PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 981–985 (1975)

Correlation of the Critical Micelle Concentrations of Surfactants with Their Effects on a Bacterial Demethylase[1] (38672)

JOHN T. PINTO AND WILHELM R. FRISELL

Department of Biochemistry, College of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, New Jersey 07103

In the course of recent studies concerned with the purification and characterization of a bacterial sarcosine dehydrogenase (1), it was observed that enzyme activity measured as the rate of reduction of 2,6-dichlorophenolindophenol (DCPIP) could be enhanced in the presence of nonionic surfactants such as Triton X-100, Brij 35, and Tween 80. Titration with each surfactant indicated that the increase in dehydrogenase activity was maximal at detergent concentrations corresponding, within experimental error, to values reported by other investigators for critical micelle concentrations (CMC) (2). It was observed also that admixture of the nonionic detergents with DCPIP alone resulted in a 40–50 nm bathochromic shift as well as a hyperchromicity in the absorption spectrum of the dye. Similar spectral shifts have been observed in the micellar interaction of azo-dyes with anionic (3), cationic (4), and nonionic detergents (5). Although spectrophotometric changes in absorbance of indophenol dyes have been used for determining CMC of cationic surfactants (6, 7), DCPIP has not been employed for CMC determination of the nonionic surfactants and micelle complexes of this dye have not been studied under conditions normally optimal for enzyme analyses. The dual function of DCPIP as an electron acceptor in the sarcosine dehydrogenase system and as an indicator for surfactant–micelle formation made it possible to correlate micelle formation and enzyme activation.

Methods and Materials. The nonionic detergents were obtained from the following sources: Tween 80 (polyoxyethylene-20-sorbitan mono-oleate) and Brij 35 (polyoxyethylene-23-lauryl alcohol), Atlas Chemical Co.; Triton X-100 (isooctylphenoxypolyethoxyethanol), Rohm and Haas. The anionic detergents, deoxycholate and Sarkosyl NL-97 (*N*-dodecanoyl-*N*-methylglycine), were purchased from Sigma Chemical Co. and Ciba-Geigy, respectively. 2,6-Dichlorophenolindophenol (DCPIP) was obtained from Sigma Chemical Co. The other reagents were of highest purity commercially available. All solutions were made with 75 m*M* potassium phosphate buffer, pH 7.5, prepared with glass distilled water.

Sarcosine dehydrogenase was isolated from a sarcosine-grown organism according to procedures described earlier (1).

Sarcosine dehydrogenase activity was measured spectrophotometrically with DCPIP as the electron acceptor in the presence of phenazine methosulfate (8). Oxidase assays were carried out with a Gilson differential respirometer at 30°. Protein was analyzed by the procedure of Lowry as modified by Oyama and Eagle (9).

Difference spectra of DCPIP with increasing concentrations of surfactant were obtained with a Cary 17 recording spectrophotometer. Titrations were carried out in optically matched tandem cells, each compartment of which had a 0.436 cm path length. The reference cell contained the following: first compartment, 0.2 ml of 1.6 m*M* DCPIP and 5.0 ml of buffer; second compartment, 0.2 ml of buffer and 5.0 ml of surfactant solution. The sample cell contained the following: first compartment, 0.2 ml of DCPIP and 5.0 ml of surfactant solution; second compartment, buffer only. All measurements were made at 28°. The range of concentrations of detergents was ±10 times their reported CMC. The concentrations of the stock solutions, in 75 m*M* potassium phosphate buffer, pH 7.5,

[1] Supported in part by USPHS Grant No. AM 14315. The skilled assistance of Mrs. Verrell Randolph is gratefully acknowledged.

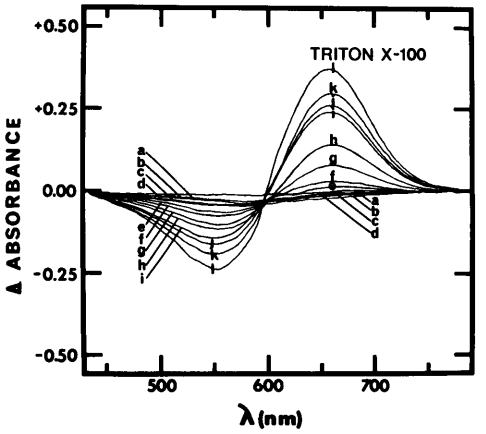

FIG. 1. Difference spectra of DCPIP with Triton X-100. In all of the spectral analyses, the molarity of the DCPIP was 61 μM. Curves a-l represent the concentrations of Triton X-100: 0.5, 1, 2, 3, 4, 5, 10, 20, 40, 50, 80, and 100 $\times$ 10^{-2} g/100 ml, respectively.

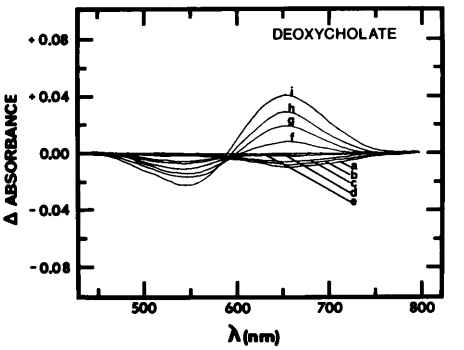

FIG. 2. Difference spectra of DCPIP with deoxycholate. Curves a–i represent the following concentrations of deoxycholate: 0.39, 1.96, 3.92, 7.85, 9.82, 11.78, 23.55, 31.41, and 39.36 $\times$ 10^{-2} g/100 ml, respectively.

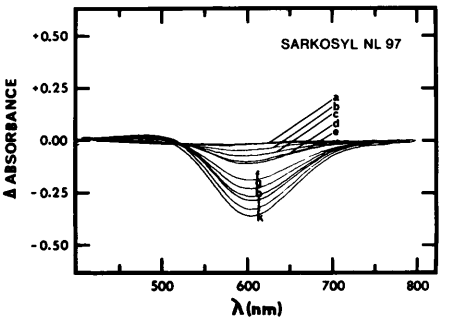

FIG. 3. Difference spectra of DCPIP with Sarkosyl NL-97. Curves a–k represent the following concentrations of Sarkosyl NL-97: 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1.0, 1.25, 1.50, 1.75, and 2.0 g/100 ml, respectively.

were: Triton X-100, Brij 35, and Tween 80, all 1.0% (w/v); deoxycholate, 0.393% (10 mM); and Sarkosyl, 0.5% (w/v). All surfactant concentrations in the Figures and Tables are expressed as grams per 100 ml.

Results and Discussion. Figure 1 shows the difference spectrum of the titration of DCPIP with Triton X-100. Similar spectra with the same maximum as for Triton are obtained with Tween 80 and Brij 35. These spectral shifts are similar to those reported for the interaction of azo dyes with anionic, cationic, and nonionic surfactants (3–5). Although charge-transfer complexing has been implicated in reactions of nonionic detergents with dyes such as 7,7,8,8-tetracyanoquinodimethane (10), there is no evidence for such complexes in the present studies with DCPIP. No new absorption bands can be detected. It is reasonable to assume, however, that the spectrum of DCPIP should be sensitive to solvent perturbations and that the observed bathochromic shifts can be the result of some type of molecular complexing between the dye and the surfactant. In the complexing of phenolic compounds with detergents possessing polyoxyethylene groups, for example, hydrogen-bonding within the side chains of the micelles has been demonstrated (11). The red shift observed in the spectra of the DCPIP-nonionic surfactant complexes, which appear to be ionic in nature, is consonant with the decrease in the polarity of the solvent when the concentration of detergent is increased.

Spectral shifts similar to those obtained with nonionic detergents are also observed in the reaction of DCPIP with the anionic surfactant deoxycholate (Fig. 2). The Sarkosyl system, on the other hand, is characterized by a decrease in absorbancy as shown by the spectra in Fig. 3. The structure of this detergent, consisting of a dodecanoyl moiety in amide linkage with *N*-methylglycine, could facilitate a partition of the dye between the phosphate buffer and the micelle matrix and thereby decrease the free DCPIP concentration in the system. Under these conditions, the light scatter

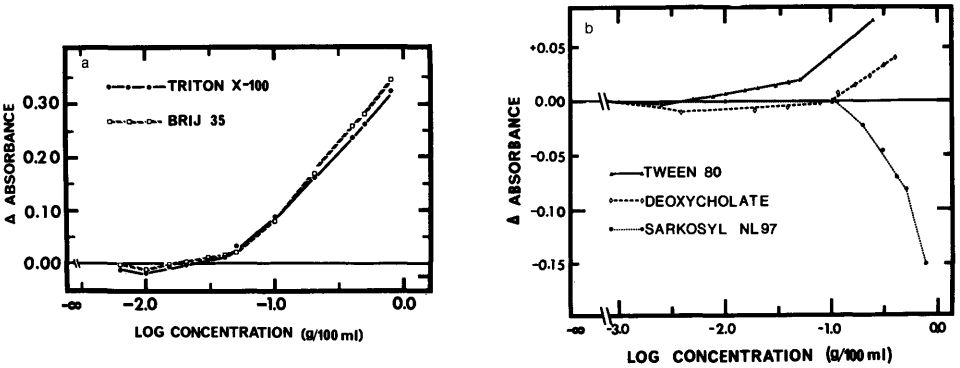

FIG. 4a. Differential absorbance of DCPIP as a function of log concentration of surfactant. The maximum absorbance readings of both Triton and Brij were obtained at 658 nm. b. Differential absorbance of DCPIP as a function of log concentration of surfactant. Maximum absorbance readings of DCPIP were obtained at 658, 652, and 600 nm for Tween 80, deoxycholate, and Sarkosyl, respectively.

TABLE I. CRITICAL MICELLE CONCENTRATIONS DETERMINED WITH 2,6-DICHLOROPHENOLINDOPHENOL

Surfactant	Critical micelle concentrations		
	2,6-Dichlorophenolindophenol method (g/100 ml)	Literature Value (g/100 ml)	Method
Triton X-100	0.0133	0.0150	Surface Tension[a]
Brij 35	0.0150	0.0110	Photometric-I_2[a]
Tween 80	0.0036	0.0045[b]	—
Deoxycholate	0.1210	0.1200	Spectral Change Methyl orange
Sarkosyl NL-97[c]	0.1410	—	—

[a] See Reference 2.

[b] Atlas Chemical Co., Wilmington, Del. (personal communication).

[c] *n*-Dodecyl-sarcosinate. Although the CMC for Sarkosyl has apparently not been reported, the values for two closely related compounds, *N*-dodecyl-beta alaninate and *N*-dodecyl-beta alanine HCl, are 0.089 and 0.296 g/100 ml, respectively (See Reference 2).

would be sufficiently strong to decrease light absorption by the dye within the micelle.

The CMC values of Triton, Brij, Tween, and deoxycholate were determined by plotting the change in absorption at 658 nm against the log concentration of the surfactant (Figs. 4a and 4b). The CMC has been defined as the point at which the spectrum begins to undergo a bathochromic shift. For purposes of comparison with the other detergents, the CMC of Sarkosyl has been assumed to correspond to the log concentration at which the negative change in absorbance becomes evident. As indicated in the data in Table I, there is an excellent correlation between the CMC values obtained with DCPIP titration and those determined by other methods. The sensitivity of the DCPIP procedure makes it possible to employ extremely low molar ratios of dye to surfactant, thereby minimizing secondary perturbation effects within the micelle structures.

Having established the validity of the DCPIP method for determining the surfactant micelle concentrations, it became relevant to determine whether there was any relationship between the CMC of each detergent and its stabilizing, stimulatory or inhibitory effect on enzymatic activity. The results summarized in Fig. 5 illustrate the activation and inhibitory effects of nonionic and anionic surfactants, respectively, on sarcosine dehydrogenase activity. The data in Table II demonstrate the correlation be-

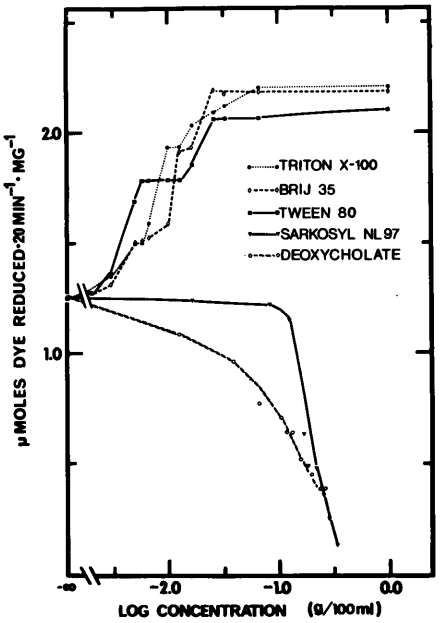

FIG. 5. Correlation of sarcosine dehydrogenase activity with the log concentrations of anionic and nonionic surfactants. The specific activity of sarcosine dehydrogenase was 1.25 μmoles dye reduced per 20 min per mg protein. The concentration of protein was 100 μg/ml per assay.

TABLE II. CORRELATION OF CMC WITH ACTIVATION OR INHIBITION OF SARCOSINE DEHYDROGENASE ACTIVITY.[a]

Surfactant	Concentration of surfactant at midpoint activation (g/100 ml)	Concentration of surfactant at 50% inhibition (g/100 ml)	CMC DCPIP method (g/100 ml)
Triton X-100	0.0095	—	0.0133
Brij 35	0.0127	—	0.0150
Tween 80	0.0047	—	0.0036
Deoxycholate	—	0.1210	0.1210
Sarkosyl NL-97	—	0.1830	0.1410

[a] Sarcosine dehydrogenase activity was measured by DCPIP reduction as described elsewhere (8).

tween the CMC values of the nonionic surfactants and the concentration at which enzyme activation becomes maximal. The CMC of the inhibitory anionic surfactants, on the other hand, were found to correspond to the concentrations required for 50% inhibition of the enzyme.

The activation of sarcosine dehydrogenase by Triton X-100 (0.0095 g/100 ml) and its inhibition in the presence of deoxycholate (0.1210 g/100 ml) is observed, whether enzyme activity is determined with DCPIP (plus phenazine methosulfate) as the terminal electron acceptor or with oxygen as the terminal electron acceptor. If the primary action of the detergents is to effect a conformational change in the micellar state(s) of the dehydrogenase–oxidase system, it is reasonable that both substrate-binding at the active site and subsequent electron transfer could be influenced (12, 13).

Summary. The activity of a sarcosine dehydrogenase isolated from a strain of Pseudomonas is enhanced by the addition of Triton X-100, Brij 35, and Tween 80, and is inhibited by deoxycholate and Sarkosyl NL-97. 2,6-Dichlorophenolindophenol, which is used as the oxidant in the dehydrogenase assay, has also been employed as an indicator in the spectrophotometric determination of the critical micelle concentrations (CMC) of both the nonionic and anionic detergents under conditions optimal for the enzyme analyses. A correlation between the activation or inhibitory activities of the surfactants and their CMC values has been established.

A portion of these studies are from the thesis of J. T. Pinto, submitted in partial fulfillment of the requirements for the Ph.D. degree, The Graduate School of Biomedical Sciences, College of Medicine and Dentistry of New Jersey.

1. Frisell, W. R., Arch. Biochem. Biophys. **142,** 213 (1971).
2. Mukerjee, P., and Mysels, K. J., National Standard Reference Data Series, United States Department of Commerce Publication, National Bureau of Standards. Critical Micelle Concentration of Aqueous Surfactant Systems (February, 1971).
3. Benzonana, G., Biochim. Biophys. Acta **176,** 836 (1969).
4. Duff, D. G., and Giles, C. H., J. Coll. Interface Sci. **41,** 407 (1972).
5. Becher, P., J. Phys. Chem. **66,** 374 (1962).
6. Corrin, M. L., and Harkins, W. D., J. Amer. Chem. Soc. **69,** 679 (1947).
7. Tong, L. K. J., Reeves, R. L., and Andrus, R. W., J. Phys. Chem. **69,** 2357 (1965).
8. Mackenzie, C. G., and Hoskins, D. D., *in* "Methods of Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. V, p. 738, Academic Press, New York (1962).

9. Oyama, J. I., and Eagle, H., Proc. Soc. Exp. Biol. Med. **91**, 305 (1961).
10. Deguchi, K., and Meguro, K., J. Coll. Interface Sci. **38**, 596 (1972).
11. Mulley, B. A., and Metcalf, A. D., J. Pharm. Pharmacol. **8**, 274 (1956).
12. Cerletti, P., Giovenco, M. A., Giordano, M. G., Giovenco, S., and Strom, R., Biochim. Biophys. Acta **146**, 380 (1967).
13. Rothfield, L., and Finkelstein, A., Ann. Rev. Biochem. **37**, 463 (1968).

Received September 23, 1974. P.S.E.B.M. 1975, Vol. 148.