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Microheterogeneous surfactant-based systems as the media for enzymatic reactions

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Abstract - Surfactant aggregates (micelles) of various structure, size and shape are the systems with the media mostly close to those in natural (cell) systems. Hence, they are unique in their abilities for enzymologists whose task is to disclose the potentiality of enzymatic catalysis *in vivo*. By example of enzyme-containing systems of reverse micelles of surfactants in organic solvents, principal ways of regulation of enzyme activity are discussed. Apart from the effects of reverse micelles as an enzyme supports, they can be used as the matrices for constructing rather sophisticated biocatalysts and, in the first turn, those compatible with organic solvents.

INTRODUCTION

High effectivity and substrate specificity of enzyme catalysis are the matters of constant interest drawn by synthetic chemists. However, traditional enzymology is based on experiments with water or aqueous solutions as the media for enzymatic reactions. This item limits to an appreciable extent the possibilities of application of enzymes in systems without water or with small content of water. For the reason of actual necessity in such systems, a number of approaches has been put forward that enable to fit biocatalytic systems to the media with low water content; see ref.1 for the review. A particular place in the line is occupied by the microheterogeneous systems on the basis of aggregates of surfactants (micelles) in organic solvents. Investigations of enzymes in the systems made the basis for a new challenging and rapidly developing trend of biochemistry named micellar enzymology; see refs. 2-7 for reviews. The main objective of the trend is to understand the principles of regulation of enzyme structure and function in the systems. The works in the area of micellar enzymology are mostly interesting for fundamental biochemistry. At the same time, their practical significance is not to date properly evaluated although obviously fascinating; for reviews, see refs. 8-10.

Here we will consider the basic ideas of micellar enzymology and enzymatic catalysis in micellar systems.

APPLICATION OF MICROHETEROGENEOUS SYSTEMS OF SURFACTANT AGGREGATES IN ENZYMOLOGY

Classical "in-water enzymology" usually deals with "purified" enzyme preparations that can lack on isolation from living matter some of the components essential for exposition of real catalytic properties *in vivo*. In nature enzymes function in microheterogeneous systems, for example interacting with different surfaces composed from lipid membranes or being incorporated into biomembranes. Even in cytoplasm, water is not a dominating component and is playing a structural role as well by participating in formation of biocatalytic complexes (generally of glycolipoprotein origin). It is just the time to come to a step by step reconstruction of the complexes started from the well-studied enzyme preparations in order to elucidate the role of different components of natural media in biocatalysis.

From the point of view of classical colloid chemistry, phospholipids, the major components of biomembranes, are typical surfactants. They spontaneously form aggregates of different structure, e.g. spherical, cylindrical and lamellar micelles, that, in turn, can be packed in more complex liquid crystalline structures. A great amount of ternary systems of the type "surfactant - water - organic solvent" has been studied in colloid chemistry up to date. Depending on the particular ratio between the components, surfactant aggregates with different structure and properties can be obtained at investigators' will. Fig. 1 gives an example of phase diagram for the system.

Hence, the task of "reconstructing enzymology" seems quite obvious - to incorporate enzymes in different surfactant aggregates and to study their properties in the systems. The final task is to obtain an "enzogram" that shows how catalytic activity of the enzyme studied is changed in response to alterations in its microenvironment in the range of structural changes in the ternary systems "surfactant - water - organic solvent". Fig. 2 shows an example of the enzogram.

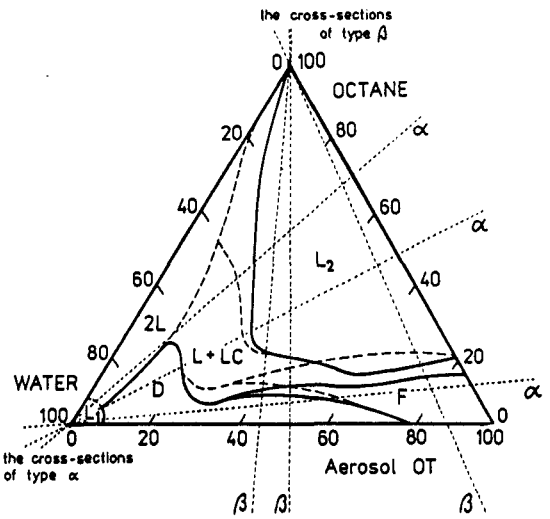

Fig.1. Phase diagram for Aerosol OT (AOT)-water-octane system. The concentration of components are expressed in % w/w. L_2 - reverse micelles, D and F - liquid-crystal line mesophases with lamelar and and reverse hexagonal packing of AOT molecules, respectively. The cross-section of α and β types shows the examples of ternary mixtures used as media for enzyme reactions to obtain profiles of types **a** and **b** shown in Fig.2. For details, see refs. 11 and 12.

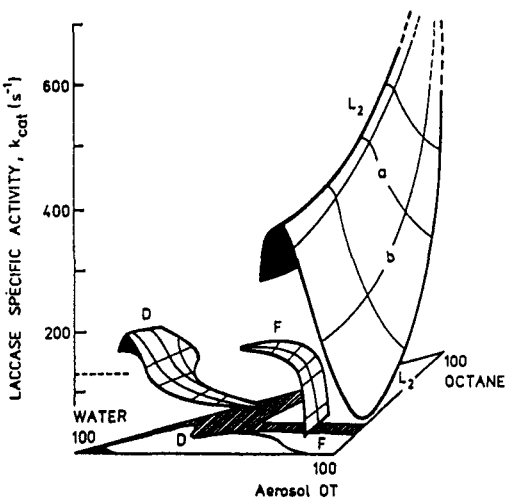

Fig.2. Dependence of laccase activity in AOT-water-octane system on the composition of the system; for experimental conditions, see ref.12. Catalytic activiity of laccase in aqueous solution is shown by the dashed line. The activity profiles "**a**" and "**b**" were measured in the ternary mixtures corresponding to cross-sections of types α and β in Fig.1, respectively.

As seen from Fig.2, enzymes (laccase in this case) are spontaneously included and display catalytic activity in all types of aggregates. However, the activity changes either on transition from one type of aggregates to another or on changing the concentration of the components and, consequently, dimensions and structure of the aggregates within each phase. At the same time, the whole area of classical "aqueous enzymology" is represented by the only point in the left corner of the diagram in Fig.2. It is necessary to mention that the level of catalytic activity in water is substantially less than that in the microheterogeneous systems, especially in reverse micelles (phase L_2).

CHOICE OF THE OPTIMAL MICELLAR MATRIX

The geometrical factor is the most important for enzyme regulation in micellar systems. The highest enzymatic activity is attained under the conditions when the size (and shape) of enzyme molecule is similar to that of micellar matrix. For example, for horse liver alcohol dehydrogenase which molecule is approximated by ellipsoid, cylindrical micelles with a diameter equal to the middle axis of the molecule is the best matrix (ref.7). For spherical enzymes, the geometrical fit is well achieved in spherically-shaped surfactant aggregates, i.e. in reverse micelles. The size of the inner cavity can be smoothly changed by varying the hydration degree of a surfactant, i.e. $w_0 = [H_2O]/[surf]$. In Fig.1 this operation corresponds to a cross-section of the type α. In Fig.2 cross-section of the type **a** shows the "activity - w_0" profile, i.e. how catalytic activity of the enzyme solubilized in the system of reverse micelles is regulated by the hydration degree (by the size of micelles). Similarly to laccase (Fig.2), "activity - w_0" profiles for most spherical enzymes are bell-shaped with the optimum of activity at the values of w_0 corresponding to the size of the inner micellar cavity equal to a diameter of protein molecule. In other words, the optimal enzyme-containing micelle is constructed by addition of surfactant molecules to a protein molecule to form a densely packed monolayer. The micelles are in a dynamic equilibrium as a result of exchange by surfactant molecules by collisions between different aggregates. The collisions between empty and protein-containing micelles are detrimental for the optimal microenvironment of an enzyme and, hence, for its catalytic activity. Elimination of the unfavorable interactions is achieved by dissolution of a micellar system by organic solvent; see profile of type **b** in Fig.2.

In the tailor-made micellar matrix, conformational mobility of enzyme molecule is decreased that may stabilize the catalytically active conformation of enzyme as in the case of α-chymotrypsin (ref.13). Additional increase in rigidity of a micellar matrix that is experimentally achieved by a substitution of water by water-miscible organic solvents (polyols or dimethyl sulfoxide) in the systems leads to an increase of catalytic activity of the enzyme (ref.14). In surfactant-free aqueous solutions of enzymes containing water-miscible organic solvents, the catalytic activity decreases abruptly at relatively low concentrations (usually 20-50 v/v %) of the solvent. At the expense of the stabilizing action of the optimal (tailor-made) micellar matrix, high enzyme activity is retained in practically dry (without water) systems of the type: surfactant - water/water-miscible organic cosolvent - water-immiscible organic solvent. However, an increase in catalytic activity is displayed in parallel with the loss of substrate specificity (ref.15) that can be beneficial from the synthetical point of view. For example, the highly active "tense" form of α-chymotrypsin efficiently catalyzes hydrolysis of the substances which are not the substrates of the enzyme neither in aqueous solutions nor in hydrated reverse micelles (ref.15). On the other hand, the lack of water in the reaction medium leads to a substantial shift of thermodynamic equilibrium in hydrolytic processes that allows to carry out effective synthesis of peptide and amide bonds (ref.16).

CONCLUSION

Any description of the properties of reverse micelles cannot be considered as complete without a discussion of their application as a tool for construction of new assemblies. The application is based on a notion that a micelle is a *nanoreactor of a variable molecular size*. As seen from the Scheme in Fig.3, a micelle of the size that is optimal for solving the particular task, can be easily obtained, and all the necessary high- and low-molecular weight components including water-soluble substances, can be incorporated to produce the aggregate needed. By using well-known chemical methods such as cross-linking with bifunctional reagents, (co)polymerization etc., one can fix the needed assembly inside the micelle. By further removing of the surfactant, the assembly can be obtained in a "pure" form. This method has been used with a success for creation of conjugates of proteins with synthetic polymers of needed

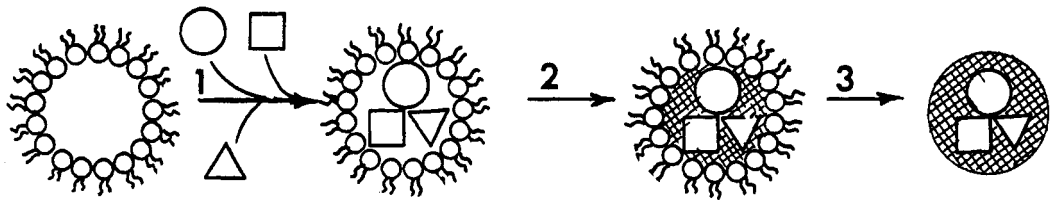

Fig.3. Scheme of preparation of nanoparticles and macromolecular conjugates by using reverse micelles of surfactants.
1 - solubilization-construction,
2 - chemical fixation (cross-linking by bifunctional reagents or polymerization,
3 - washing from surfactant.

stoichiometry (refs.17,18), of polymeric nanogranules containing highly active and stable enzymes that are soluble both in water and organic solvents (refs.19,20). The latter seems to be specially promising in organic synthesis.

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