VI. SUBSTRATE SPECIFICITY OF SOLUBILISED ENZYMES

The change in the specificity of the enzyme as a result of its solubilisation can be both true, i.e. associated with a change in the catalytic properties of the enzyme itself, and apparent, i.e. caused by a change in the observed Michaelis constant, which depends, as can be seen from Eqn.(13), on the distribution of the substrate in the inverted micelle system (i.e. on the local concentration of the substrate around the solubilised enzyme molecule.

A change in the true substrate specificity has been established for pancreatic lipase, which catalyses the hydrolysis of the glycerol esters of aliphatic acids[61]. Namely, whereas in aqueous solution k_{cat} for the hydrolysis of tributyrin is greater by a factor of ~2 than for triolein[93], in the AOT inverted micelle system in octane k_{cat} for triolein actually exceeds k_{cat} for tributyrin by a factor greater than 15.[61] This means that the transition from an aqueous solution to the inverted micelle system is accompanied by a significant shift of the true substrate specificity of the lipase in favour of high-molecular-weight substrates.

A striking example of a change in the substrate specificity of an enzyme due to the substrate distribution effects is provided by the oxidation of aliphatic alcohols catalysed by horse liver alcohol dehydrogenase in the AOT inverted micelle system in octane[53]. Fig.5 shows that the transition from an aqueous solution to the inverted micelle system is accompanied by a shift of the maximum on the curve describing the dependence of the second-order rate constant for this reaction $k_{cat}/K_{M,app}$ on the length of the hydrocarbon chain in the molecule of the alcohol substrate n; it is seen that octanol ($n = 8$) is the optimum substrate in water, while butanol ($n = 4$) is the optimum substrate in a colloidal solution of water in octane. In other words, the substrate specificity of alcohol dehydrogenase, whose measure is in fact the quantity $k_{cat}/K_{M,app}$,[94] changes sharply on passing from an aqueous to a micellar solution.

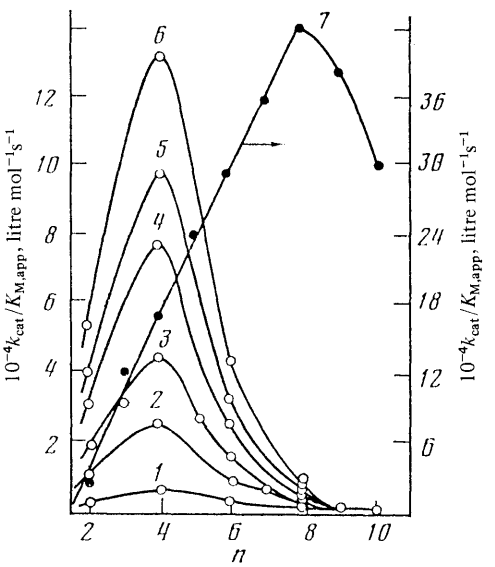


Figure 5. Dependence of the second-order rate constant $k_{cat}/K_{M,app}$ for the oxidation of aliphatic alcohols, catalysed by horse liver alcohol dehydrogenase, on the length of the hydrocarbon chain n in the alcohol substrate molecule[53]. *1*)–*6*) AOT (0.1 M)–octane–water (0.02 M phosphate buffer, pH 8.8) inverted micelle system; $[H_2O]/[AOT]$: *1*) 12.2; *2*) 23.0; *3*) 29.0; *4*) 37.7; *5*) 42.3; *6*) 48.9; aqueous solution (0.02 M phosphate buffer, pH 8.8); temperature 25 °C.

On the other hand, the conformation of the active centre of alcohol dehydrogenase hardly changes in such a transition, which is indicated by spectroscopic data[39] and the fact that the character of the dependence of k_{cat} on n is the same in aqueous solution and in the inverted micelle system[53]. The observed change in substrate specificity is caused[53] by the change in the character of the dependence of $K_{M,app}$ on n

associated with the effects due to the distribution of the alcohol substrate between the micellar and organic phases.

VII. REGULATION OF THE CHEMICAL EQUILIBRIUM IN INVERTED MICELLE SYSTEMS

The system of hydrated inverted surfactant micelles in an organic solvent in the presence of a not unduly high water content constitutes in essence a two-phase water–organic solvent phase, in which, however, the aqueous phase is "uniformly" distributed (by virtue of the presence of the surfactant) in the bulk of the organic solvent. The characteristics of the shifts in the equilibria of the chemical reactions (including enzymic reactions) in two-phase water–organic solvent systems in the absence of surfactants (i.e. in systems where there is a macroscopic interface between the phases) have been investigated in fair detail[95–102]. The approach[95–100] taking into account the distribution of the reactants between the organic and aqueous phases proved fruitful in the analysis of such systems. In particular, for the simplest reaction A ⇌ B in a two-phase system, the kinetic scheme assumes the following form:

$$\begin{array}{ccc} & \overset{K_w}{} & \\ (A \rightleftharpoons B)_w & & \\ {\scriptstyle P_A}\downarrow\uparrow & & \downarrow\uparrow{\scriptstyle P_B} \\ & \underset{K_{org}}{} & \\ (A \rightleftharpoons B)_{org} & & \end{array} \qquad (15)$$

where K_w and K_{org} are the equilibrium constants of reaction (15) in water and in the organic solvent respectively and $P_A = [A]_{org}/[A]_w$ and $P_B = [B]_{org}/[B]_w$ are the partition coefficients of the reactants between the phases, while the subscripts "w" and "org" refer to the aqueous and organic phases respectively. The expression for the effective equilibrium constant of reaction (15), which is equal to the ratio of the concentrations of the reactants A and B relative to the overall volume of the system, i.e. $K_{eff} = [B]_{tot}/[A]_{tot}$, can be formulated[95–98] as follows:

$$K_{eff} = K_w(1+\alpha P_B)/(1+\alpha P_A) \qquad (16)$$

where $\alpha = V_{org}/V_w$ is the ratio of the volumes of the organic and aqueous phases. In conformity with Eqn.(16), the quantity K_{eff} varies monotonically with increase of α—from $K_{eff} = K_w$ for $\alpha = 0$ to $K_{eff} = K_w P_B/P_A = K_{org}$ for $\alpha \to \infty$ (see, for example, curve *1* in Fig.6).

In the more complex case of a bimolecular reaction A + B ⇌ C + D in a two-phase system, the dependence of K_{eff} on α can have the non-trivial form of a curve with an extremum[96–98]. In other words, in a system of two immiscible solvents the effective equilibrium constant can be higher (or correspondingly lower) than the limiting values characterising the equilibrium in each of the phases.

On passing from a truly two-phase water–organic solvent system to a pseudo-two-phase system of hydrated inverted surfactant micelles in an organic solvent, one may expect, in principle, the appearance of effects associated with the specific influence of the surfactant on the equilibrium position. Effects of this kind have been observed[103] in the oxidation of isobutyl alcohol to isobutyraldehyde catalysed by horse liver alcohol dehydrogenase in a system of inverted AOT micelles in octane:

$$RCH_2OH + NAD^+ \overset{K}{\rightleftharpoons} RCHO + NADH + H^+ . \qquad (17)$$

The pH-dependent equilibrium constant of reaction (17) was investigated:

$$K = \frac{[RCHO]_{eq}\,[NADH]_{eq}}{[RCH_2OH]_{eq}\,[NAD^+]_{eq}} . \qquad (18)$$

The results are presented in Fig.6 in the form of plots of $\lg(K/K_w)$, where $K_w = 2.2 \times 10^{-3}$, against the ratio of the volumes of the organic and aqueous phases V_{org}/V_w.

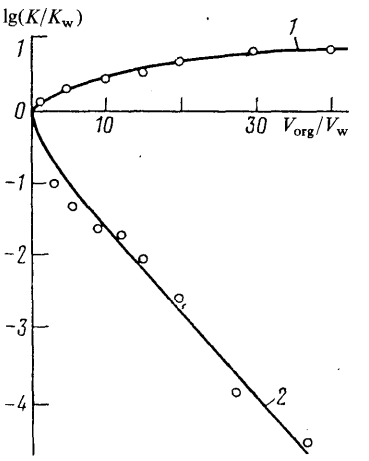


Figure 6. Dependence of the equilibrium constant of reaction (17) on the ratio of the volumes of the organic and aqueous phases[103]; *1*) water (0.02 M phosphate buffer, pH 8.8)–octane two-phase system; *2*) inverted AOT micelle–octane–water (0.02 M phosphate buffer, pH 8.8) system; initial AOT concentration in octane 0.7 M; temperature 20 °C.

In the truly two-phase water–octane system (in the absence of a surfactant), the equilibrium position of reaction (17) can be described by an equation analogous to Eqn.(16), because the coenzymes NAD^+ and NADH are insoluble in octane (their partition coefficients are zero).[96,97] Indeed in this case the dependence of $\lg(K/K_w)$ on the phase volume ratio V_{org}/V_w has the form of a curve with a plateau in conformity with Eqn.(16) (curve *1* in Fig.6). In other words, as the water content in the system diminishes, the equilibrium constant of reaction (17) increases, i.e. the equilibrium shifts to the right (in the direction of the aldehyde). On passing from the two-phase water–octane system to a homogeneous (micellar) solution of water solubilised in octane with the aid of AOT, the character of the dependence of the equilibrium constant on V_{org}/V_w changes sharply (cf. curves *1* and *2* in Fig.6 respectively). Namely an increase of the content of the organic component (octane) in the system leads to a more significant shift of the equilibrium in the presence of the surfactant than in its absence and in addition the shift is in the opposite direction (towards the formation of the alcohol).

The specific influence of AOT on equilibrium (17) is due to the differences between the capacities of the starting materials and reaction products for binding onto the inner surfaces of the inverted micelles formed by the ionic groups of the AOT molecules[103].

It is important to emphasise that the degree of the alcohol–aldehyde conversion in the micellar system can be regulated reversibly by varying the composition of the reaction medium, for example, the water content[103]. Even a slight change in the water content in the system (several tenths vol.%) can then lead to a significant (by an order of magnitude and more) shift of the equilibrium constant (see curve *2* in Fig.6).

VIII. MODELLING OF THE STRUCTURE AND FUNCTION OF BIOLOGICAL MEMBRANES

The idea that inverted micelles constitute an extremely realistic model of biological membrane structures has been frequently stated in the literature[5,104-106]. This hypothesis has been recently confirmed experimentally by Dutch[107-117] and English[118,119] investigators. It was observed that the bilayer membranes frequently contain nonbilayer structures, the so-called lipid particles, which can be clearly distinguished in electron photomicrographs. Investigations showed that these particles consist of associated lipid molecules constructed in the form of inverted micelles and incorporated between the monolayers of the bilayer membrane (Fig.7*a*).

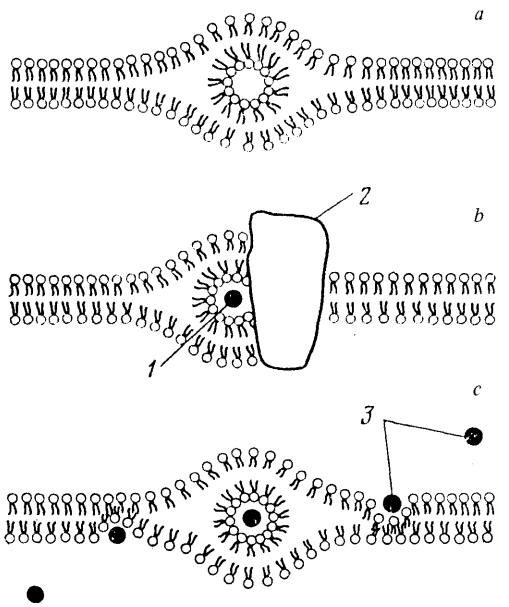


Figure 7. Some of the possible functions of the intramembrane lipid particles (*a*) in a biological membrane[107-122]; *b*) interaction of cytochrome *c* (*1*) with cytochrome oxidase (*2*); c) transmembrane transfer of a protein molecule (*3*).

The intramembrane lipid particles together with the intramembrane particles of another kind (for example, protein particles) apparently play an important role in the functioning of biological membranes. This conclusion is based on the fact that membranes characterised by a high metabollic activity (for example, the membranes of mitochrondria and chloroplasts) contain a large number of intramembrane particles, while the relatively inert membranes contain none at all[117]. The most important cell processes such as the coalescence and compartmentalisation of the membranes[110-112,120,121], exocytosis[111], the transport of lipids through membranes via the "flip–flop" mechanism[113,114], and the transmembrane transport of ions[107,111] proceed via a mechanism similar to that illustrated in Fig.7*c* for protein molecules.

Data[115,116,122] showing that certain proteins, for example, cytochrome *c*, are capable of inducing the formation of inverted micelles in a bilayer membrane, being incorporated at the same time in the inner cavity of such a micelle, are of fundamental importance. It may be that this mechanism plays a significant role in the interaction of cytochrome *c* with the membrane enzyme cytochrome oxidase[116] (Fig.7*b*) and in the transmembrane transport of proteins[115,116,122] (Fig.7*c*).

The functions of the lipid particles and related structures enumerated above have served as a basis of a recently proposed new metamorphic mosaic model of biological membranes[111].

Studies designed to elucidate the role of the nonbilayer lipid particles in membrane processes have only just begun. Nevertheless their first results already show how fruitful can be the employment of inverted micelles as a model of biological membrane structures. We believe[123] that micellar enzymology is fully capable of reflecting the true picture of the functioning of enzymes *in vivo* (particularly when natural lipids are used for the formation of inverted micelles in an organic solvent).

Thus the enzyme "superactivity" [58,59] in inverted micelles compared with the enzyme activity in aqueous solution (see Figs.3 and 4) shows that the catalytic potential of enzymes cannot always be fully elucidated in classical *in vitro* experiments. The most significant differences between catalytic activities will probably be found for the membrane enzymes.

The results obtained in the study of the substrate specificity of pancreatic lipase and alcohol dehydrogenase in the inverted AOT micelle system in octane (Fig.5) justify the expectation of significant differences between the substrate specificities which the enzyme exhibits in a living cell and in aqueous solution in experiments carried out *in vitro*. Furthermore it is possible that the regulating effects observed in the study of the characteristic features of the equilibrium of an enzymic reaction in a micellar system (Fig.6) occur also in a living cell where the content of metabolites might be regulated (with participation of enzymes) by varying the degree of hydration of biological membranes.

IX. APPLIED ASPECTS OF MICELLAR ENZYMOLOGY

The science of chemical reactions and their catalysis by enzymes in inverted micelles is at present in its initial stage and a wide range of problems in this field still awaits solution. However, the experimental data already accumulated indicate the exceptional fruitfulness of this field, which extends and deepens the fundamental knowledge about enzymes and biological membranes.

Micellar enzymology leads to new prospects for the employment of biocatalysis in chemical practice. Thus micellar systems find extensive applications in **organic synthesis**, in the first place for the enzymic transformation of water-insoluble compounds, such as fats, steroids, petroleum products, etc. The possibility of the enzymic hydrolysis of fats has been demonstrated for the action of pancreatic lipase on triglycerides in the "AOT–water–octane" system[61] and a higher catalytic activity of the solubilised enzyme relative to a long-chain less water-soluble substrate was noted. A striking example of the employment of enzymes in inverted micelles for organic synthesis is provided by the work of Dutch investigators[124], who proposed a relatively simple and effective system for the stereospecific production of steroids. This communication should apparently arouse special interest in chemists specialising in synthesis, because, by virtue of the cofactor regeneration system, ordinary molecular hydrogen was used as the reducing agent.

Studies designed to create an energy conversion system have begun to develop in recent years (Berezin and Martinek[2], Chapter XII). A process for the photochemical

formation of hydrogen with participation of an enzyme (a hydrogenase) solubilised by inverted micelles in an organic solvent has been proposed[125].

Enzymes are already used in **analytical chemistry**[2]. Inverted micelle systems lead to new possibilities for the employment of enzymes in the detection of water-insoluble compounds. This is particularly important for the monitoring of the contamination of the environment, which is frequently due to hydrophobic substances such as pesticides and other chemical poisons, the products of incomplete fuel combustion, the waste from chemical industry, etc. The use of enzymes as analytical reagents can lead to the solution also of a more general problem—the creation of reliable test systems for the biological activities of various compounds both water-soluble and water-insoluble. Examples of such analytical systems for the investigation of water-insoluble derivatives of vitamin B_2 have already been successfully realised on the basis of micellar solutions of D-aminoacid oxidase and lipoxygenase[126].

Inverted surfactant micelle systems in organic solvents supplement and extend the methodology of **protein** (enzyme) **chemistry**. They have been used successfully to determine the molecular weights and effective sizes of protein globules[127,128] and also in the thin-layer chromatography of aminoacids[129]. Inverted micelle systems can serve as a universal medium for reactions involved in the **chemical modification of proteins** (enzymes). An envelope of surfactant molecules protects the protein from uncoiling (denaturation) and the presence of the aqueous and organic phases makes it possible to introduce into the reaction both water-soluble and water-insoluble reactants.

In order to impart membranoactive properties to water-soluble proteins, it is necessary to introduce one–two hydrophobic (anchor) groups into the molecule: this procedure has been applied successfully in the treatment with the water-insoluble stearoyl chloride of α-chymotrypsin solubilised by inverted AOT micelles in octane[130]. The converse problem can probably be solved analogously—water-insoluble membrane enzymes can be made hydrophilic by chemical modification.

Although we have consistently emphasised the advantages of employing micellar systems for reactions involving water-insoluble reactants, these systems can prove useful also in many cases regardless of the nature (solubility) of the reactants. From the standpoint of **organic synthesis**, the possibility of regulating the equilibrium position in the catalysed reaction, which should make it possible to carry out reactions whose equilibria in aqueous solution are displaced towards the starting materials (see the relevant reviews[2,100–102]), must be regarded as an important property of inverted micelle systems. The concentration effect and the effects involving an increase of the catalytic activity of enzymes in inverted micelles can increase significantly the sensitivity of **analytical systems**. Indeed it has been shown[131] that, on passing from a traditional aqueous solution to the Brij 96–water–octane system, the sensitivity of the bioluminescence analysis of ATP increases by more than an order of magnitude. Furthermore, the stabilisation of the level of the detected signal is observed in the micellar system, which makes it possible to simplify significantly the entire analytical procedure[131].

The present state and prospects for the practical utilisation of biocatalysis are closely related to the development and improvement of methods for the stabilisation and immobilisation of enzymes[2]. "Molecular immobilisation" has been achieved[132] by the polymerisation of a micellar matrix and/or the components of the inner cavity of the inverted micelle made up of reactants containing a double bond. Protein preparations with an envelope having a specified thickness around each protein molecule were obtained as a result.

It is clear from the achievements and prospects in micellar enzymology that this field of science merits new efforts and investigations. There is every reason to believe that in the course of such investigations new possibilities for the application of micellar enzymology in other fields, not considered in this review, will be discovered and developed.

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