ENZYMES ENTRAPPED INTO REVERSED MICELLES OF SURFACTANTS IN ORGANIC SOLVENTS:
Key Trends in Applied Enzymology (Biotechnology)

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This paper discusses applications of enzymes solubilized by surfactants in organic solvents to fine organic syntheses, in clinical and chemical analysis, and in therapy, as well as some future trends in biotechnology.

KEY WORDS Enzymes, reversed micelles, surfactants.

Dedication

This paper is dedicated to Professor Dr Georg Manecke on the occasion of his 70th birthday.

INTRODUCTION

Micellar enzymology studies the catalysis by enzymes entrapped in hydrated reversed micelles of surfactants (detergents, phospholipids etc) in organic solvents. The retention of the enzymatic function in such microheterogeneous media (Figure 1) is not surprising for lipolytic enzymes (Hanahan, 1952; Misiorowski and Wells, 1974), since the presence of an interface is an obligatory condition for their functioning. The enzymes of traditional “in-water” enzymology are quite a different matter. That is why the first report (Martinek et al., 1977c) on the catalytic activity of chymotrypsin and peroxidase solubilized by Aerosol OT in octane (or benzene) initiated subsequent research in this field; to date about 30 enzymes have been involved in catalytic activity studies, as recently reviewed by Luisi (1985) and Martinek et al. (1986).

The key research problems of micellar enzymology and its relation to enzyme membranology have been discussed extensively (Martinek et al., 1986). One of

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the most striking effects is the apparent enhanced activity ("superactivity") of the entrapped enzymes. For instance, the catalytic activity of peroxidase entrapped in reversed micelles of Aerosol OT (in octane), of Brij 96 (in cyclohexane), of dodecylammonium propionate (in a mixture of diethyl ether and benzene), or of lecithin (in a mixture of methanol, pentanol and octane) is more than 100 times higher than that characterizing the enzymatic reaction (oxidation of pyrogallol) in water (Klaychko et al., 1984). Other enzymes possessing similarly high catalytic activity in the micellar medium (compared with the value of $k_{cat}$ in aqueous solution) are acid phosphatase and laccase (Levashov et al., 1986). In addition to the enhanced activity, entrapped enzymes may also possess an enhanced stability. For instance, after cytochrome P-450 is isolated from microsomal membranes, it becomes rather labile in aqueous buffer. However, Erjomin and Metelitsa (1982) have shown that its lifetime in the micellar medium at elevated temperatures is equal to that in microsomes themselves. Thus, in colloidal solution of water in organic solvents, solubilized enzymes, as compared with those in aqueous solution, possess quite different, and often more useful, catalytic properties (see reviews by Luisi (1985) and Martinek et al. (1986)).

In the present paper we shall try to identify and rationalize some key trends that may prove to be important for applied enzymology (biotechnology). Let us consider some representative examples.

**ANALYTICAL AREAS**

In micellar media enzymes can interact easily with water-insoluble compounds (substrates, inhibitors, activators etc.) as is schematically presented in Figure 1. The inhibition of lipoxygenase by a hydrophobic derivative of vitamin B2 seems to occur in this way (Kurganov et al., 1985). Thus, the development of micellar media allows the theory and methodology of well-known enzymatic analytical approaches, confined so far to aqueous systems (Mosbach, 1976; Carr and Bowers, 1980), to be extended to organosoluble compounds; for instance, for monitoring environmental pollution by pesticides, chemical industry wastes and so on.

The second, but no less important, aspect of the micellar approach stems from the fact that the enzyme entrapped in a membrane-like surrounding often becomes more catalytically active and stable (see above), which results in the

![Figure 1 Schematic representation of the interaction of substrate (or other reagent) molecules ($S$) distributed in the reversed micellar system with entrapped hydrophilic ($E_1$), surface-active ($E_2$) and hydrophobic ($E_3$) enzymes (Martinek et al., 1982).]
higher sensitivity and reliability of the analysis. Such a situation is encountered in
the case of the bioluminescent assay based on firefly luciferase (Belayaeva et al.,
1983). The enzyme responds to the presence of ATP with luminescence that can
be easily detected:

\[ \text{ATP} + \text{luciferin} + O_2 \rightarrow \text{oxyluciferin} + \text{CO}_2 + \text{AMP} + \text{pyrophosphate} + \text{LIGHT}. \]

With luciferase entrapped in a Brij 96/octane/water system, it is possible to
detect concentrations of ATP as low as \(10^{-15}\) M (Klyachko et al., 1987).

**FINE ORGANIC SYNTHESIS**

The potential of the reversed micellar media containing entrapped enzymes in
fine organic synthesis was first discussed 10 years ago (Martinek et al., 1977c).
Two aspects should be highlighted.

Firstly, water-insoluble (or poorly soluble) compounds, such as steroids,
prostanoids, alkaloids, fats etc., can be subjected to biocatalytic conversion
(Figure 1, Table 1).

Secondly, in the traditional medium for enzymatic processes, water, the
equilibrium of many important reactions is shifted to a great extent toward the
initial reagents. This shift occurs, first of all, in processes where the initial
reagents are ionized and, therefore, strongly hydrated, as well as in those where
water forms as a product, for example, in the reactions of sugar or amino acid
condensation, dehydration etc. The situation, unfavourable in terms of thermo-
dynamics, can be improved by conducting the enzymatic reaction in a biphasic
water/water-immiscible organic solvent system (Klibanov et al., 1977; Martinek et
al., 1977b). Here, the equilibrium can be shifted (in order to increase the product
yield) by lowering the water content in the two-phase system and/or by selecting
a water-immiscible organic solvent that can extract the product efficiently. A
theory describing chemical equilibrium shifts was suggested (Martinek and
Semenov, 1981a, b), and recently Halling (1984) presented a more comprehensive
description of how water affects the biochemical equilibria in biphasic reaction
mixtures.

Colloidal solutions of water in organic solvents represents, in fact, a variation
of the biphasic liquid system (Martinek and Semenov, 1981a, b). The micellar
approach succeeded in changing the equilibrium constant of the reaction of
alcohol oxidation to the corresponding aldehyde by a factor of \(10^6\) (Martinek et
al., 1981). In the framework of the above-mentioned “biphasic” approach
(Klibanov et al., 1977; Martinek et al., 1977b; Martinek and Semenov, 1981a, b;
Khmelnitski et al., 1984), peptide synthesis has been recently performed in a
micellar medium (Lüthi and Luisi, 1984).

**NANOGRANULATED ENZYMES**

Reversed surfactant micelles in nonpolar solvents have been used successfully by
Speiser (1984) for the production of new polymeric carriers for drug delivery. In
our laboratory (Abakumova et al., 1985), polymeric nanogranules (particles of
Table 1 Applications of colloidal solution of water in organic solvents

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colloidal dimensions with a radius of less than a few dozen nanometers) with entrapped enzymes were developed. The enzymes in the interior of the polymeric particles revealed enhanced thermostability. For example, chymotrypsin copolymerized with acrylamide (Martinek et al., 1977a; Mozhaev et al., 1983) retained catalytic activity up to 80–90°C, both in the system of surfactant reversed micelles in octane and in aqueous solution (Abakumova et al., 1985).

**CONCLUSION**

Applications of reversed micellar media are not confined to the above examples. Examples in many other applied areas are listed in Table 1. Generally speaking, solubilized (or nanogranulated) enzymes in organic solvents might be successfully employed in all the fields where aqueous biocatalytic systems have been used so far. In our opinion, the most promising results are conversions of progesterone by a multi-enzyme system (Hilhorst et al., 1983) and prostanoids by prostaglandin synthetase (Mevkh et al., 1985). Moreover, the apparent solubilization of bacteria in reversed micelles was recently reported (Häring et al., 1985).

**References**


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ENZYMES IN REVERSED MICELLES