

## ENZYMIC STUDIES IN MICROEMULSIONS. EFFECT OF REVERSE MICELLES ON THE ACTIVITY OF TRYPSIN

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It is well established that enzymes may keep their catalytic activity when hosted in water in oil microemulsions (1, 2). These colloidal systems, which are thermodynamically stable and optically isotropic water dispersions in a nonpolar organic solvent stabilized by surfactants, may be considered as microreactors, where the enzyme transforms the substrates under restricted conditions mimicking the *in vivo* conditions.

It has been shown that proteases are good model enzyme systems that can function in microemulsions (3, 4, 5). We have recently reported the catalytic behavior of  $\alpha$ -chymotrypsin in various types of microemulsion systems formulated either with anionic (bis-ethylhexylsulfosuccinate sodium salt, AOT) or cationic (cetyltrimethylammonium bromide CTAB) surfactants (6).

We present here the study of the catalytic activity of trypsin in various microemulsion systems. We have examined the effect of many parameters, related to the enzyme properties (pH, T) and to the microemulsion characteristics (amphiphile used, water content, presence and nature of cosurfactant).

The hydrolysis of a model substrate such as L-lysine-p-nitroanilide (LPNA) was carried out in both anionic and cationic systems. The reaction was followed spectrophotometrically in the microemulsion by monitoring the release of p-nitroanilide. In the anionic AOT/isooctane/water systems, the reaction followed a Michaelis-Menten pattern, while the catalysis strongly depended on the hydration ratio  $w_0 = [H_2O]/[AOT]$  of the reverse micelles and on the initial pH of the aqueous dispersed phase. The catalysis was

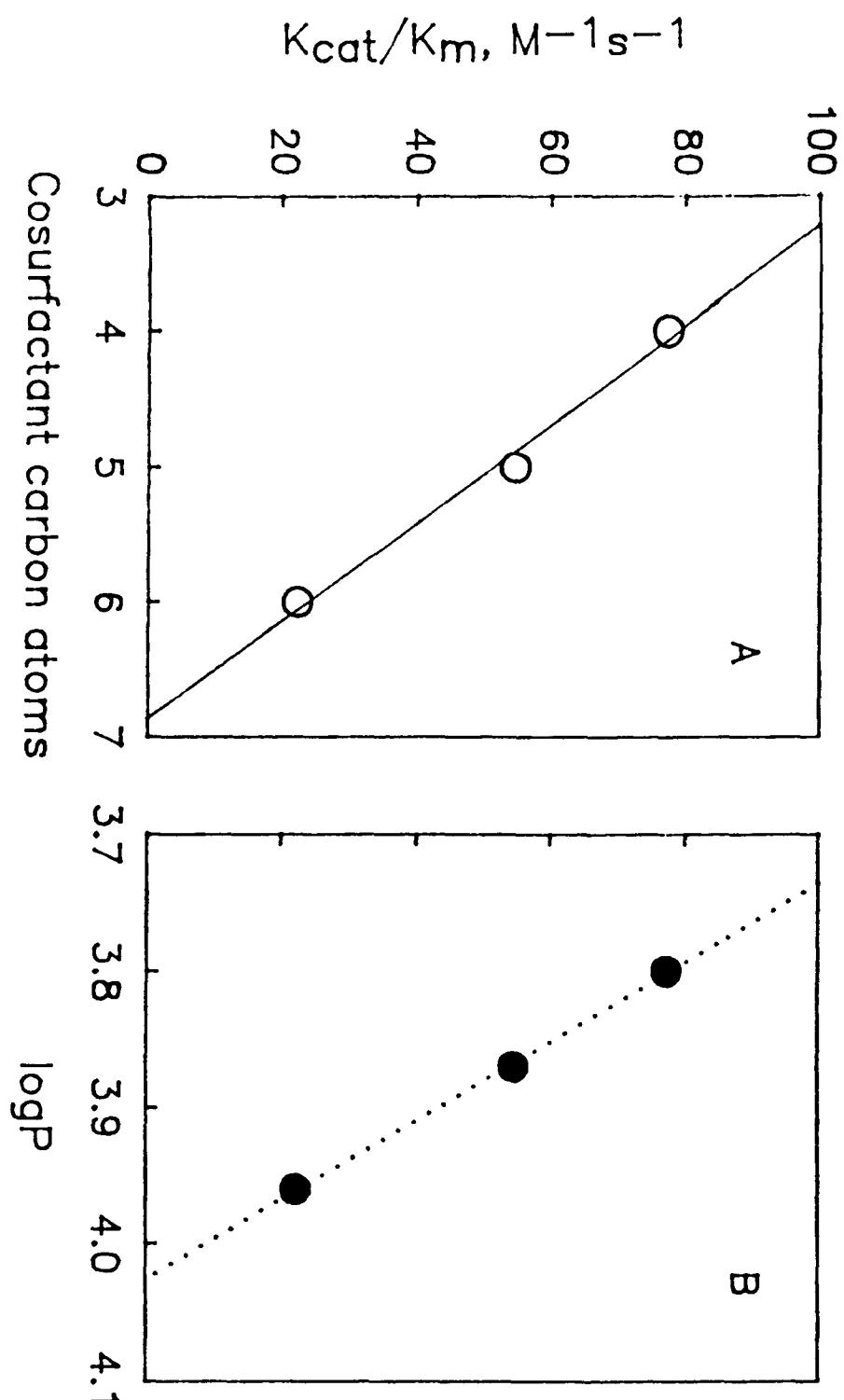
found to follow a bell-shaped pattern with increasing  $w_0$  with an optimum activity at  $w_0 = 9$ .

The same reaction was performed in an another series of microemulsion systems using the cationic amphiphile CTAB in the presence of a series of short-chained aliphatic alcohols as cosurfactants. The presence of cosurfactants in this type systems is indispensable in order to separate the charged polar heads of the amphiphile, increasing thus, the stability of the reverse micelles. It was found that the chain lenght of the cosurfactant used affected the kinetics, expressed in terms of the ratio  $K_{cat}/K_m$ .

Figure 1A shows that increasing the number of the cosurfactant carbon atoms, the rate of catalysis decreases linearly. These results are related to the variation of the polarity of the medium induced by the nature of the amphiphiles. In Fig. 1B the rate of catalysis is plotted versus  $\log P$ , a parameter related to the hydrophobicity of the reaction medium. As it can be noticed the increase of  $\log P$ , which indicates a decrease of the polarity, slows down linearly the rate of catalysis.

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**Fig. 1.** Dependence of the reaction rate of the hydrolysis of L-lysine-p-nitroanilide in cationic microemulsion systems formulated with CTAB, versus (A) the number of carbon atoms of the alcohols used as cosurfactant and (B) the polarity of the reaction medium in terms of  $\log P$ , with  $\log P = \sum x_i \log P_i$ .