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RESEARCH ARTICLES

Influence of the State of Molecular Aggregation on the Enzymic Hydrolysis of Arylsulfate Esters

THOMAS H. BAXTER* and H. B. KOSTENBAUDER†

Abstract □ While the acid-catalyzed hydrolysis of potassium dichloronaphthyl sulfate is considerably enhanced when the substrate is bound to the surface of a micelle-forming surfactant, the enzyme catalyzed hydrolysis of this substrate exhibits a marked overall inhibition when the substrate is bound to the same surfactant. A plot of the rate of enzymic hydrolysis of that fraction of the substrate bound to the surfactant micelle *versus* surfactant concentration exhibits a sharp maximum. It is postulated that this maximum can be attributed to a gross change in the composition or structure of the surfactant-substrate micelle, such that the required "fit" of the bound substrate on the enzyme surface is sterically blocked. This "critical" surfactant concentration is also evidenced by an abrupt change in the extent of substrate-surfactant interaction under similar conditions. The rate of hydronium ion-catalyzed hydrolysis of potassium dichloronaphthyl sulfate bound to the same surfactant exhibits no such maximum.

Keyphrases □ Arylsulfate esters—synthesis, enzymic hydrolysis □ Enzymic hydrolysis—molecular aggregation effect □ Critical micelle concentration—conductivity measurements □ Surfactants, micellar—arylsulfate binding □ UV spectrophotometry—analysis

The micelle-forming nature of many compounds of biochemical interest and the involvement of some of these compounds in biologically important reactions suggest that there may be similarities between chemical reactions occurring in micellar systems and enzymic reactions occurring when the substrate is in micellar solution or bound to a macromolecule.

It has been known for a considerable time (1, 2) that the addition of bile salts increases the rate of hydrolysis of fat by pancreatic lipase. The mechanism by which

bile salts exert this activation effect has been postulated as due either to an emulsification of the fatty substrate, thereby increasing available substrate surface area, or to activation of the enzyme.

Wills (3) investigated the effect of synthetic anionic, cationic, and nonionic surface-active agents on pancreatic lipase in an attempt to determine the major factor of bile salt stimulation of lipase activity. He noted that all the anionic and nonionic detergents tested had no effect at low concentrations, but inhibited enzymic hydrolysis of triolein by pancreatic lipase when present in high concentrations. However, for each cationic detergent, a critical concentration was found which produced maximum rate enhancement; at higher surfactant concentrations inhibition usually occurred. Wills attributed this maximum to a critical concentration at which the detergent is closely bound to oil globules in the emulsion and thus prevented from inhibiting the enzyme.

Hoffman and Borgstrom (4) studied the pancreatic lipase hydrolysis of long-chain monoglycerides in micellar solution. They reported a marked increase in the extent of hydrolysis of 1-monoolein with a low concentration of sodium glycodeoxycholate and sodium taurodeoxycholate. However, with higher bile salt concentrations the authors noted increasing inhibition.

The present study was designed to evaluate the rate of enzymic hydrolysis of potassium octylphenyl sulfate at substrate concentrations above and below the critical micelle concentration of this ester. The effect of a nonionic surfactant, polysorbate 80, upon the arylsulfatase and acid-catalyzed hydrolysis of potassium dichloronaphthyl sulfate, a substrate which interacts with polysorbate 80, and upon the arylsulfatase-catalyzed hydrolysis of potassium phenylsulfate, a substrate which does not interact with polysorbate 80, was also investigated.

EXPERIMENTAL

Reagents—Octylphenol,[1] recrystallized five times from *n*-heptane to yield pure 1,1,3,3-tetramethylbutylphenol, *para*-isomer, m.p. 84.6° (5), 2,4-dichloro-1-naphthol,[2] phenol USP, *N,N*-dimethylaniline,[3] potassium hydroxide, USP, and chlorosulfonic acid.[4]

The surfactant employed was polysorbate 80, USP.[5] The enzyme used was analytical grade,[6] which has been determined by Abbott (6) to exhibit considerable arylsulfatase activity. Other materials were reagent grade.

Synthesis of Arylsulfates—Potassium phenylsulfate (PPS), potassium octylphenyl sulfate (POPS), and 2,4-dichloro-1-naphthyl sulfate, potassium (PDNS) were synthesized using a modification of the method described by Richmond (7). The general method utilized is outlined below.

The chlorosulfonic acid, 0.39 mole, was slowly added to 140 ml. of *N,N*-dimethylaniline while maintaining the temperature below 10° and stirring constantly. The mixture was then allowed to warm to room temperature and a solution of 0.33 mole of the phenol, or naphthol, in 350 ml. of *N,N*-dimethylaniline was added. The mixture was then stirred at room temperature for 1 hr. At the end of this time the reaction mixture was poured into a cold solution of 50% potassium hydroxide containing 0.61 mole of potassium hydroxide. If necessary, additional potassium hydroxide was added to render the mixture alkaline. The insoluble material which formed was collected on a Büchner funnel, pressed dry with filter paper, and extracted twice with hot 95% ethanol. The alcohol extracts were filtered and the filtrate was allowed to crystallize at 5°. The combined crystalline product from the two extracts was then recrystallized four times from hot 95% ethanol. The product was vacuum-dried using a water aspirator and the dried product stored at 5° under vacuum. Microanalytical results[7] for carbon, hydrogen, potassium, and chlorine for the arylsulfate products were in good agreement with the theoretical values. The IR absorbtion spectra of the products are typical of arylsulfate esters (8).

Determination of Critical Micelle Concentration—To establish the critical micelle concentration (CMC) of the arylsulfates synthesized, conductivity measurements were obtained for aqueous solutions at 48°. A dipping-type conductivity cell with a cell constant of 1.00 $cm.^{-1}$ was employed. The water used was that used throughout the study and had a specific conductivity of approximately 5×10^{-6} ohm^{-1}. The CMC for POPS was 33 m*M* (10.7 mg./ml.); under these conditions PPS and PDNS did not form micelles at concentrations used in these studies.

Binding of Arylsulfates to Micellar Surfactants—The extent of PDNS and PPS interaction with polysorbate 80 was determined by assaying for the amount of arylsulfate in solution on either side of a nylon membrane[8] which was permeable to the arylsulfate but impermeable to polysorbate 80. Specially designed dialysis cells were utilized throughout the study. The cells consisted of two separate methyl methacrylate[9] units which when bolted together formed two cavities separated by the nylon dialysis membrane. The general procedure entailed placing 10 ml. of the surfactant solution and 10 ml. of the arylsulfate solution into the respective sides of the cell, closing the cells with threaded methyl methacrylate plugs fitted with polytetrafluoroethylene[10] gaskets, and placing the cells in a shaker in a constant-temperature oven at 48 ± 0.5° where they were allowed to remain until equilibrium was attained.

Because of the extremely slow dialysis of PPS across the nylon membrane, requiring in excess of 30 days to equilibrate, all studies involving PPS binding were carried out using initially equivalent concentrations of PPS on both sides of the dialysis membrane.

The concentration of the arylsulfate was determined by removing an aliquot from each side of the dialysis cell and assaying spectrophotometrically for the arylsulfate. Appropriate blank solutions were used in each case. The molar absorptivity values and wavelengths of maximum absorbance of PDNS and PPS are 7.453×10^3 at 291 mμ and 1.093×10^3 at 267.5 mμ, respectively.

pH Stat Studies—Burkhardt *et al.* (9) have shown that the hydrolysis of arylsulfates proceeds in accord with the equation:

$$R{-}O{-}SO_2{-}O^- + H_2O \rightarrow R{-}OH + HSO_4^-$$

The course of the hydrolysis can be followed by titrating with alkali the hydrogen sulfate which is formed. Burkhardt *et al.* (9) also demonstrated that arylsulfates are the salts of a strong acid and exhibit no buffering action. The rate of enzymic hydrolysis of POPS and PPS was determined by maintaining the pH of the system, in the titration vessel, at the desired pH by constant monitoring of pH and manual addition of sodium hydroxide solution from a 1-ml. micropipet-buret (Gilmont), which could be read directly to 0.001 ml. At no time did the volume of sodium hydroxide solution added exceed 0.14 ml. (0.56% of the total volume); therefore, no corrections were made to account for volume changes during the reaction. The jacketed titration vessel was thermostated at 48.0°. The substrate solution, adjusted to an ionic strength of 0.44 with sodium chloride, was thermally equilibrated prior to adding the analytical grade enzyme solution. Following the addition of the enzyme, the pH was adjusted to 7.40 and the pH of the system maintained constant within ±0.01 pH unit by manual addition of 0.50 *N*

[1] Technical grade, Rohm and Haas Co., Philadelphia, Pa.
[2] Reagent grade, Distillation Products Industries, Eastman Organic Chemicals Department, Rochester, N. Y.
[3] *Fisher Scientific* Company, Fairlawn, N. J.
[4] Matheson, Coleman and Bell, East Rutherford, N. J.
[5] Polyoxyethylene (20) sorbitan monooleate (Tween 80), Atlas Chemical Industries, Wilmington, Del.
[6] Mylase P, a concentrated, highly active preparation of many enzymes derived from *Aspergillus oryzae*, Wallerstein Company, Division of Baxter Laboratories, Inc., Staten Island, N. Y.
[7] Microanalytical data provided through the courtesy of the Microanalytical Department, Wyeth Laboratories, Radnor, Pa.
[8] Plaskon 8200 Nylon, 0.5 mil, FN 94Cl, Central Research Lab., Allied Chemical Corp., Morristown, N. J.
[9] Plexiglas, Rohm & Haas Co., Philadelphia, Pa.
[10] Teflon, E. I. du Pont de Nemours and Co., Inc., Wilmington, Del.

sodium hydroxide solution from the micropipet-buret. The rate of consumption of alkali is equal to the rate of enzymic hydrolysis. In the case of PPS, systems being titrated contained various concentrations of polysorbate 80. Polysorbate 80 and analytical grade enzyme were demonstrated to exhibit no buffering effect nor base-consuming action over the concentration ranges studied.

Spectrophotometric Studies—By utilizing the spectral shift exhibited by dichloronaphthol when dissolved in 1 *N* sodium hydroxide solution, it is possible to follow the course of hydrolysis of PDNS without interference by the intact arylsulfate by spectrophotometrically determining the concentration of dichloronaphthol at a wavelength of 343 mμ. The molar absorptivity of dichloronaphthol in 1 *N* sodium hydroxide solution is 8.629×10^3 at 343 mμ.

The following general procedure was used: the substrate solution and the required volume of surfactant solution were added to the 40-ml. jacketed glass beaker which was thermostated at 48.0°. Sufficient buffer solution was then added to the thermostated beaker to bring the total volume to 15 ml. Ten milliliters of an analytical grade enzyme solution containing the required amount of enzyme per final 25-ml. volume was then added. Following addition of the enzyme solution the system was equilibrated at 48.0 ± 0.2°. Agitation was provided by a magnetic stirring bar. A 2-ml. sample, the zero-time sample, was withdrawn from the system and immediately discharged into a volumetric flask containing 1 *N* sodium hydroxide solution. The solution was then brought to volume with 1 *N* sodium hydroxide solution and the concentration of dichloronaphthol determined by the absorbance of the solution at 343 mμ. The solution was also scanned over the range of 270 mμ to 360 mμ on a spectrophotometer[11] to detect any abnormal spectral shifts and also to confirm that as the absorbance of the solution at 343 mμ increased, corresponding to an increase in the concentration of the hydrolysis product, the absorbance at 291 mμ, corresponding to the intact arylsulfate, decreased.

Neither the enzyme nor the intact arylsulfate interfere at 343 mμ The spectrum of the intact arylsulfate in 1 *N* sodium hydroxide solution exhibited no changes over a period of several hours. To avoid the possibility of inaccuracies due to loss of solvent from the thermostated beaker during the course of the study, two studies were duplicated in sealed 5-ml. glass vials, each containing exactly 2 ml. of the initial solutions. The results of the two methods were in good agreement. The pH of the systems was monitored for each run and remained essentially unchanged throughout the course of the experiment. This spectrophotometric method was also employed to follow the acid-catalyzed hydrolysis of PDNS.

The buffers employed in the binding and kinetic studies were 0.2 *M* with respect to total phosphate and covered the pH range from 5.0 to 8.4. The ionic strength of each buffer was adjusted to 0.44 with sodium chloride.

Where the initial substrate concentration was varied the ratio of initial enzyme concentration to initial substrate concentration was maintained constant at 2.119 mg. of analytical grade enzyme per millimole of substrate.

Blank runs, omitting the enzyme, but with and without the surfactant, demonstrated no hydrolysis of any substrate over periods of time far in excess of the actual study conditions.

RESULTS AND DISCUSSION

Binding of Arylsulfates to Micellar Surfactants—Figure 1 is a Langmuir-type plot of the interaction of PDNS with polysorbate 80 at pH 7.4. Figure 1 exhibits two linear regions of significantly different slopes, indicating that the nature of the binding of PDNS by polysorbate 80 changes markedly with concentration of free arylsulfate and polysorbate 80 concentration.

Nakagawa *et al.* (10) have studied the influence of solubilized components, particularly long-chain alkanes and alcohols (such as *n*-decane and *n*-decanol), on the micellar molecular weights and aggregation numbers of two methoxypolyoxyethylene glycol monodecyl ether surfactants by light-scattering techniques. They reported increases in micelle molecular weight and aggregation numbers ranging up to fourfold that in the absence of any additive. The greatest changes were effected by the more polar solubilizate, decanol. These authors have shown that the micellar weights increased with the addition of solubilizates up to the saturation limit of the solubilizates, the increase originating not only from the simple incorporation of solubilizate molecules in the existing micelle, but also from the number of detergent molecules in the micelle increasing with added solubilizate. In these cases it seems plausible to consider the additive and the surfactant interacting to form a new micelle considerably increased in size and possibly modified in structure.

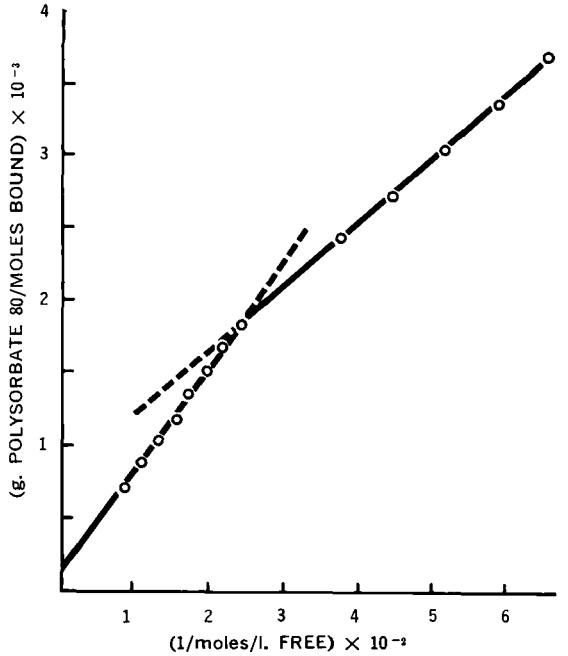

Figure 1—*Langmuir-type plot for the interaction of potassium dichloronaphthyl sulfate with polysorbate 80 in 0.2* M *phosphate buffer, pH 7.4, ionic strength 0.44, temperature 48°.*

Considering these factors when appraising Fig. 1, it is possible to conclude that the inflection in the slope of the plot is due to a change in micelle structure or size. The positive intercept on the ordinate of the Langmuir-type plot indicates a limiting binding capacity or saturation of polysorbate 80 at infinitely high PDNS concentration. Supportive of this concept of Langmuir-type absorption of PDNS, a highly dissociated salt, by polysorbate 80 are the generally accepted theories of solubilization expressed by Elworthy and MacFarlane (11).

Binding studies were also conducted at varying pH values over the pH range from 5.0 to 8.4. The extent of interaction was found to be relatively pH-independent.

Equilibrium dialysis studies run for as long as 25 days at 48°, pH 7.4 and ionic strength 0.44, indicate that no binding occurs between polysorbate 80 and PPS.

Enzymic Hydrolysis of Potassium Octylphenyl Sulfate Above and Below the CMC—First-order rate constants for the enzymic hydrolysis of POPS were determined for initial POPS concentrations both above and below the CMC (33 m*M*) of the compound. Studies at five initial substrate concentrations ranging from 18.5 to 43.1 m*M* yielded first-order rate constants of $14.8 \pm 1.7 \times 10^{-4}$ min.$^{-1}$, with no observable effect resulting from micellization of POPS.

The apparent lack of effect of micellization on rate of enzymic hydrolysis can probably be attributed to a low aggregation number for the POPS micelle. Results of studies on the acid-catalyzed hydrolysis of POPS also support the suggestion that an appreciable concentration of nonassociated substrate exists at concentrations above the CMC. Seltzer (12) found that the rate of acid-catalyzed hydrolysis of POPS was increased only 1.5-fold at concentrations above the CMC. This is in marked contrast to the increased rate of acid-catalyzed hydrolysis of micellar sodium dodecyl sulfate, shown by Motsavage and Kostenbauder (13) to be more than 30 times that for sodium dodecyl sulfate solutions at concentrations below the CMC.

Effect of Polysorbate 80 on the Rate of Enzymic Hydrolysis of Potassium Phenyl Sulfate—The initial zero-order rate of enzymic

[11] Beckman model DK2, Beckman Instruments, Inc., Fullerton, Calif.

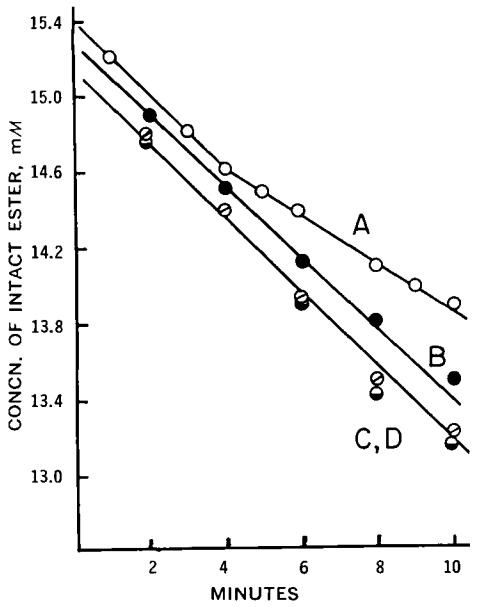

Figure 2—*Effect of polysorbate 80 on the initial zero-order rate of enzymic hydrolysis of potassium phenyl sulfate. Key:* A, *0;* B, *0.8%;* C, *1.6%;* D, *4.0% polysorbate 80. Conditions: pH 7.4, ionic strength 0.44, temperature 48°, analytical grade enzyme concentration 32 mg./ml. Nonlinearity in absence of surfactant is indicative of enzyme inhibition by the hydrolysis product, phenol.*

hydrolysis of potassium phenylsulfate is essentially independent of the concentration of polysorbate 80 as illustrated by Fig. 2. However, the overall first-order hydrolysis rates are apparently increased by polysorbate 80 as indicated in Fig. 3. While this apparent activation effect of polysorbate 80 was not specifically studied, it is postulated that the polysorbate 80, by complexing with the hydrolysis product, phenol, may afford the enzyme protection from inactivation by this product of hydrolysis and thus apparently accelerate the overall rate of enzymic hydrolysis. The interaction of polysorbate 80 with phenol and phenol-type preservatives is well-

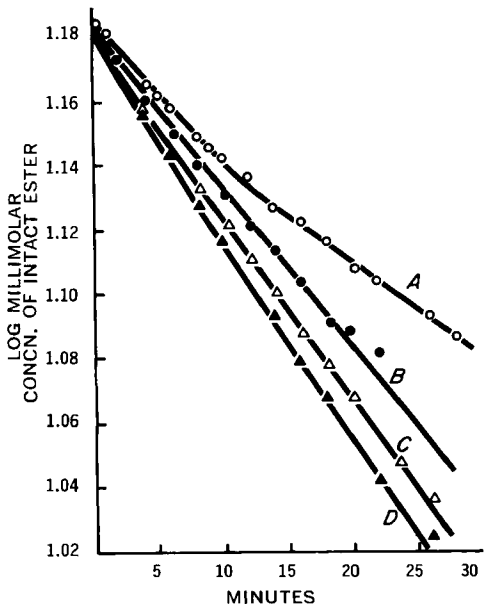

Figure 3—*Effect of polysorbate 80 on the observed first-order rate of enzymic hydrolysis of potassium phenyl sulfate. Key:* A, *0;* B, *0.8%;* C, *1.6%;* D, *4.0% polysorbate 80. Conditions: pH 7.4, ionic strength 0.44, temperature 48°, analytical grade enzyme concentration 32 mg./ml. Nonlinearity in absence of surfactant is indicative of enzyme inhibition by the hydrolysis product, phenol.*

Table I—Effect of Polysorbate 80 on the Observed Zero- and First-Order Rate Constants for the Enzymic Hydrolysis of Potassium Phenyl Sulfate[a]

Polysorbate 80 Concn., mg./ml.	Observed Rate Constants Zero-Order, mmole min.$^{-1}$	First-Order, $k \times 10^3$ min.$^{-1}$	Relative Rates[b] Zero-Order	First-Order
0	0.190	9.8	—	—
8	0.182	11.1	0.96	1.13
16	0.210	12.9	1.10	1.32
40	0.210	15.3	1.10	1.56

[a] Conditions: pH 7.4, ionic strength 0.44, temperature 48°, analytical grade enzyme concentration, 32 mg./ml. [b] Relative rate = rate constant in presence of polysorbate 80/rate constant in absence of polysorbate 80.

documented (14). The observed zero- and first-order rate constants for enzymic hydrolysis in the presence of varying amounts of polysorbate 80 are listed in Table I.

Enzymic Hydrolysis of Potassium Dichloronaphthyl Sulfate in Presence of Polysorbate 80—PDNS does not exhibit self-micellization at practical concentrations, but does readily adsorb to polysorbate 80, permitting a study of the rate of enzymic hydrolysis of a substrate adsorbed to a nonionic surfactant.

The rate of enzymic hydrolysis of PDNS is depressed by increasing concentrations of polysorbate 80, as illustrated in Fig. 4. The observed zero-order rate constants and degree of enzyme inhibition are listed in Table II.

To establish that the study was conducted in a substrate concentration range at which rate of enzymic hydrolysis was proportional to substrate concentration, studies were conducted in absence of polysorbate 80 for substrate concentrations of 3 to 15 m*M*, and a plot of zero-order rate, *V*, *versus* substrate concentration, *S*, was linear with intercept of zero and slope of 0.0066 l./min. The maximum velocity, V_m, and the value of K_s for the Michaelis-Menten (15) equation (Eq. 1) were determined by the Lineweaver-Burk technique (16).

$$V = \frac{V_m(S)}{(S) + K_s} \qquad \text{(Eq. 1)}$$

The values so obtained are K_s = 55.0 m*M* and V_m = 0.444 mmole/min.

For studies in absence of polysorbate 80 a plot of rate of hydrolysis *versus* enzyme concentrations up to 66 mg./ml. also was linear

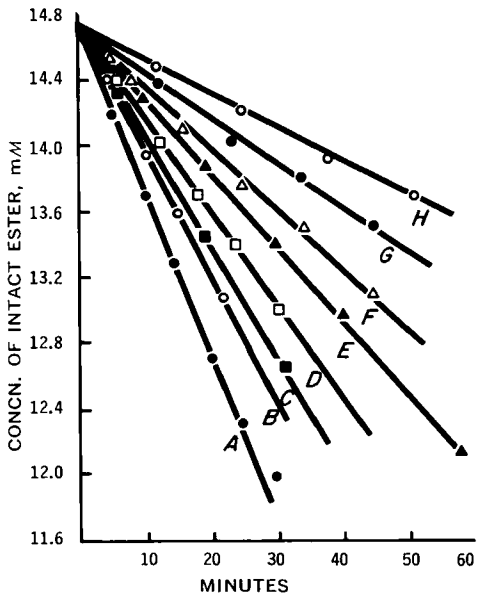

Figure 4—*Effect of polysorbate 80 on the rate of enzymic hydrolysis of potassium dichloronaphthyl sulfate. Key:* A, *0;* B, *0.5%;* C, *1.0%;* D, *1.5%;* E, *2.0%;* F, *2.4%;* G, *3.0%;* H, *4.0% polysorbate 80. Conditions: pH 7.4, ionic strength 0.44, temperature 48°, analytical grade enzyme concentration 32 mg./ml.*

Table II—Effect of Polysorbate 80 on the Observed Zero-Order Rate Constants for the Enzymic Hydrolysis of Potassium Dichloronaphthyl Sulfate[a]

Polysorbate 80 Concn., mg./ml.	Zero-Order Rate Constant, mmole/min.	Degree of Inhibition[b]
0	0.097	
5	0.076	0.78
10	0.066	0.68
15	0.054	0.56
20	0.043	0.44
24	0.037	0.38
30	0.026	0.27
40	0.019	0.20

[a] Conditions: 0.2 *M* phosphate buffer, pH 7.4, ionic strength 0.44, temperature 48°, substrate concentration 15.1 m*M*, analytical grade enzyme concentration 32 mg./ml. [b] Degree of inhibition = observed rate in presence of polysorbate 80/observed rate in absence of polysorbate 80.

with intercept of zero. Studies with potassium phenyl sulfate demonstrated that the presence of polysorbate 80 has no effect on the activity of the enzyme when substrate is not adsorbed to the surfactant.

These observations suggest that the observed inhibition of enzymic hydrolysis of PDNS in presence of polysorbate 80 can be attributed to depletion of available substrate.

Correlation of Polysorbate 80—Potassium Dichloronaphthyl Sulfate-Binding Data with Rate of Enzymic and Acid-Catalyzed Hydrolysis of PDNS—If the inhibition of the enzymic hydrolysis of PDNS by polysorbate 80 occurs by virtue of depletion of available substrate, it should be possible to correlate the degree of inhibition with the extent of binding. From the binding data presented in Fig. 1, the concentration of free and bound PDNS in presence of polysorbate 80 may be calculated, and a plot of free substrate concentration *versus* rate of hydrolysis would be expected to be linear and correspond to a similar plot in absence of polysorbate 80. Such data are presented in Fig. 5, and it is evident that the two plots are not identical; the observed initial zero-order rate of hydrolysis of PDNS in presence of polysorbate 80 is greater than that predicted on the basis of only free substrate being hydrolyzed.

If it is assumed that bound substrate is also subject to enzymic hydrolysis, at a rate equivalent to the difference between the observed overall rate and the rate for free substrate hydrolysis, a plot of rate of hydrolysis of bound substrate *versus* concentration of bound substrate can be constructed. Such a plot is presented in Fig. 6, and it exhibits a pronounced maximum in rate of enzymic hydrolysis at a bound substrate concentration of 8 m*M* (53% of the total substrate concentration) and corresponds to a polysorbate 80 concentration of 15 mg./ml. At bound substrate concentrations greater than 8 m*M* (and correspondingly higher polysorbate 80

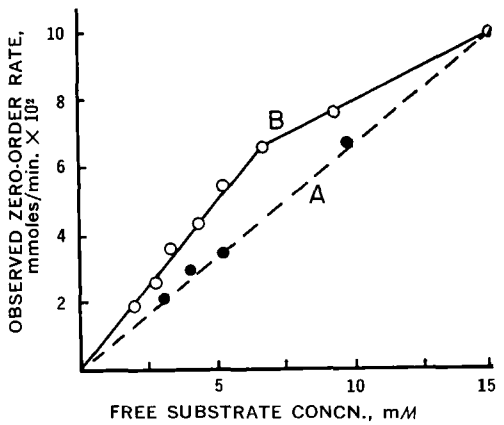

Figure 5—A, *observed rate of enzymic hydrolysis of potassium dichloronaphthyl sulfate* versus *substrate concentration in absence of polysorbate 80;* B, *observed rate of enzymic hydrolysis* versus *free substrate concentration in presence of polysorbate 80. Initial total substrate concentration 15.1* mM.

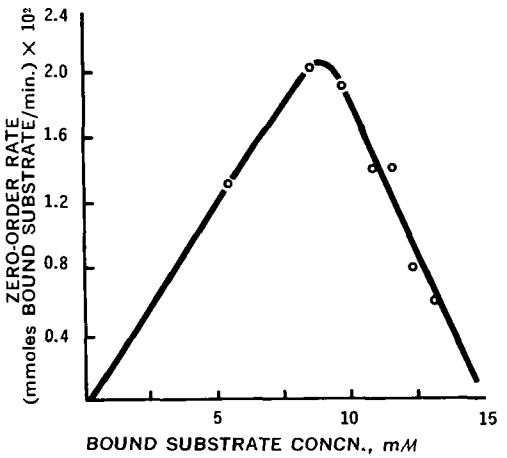

Figure 6—*Rate of enzymic hydrolysis of bound potassium dichloronaphthyl sulfate* versus *bound substrate concentration. Initial total substrate concentration 15.1* mM.

concentrations), the rate of enzymic hydrolysis of bound substrate decreases sharply and approaches zero. Of particular interest is the fact that this maximum in rate of hydrolysis of bound substrate occurs at a surfactant concentration which corresponds to a marked change in slope for the PDNS-polysorbate 80 binding data as exhibited in Fig. 1.

Contrary to the effect of increasing concentration of surfactant on enzymic hydrolysis of bound PDNS, data presented in Table III for the specific acid-catalyzed hydrolysis of PDNS in presence of polysorbate 80 indicate that the fraction of the overall rate attributed to hydrolysis of bound PDNS, as calculated from binding data of Fig. 1, continues to increase with increasing fraction of PDNS bound. There appears to be a modest decrease in the specific rate constant for hydrolysis of bound PDNS with increasing surfactant concentration, although the apparent decrease may well be within the range of experimental accuracy in determination of rates and the uncertainty introduced by the assumption that binding data obtained at pH 7.4 can be applied to the system at pH 1, where it is not feasible to conduct binding studies. Behme *et al.* (17) have, however, found that first-order rate constants for the acid-catalyzed hydrolysis of methyl orthobenzoate in presence of sodium dodecyl sulfate increase with increasing surfactant concentration, reach a maximum, and then decrease at higher surfactant concentrations.

It is postulated that the observed maximum in a plot of rate of enzymic hydrolysis of bound substrate *versus* bound substrate concentration is the result of a change in the structure of the polysorbate 80 micelle with which the substrate is associated. This change in micelle structure is apparently such that effective association of the bound substrate and the enzyme is prevented either as a result of hindered access of the large enzyme to the micellar region in which the substrate is bound, or by prevention of required enzyme-substrate "fit." The existence of an abrupt change in the extent of

Table III—Effect of Polysorbate 80 on the Acid-Catalyzed Hydrolysis of Potassium Dichloronaphthyl Sulfate[a]

Polysorbate 80 Concn., mg./ml.	Fraction of PDNS Bound[b]	Overall First-Order Rate, hr.$^{-1}$	Portion of Overall Rate Attributed to Bound PDNS, hr.$^{-1}$	Specific First-Order Rate Constant for Hydrolysis of Bound PDNS, hr.$^{-1}$
0	0	0.090	0	—
8	0.50	1.47	1.42	2.84
12	0.60	1.61	1.59	2.62
20	0.71	1.84	1.64	2.53
40	0.86	1.84	1.82	2.14

[a] Conditions: 0.1 *N* HCl, ionic strength 0.44, temperature 48°, initial substrate concentration 13 m*M*. [b] Calculated from binding data of Fig. 1.

polysorbate 80—PDNS interaction, as depicted in **Fig. 1**, at a concentration of surfactant and bound substrate corresponding to the concentration conditions at which the rate of enzymic hydrolysis of the bound substrate undergoes a pronounced change, is strongly supportive of the suggestion that this change in rate of enzymic hydrolysis is due to a change in micelle structure. The results of the study of hydronium ion-catalyzed hydrolysis indicate that the steric shielding of the fraction of PDNS associated with the micelle from attack by the enzyme is not evidenced upon attack by the small hydronium ion. Data of Behme *et al.* (17) for methyl orthobenzoate hydrolysis in presence of sodium dodecyl sulfate suggest, however, that in some surfactant systems it may be possible to demonstrate maxima in rate of bound substrate at high surfactant concentrations even for catalytic species such as hydronium ion.

The postulated inhibition of enzyme-substrate association by the micelle structure may result from a micelle structural change from a spherical shape to the more occlusive rod-shaped or lamellar-type micelle. Schick (18) has claimed that for nonionic surfactants exhibiting micelle molecular weights of 45,000 to 100,000, spheres appear the most probable shape, while for large micelles disks or rod-like shapes are likely. Nakagawa *et al.* (19) also suggested a spherical micelle for a series of nonionic surfactants of micellar weight from 40,000 to 60,000 on the basis of constant effective specific volume. Becher (20) pointed out that micelles of this size are so small that the light-scattering dissymmetry values do not give much idea about the shape of the micelles. His calculations, based on surface area measurements and the hydrated volume of the micelle, suggested that, for micelles containing molecules with fairly long ethylene oxide chain lengths (n_{15}–n_{30}), the assumption of a rod-like micelle was to be favored.

Winsor (21) has expressed the theory that solubility is affected by changes in the micelle form present in the solution and that solubility increases when there is any change tending to make the average form nearer the lamellar-type micelle, and decreases when changes occur which have the reverse effect. This is in accord with the postulate that the change in micelle structure that occurs in the polysorbate 80-PDNS system results in a lamellar or rod-shaped micelle, since it occurs at a polysorbate 80 concentration at which the solubility of the solubilizate in the micelle is markedly increased.

SUMMARY

These studies have demonstrated that enzyme-catalyzed reactions of substrates in micellar states are not restricted to the relatively few enzymes for which this phenomenon has previously been demonstrated, but may be of rather general occurrence. Knowledge that substrate in a micellar state or bound to a macromolecule is, under certain conditions, accessible to enzymic catalysts suggests that factors which are known to be responsible for alteration of the rate of a chemical reaction when the reactant or reactants are in micellar solution or bound to a macromolecule may also serve to alter enzymic reactions. However, the steric requirement for enzyme-substrate fit or access of enzyme to bound substrate may be of critical importance in these reactions involving macromolecular enzyme catalysts as opposed to reactions involving catalysts such as hydronium ion.

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