ACTIVITY STUDIES OF CHYMOTRYPSIN IN MICROEMULSIONS

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The interest of using water in oil microemulsions as a medium for enzymatic studies has been growing up, since it was shown that, generally, enzymes keep their biocatalytic ability which hosted in such microheterogeneous systems. In this case the enzyme molecules are not handled in classical aqueous buffer solutions, but in reverse micellar systems of water in organic solvents (microemulsions) (1,2). In these systems, which are thermodynamically stable and optically isotropic solutions (3), the dispersed water phase acts like a microreactor where the enzyme transforms the substrates under restricted conditions, miming the *in vivo* conditions.

The composition of the microemulsions seems to play an important role on the enzyme behavior. By changing the nature of the amphiphile molecules, used for the formulation of these systems, the stability and the activity of the enzyme may be altered.

Proteases have been considered as good model enzyme systems that can function in microemulsions (4,5,6). We have studied in a comparative basis the catalytic behavior of α-chymotrypsin in various types of microemulsion systems formulated either with anionic (AOT) or cationic (CTAB) surfactants. We have examined the effect of many parameters related to the enzyme properties (pH, T) and to the microemulsion characteristics (water content, presence and nature of cosurfactant).

In the anionic AOT/isooctane/water systems, the hydrolysis of N - glutaryl - L - phenylalanine - p - nitroanilide (GPNA) followed a Michaelis - Menten pattern, with a K_m value of 0.14 ± 0.01 mM,

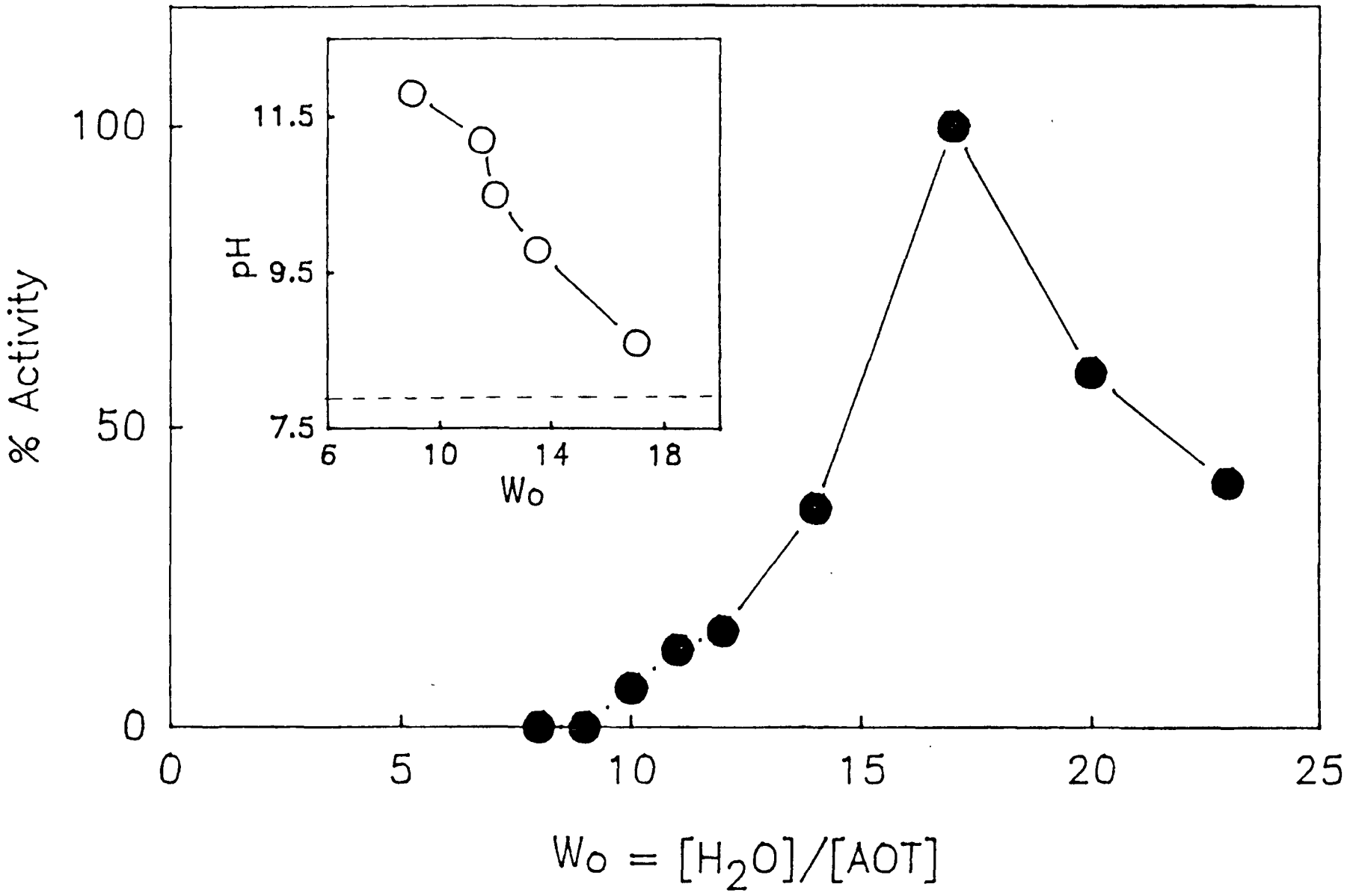

Fig. 1. Activity of a - chymotrypsin in AOT (50mM)/isooctane microemulsions as a function of the hydration ratio

which is similar to the value found in water (K_m=0.1 mM). The catalysis strongly depended on the hydration ratio w_o = [H_2O]/[AOT] of the reverse micelles and on the initial pH of the aqueous dispersed phase. A typical example is showed in Fig. 1 where the enzyme activity is plotted vs. w_o at pH = 8.6. The optimum of the bell - shaped curve shifts towards lower values when the initial pH of the enzyme is increased (Fig. 1., inset.).

The effect of the nature of the surfactant used was also tested by using the cationic amphiphile cetyltrimethylammonium bromide (CTAB) in the presence of a series of short - chained aliphatic alcohols as cosurfactants. The rate of hydrolysis of GPNA was considerably slower in this case than in the anionic systems. When the hydrolysis of N - acetyl - tryptophan - ethyl ester (ATEE) was considered, it was found that the chain length of the cosurfactant used affected the kinetics, expressed in terms of the ratio K_{cat}/K_m. Namely, this ratio varied from 4.5 10^3 M^{-1} s^{-1} for butanol, to 2.3 $10^3 M^{-1}$ s^{-1} for pentanol and to 1.1 $10^3 M^{-1}$ s^{-1} for hexanol. These results are related to the variation of the polarity of the dispersed phase induced by the nature of the amphiphiles.

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