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ALKALINE HYDROLYSIS OF A P-NITROPHENYL ESTER CATALYZED BY PROTEIN-SURFACTANT COMPLEXES

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The large enhancement in the rate of alkaline hydrolysis of p-nitrophenyl hexanoate in aqueous solutions of tetradecyltrimethylammonium bromide in the presence of bovine serum albumin was found. A maximum in the rate enhancement appeared in the vicinity of the critical micelle concentration of the surfactant. It was suggested that the rate enhancement was attributable to the formation of protein-surfactant complexes providing a new pseudo-phase for the reaction.

The rates of many reactions are affected by micellized aqueous surfactants[1] and it is generally accepted that incorporation of reactants into the micelle is of major importance. For example, cationic micelles catalyzed hydrolysis of nitrophenyl esters with hydroxide ion by incorporating both reactants into the small volume of the micellar pseudophase. On the other hand, the interaction of a small molecule with a macromolecule or a molecular aggregate has been a subject in many fields of chemistry. Such a system provides basic information on the functions of biopolymers and/or synthetic macromolecules.

In the present study, the investigation of hydrolysis of p-nitrophenyl hexanoate (PNPH) in aqueous mixed solutions of bovine serum albumin (BSA) and tetradecyltrimethylammonium bromide (TTABr) was undertaken to obtain basic information about the interaction of surfactants with proteins and about functions of protein-surfactant complexes. Enzyme like reactions of p-nitrophenyl acetate with serum albumin have been reported.[2,3] In the reaction of p-nitrophenyl acetate with serum albumin, liberation of p-nitrophenolate ion appears biphasic. For bovine serum albumin the fast initial reaction has been attributed to an acetylation of tyrosine residue. The slower phase formation of p-nitrophenolate has been studied in more detail and attributed to a catalytic hydrolysis of p-nitrophenyl acetate.[2] In the present study, the larger enhancement in the rate of alkaline hydrolysis compared to the micelle catalyzed reaction has been observed in the presence of BSA, and the rate enhancement appears in the vicinity of the critical micelle concentration of the surfactant.

The hydrolysis of PNPH, in aqueous solutions of TTABr in the presence of BSA (2 g dm^{-3}) and the absence of BSA at 25°C and pH 8.0 in 20 mM phosphate buffer, was followed spectrophotometrically at 400 nm (liberation of p-nitrophenoxide).

The substrate concentration was 0.05 mM. Pseudo-first-order rate constants were calculated in the usual manner from $\log(OD_\infty - OD_t)$ vs. time plots, which were linear over at least three half-lives. The surfactant concentration was varied from run to run, so that rate constant versus surfactant concentration profiles were obtained. The binding isotherms were measured by equilibrium dialysis at pH 8.0 (20 mM phosphate buffer), 25°C. The concentration of free surfactant was measured by a total organic-carbon analyzer. The electric conductance measurements were carried out at pH 8.0, 25°C.

In Fig. 1, the pseudo-first-order rate constant, k_{obs}, for the hydrolysis of PNPH in the presence and absence of BSA is illustrated as a function of the total concentration of the cationic surfactant. It can be seen in Fig. 1 that the larger rate enhancement is observed in the presence of the protein compared to the case of the absence of the protein (dotted line in Fig. 1). The rate enhancement appears even in the absence of the surfactant and the enhancement increases progressively with increasing concentration of the surfactant up to the vicinity of its critical micelle concentration (cmc), and the rate constant reaches a maximum around 6 mM of the surfactant. When the concentration exceeds 6 mM, the rate constant drops steeply. The cmc of TTABr in pH 8.0 phosphate buffer was found by electrical conductance method to be 2.2 mM, and in the presence of BSA plots of conductance versus concentration of TTABr revealed a break at 6.2 mM (Fig. 2). In a recent study[4] of the interaction of BSA and sodium dodecylsulfate (SDS) by electric conductometric-titration method made by Takeda et al., four breaks in the plot of conductance versus concentration of SDS were found and interpreted in terms of the conformational changes of BSA and the changes in the number of bound SDS. The break

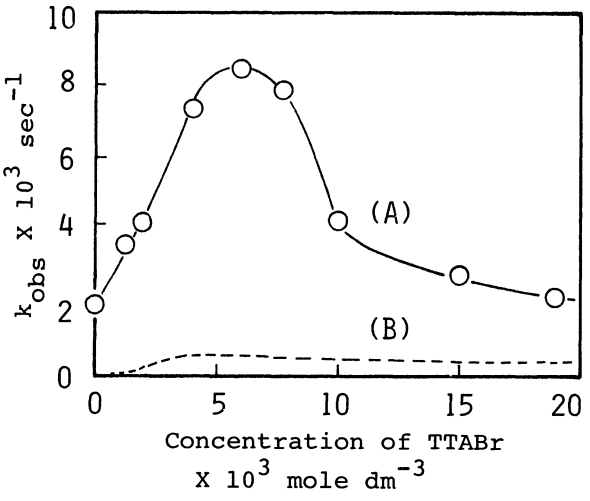

Fig. 1. Effect of TTABr on the rate of alkaline hydrolysis of PNPH in the presence (A) and the absence (B) of BSA at pH 8.0, 25°C. Concentration of PNPH was 5 X 10^{-5}mole dm^{-3} and of BSA was 2 g dm^{-3}.

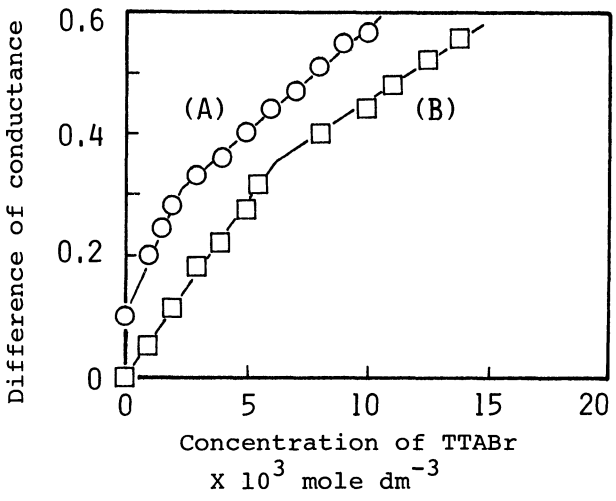

Fig. 2. Difference of specific conductance of TTABr solution in the absence (A) and the presence (B) of BSA at pH 8.0, 25°C. The scale for curve A has been displaced upward by a scale unit.

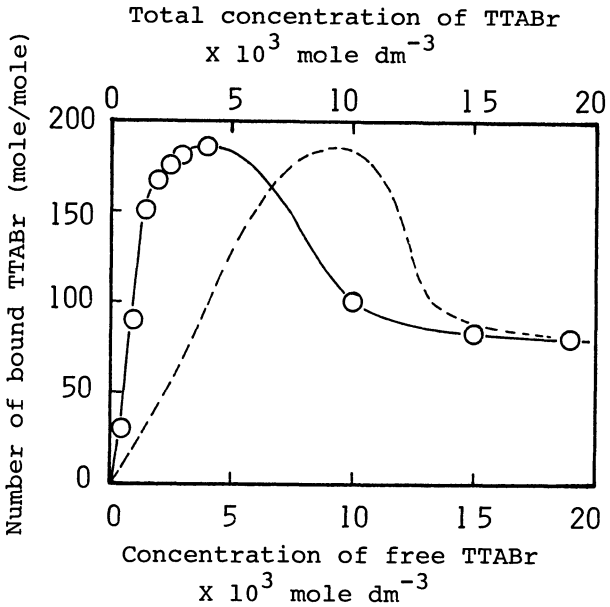

Fig. 3. Binding isotherm for TTABr on interaction with BSA at pH 8.0, 25°C. Number of bound TTABr to BSA was plotted against the concentration of free TTABr. The dotted line indicates the binding isotherm plotted against total concentration of TTABr.

appeared in the highest concentration of SDS in their study corresponds to the end of the second phase of the binding and the conformational change. The break at 6.2 mM, in the present study, may also correlate to the number of bound TTABr on BSA. Thus, an investigation was made on the binding of TTABr to BSA by the equilibrium dialysis method at pH 8.0, 25°C, and the results obtained are shown in Fig. 3. It is noteworthy that the binding curve obtained here is very similar in shape to the curve in Fig. 1. This fact suggests that compexes of BSA and TTABr play a significant role in the rate enhancement of the hydrolysis. In an investigation[5] of the influence of a nonionic surfactant (Polysorbate 80) on the Mylase P catalyzed hydrolysis of arylsulfate esters, the rate constant for the nonenzymatic acid-catalyzed hydrolysis of potassium 2,4-dichloronaphthyl sulfate was considerably enhanced by the surfactant. In general, however, the presence of surfactants in enzymatic reaction systems retards the reactions due to that a) the surfactant molecules prevent the binding of substrates and b) the binding of surfactants causes the conformational changes of enzymes. It has been well known, on the other hand, that cationic micelles and polyelectrolytes catalyze alkaline hydrolysis of esters. The catalytic reactions have been interpreted in terms of an increase of the ester concentration in the micelles and the polyelectrolytes, and an increase of cationic charge around the substrate molecules. The large enhancement of the liberation of nitrophenoxide in the present study may be attributable to an increase of the substrate concentration in the protein-surfactant complexes, an

increase of cationic charge density around the surface of the complexes and/or the acylation of amino acid residues of BSA. The large rate enhancement can not be attributed only to the increase of charge density because micelle like aggregation of the surfactant may form on the protein[6] and if so, the reaction rate in the complexes does not exceed the rate in the micellar catalysis. Moreover, if the liberation of p-nitrophenolate ion is resulted from the acylation of amino acid residues, the reaction may be retarded by the saturation of protein surface with the surfactant molecules. We have no immediate interpretation concerning the mechanism for the rate enhancement, however, an addition of guanidine hydrochloride in the reaction systems with presence and absence of the surfactant decreased the reaction rate. This fact indicates that the native like conformation of BSA might be necessary in the complexes for the rate enhancement. From the observations it is concluded that the protein-surfactant complexes provide a new pseudophase for the hydrolysis reaction.

It should also be noted in the present study that a maximum has been observed in the binding isotherm. The binding of TTABr to BSA has been studied by Nozaki and Tanford.[7] In their study, the binding experiments were performed in the concentration range of TTABr below the vicinity of its cmc. In the present study we extended the measurements of the binding isotherms to free concentration above the cmc and we observed a maximum. The observation of the maximum in binding isotherms of soluble protein with surfactants is not common. Recently, in a paper of Jones and co-workers[8] they found maxima in the binding isotherms of SDS with unreduced and reduced lysozyme, and as far as we know, it appears to be the first time adsorption maxima have been found in soluble protein-surfactant system. It is clear in Fig. 3 that the maximum occurs at free TTABr concentration slightly above the cmc as measured conductometrically (see Fig. 2) and it seems reasonable to conclude that it relates to the maximum in the surfactant cation activity which occurs in the vicinity of the cmc.

Further study concerning effects of alkyl chain length on the rate enhancement and the mechanism for the hydrolysis is in progress.

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