Interaction of Bovine Serum Albumin with Detergent Cations

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The binding isotherms of bovine serum albumin (BSA) with cationic detergents, decyltrimethylammonium bromide, dodecyltrimethylammonium bromide, and tetradecyltrimethylammonium bromide have been determined at temperatures 8 and 25 °C for pH 6.8. The isotherms have also been determined at pH 3.0 and 25 °C. The effect of KBr on the binding of dodecyltrimethylammonium bromide to BSA has been examined at 25 °C for pH 6.8. The results obtained are as follows. (a) The average number of detergent cations bound, $\bar{\nu}$, increased with the increase in the length of hydrocarbon chain, (b) the standard free energy changes $-\Delta G°$, calculated from the intrinsic association constants K, were comparable with the cohesive energy change when one methylene group is transferred from the aqueous phase to the hydrocarbon environment on micellization, (c) values of $\bar{\nu}$ at pH 3.0 differ only slightly from those at pH 6.8 in a wider range of detergent concentration, and (d) the presence of the salt shifted the binding isotherms to the lower detergent concentrations. These results are discussed as regards the nature of micellization of detergents.

Various studies have been made[1-5] on the interaction of serum albumin with anionic detergents but only a few[3,6-8] on cationic detergents.[9] So far it is known that the initial binding of anionic detergents to bovine serum albumin (BSA) involves 11 equivalent sites which do not interact, and that at higher levels of binding, at neutral pH, three stoichiometric complexes can be distinguished having the respective compositions AD_m, AD_n, and AD_{2n} (A: BSA, D: detergent anion, $m=11$, and $n=38$). Examination of the binding of different charged, cationic detergents to BSA will contribute significantly to elucidate whether the binding is attributed to the hydrophobic interaction or the electrostatic interaction. We have studied the effects of hydrocarbon chain length, pH, and added salt on the binding of cationic detergents to BSA by the method of equilibrium dialysis to elucidate the mechanism of interaction. The results obtained are compared with those of the binding of anionic detergents. The cationic detergents used are decyltrimethylammonium bromide (DeTAB), dodecyltrimethylammonium bromide (DTAB), and tetradecyltrimethylammonium bromide (TTAB).

Experimental

Materials. DeTAB, DTAB, and TTAB, prepared by the reaction of the corresponding alkylbromides with trimethylamine,[10] were purified by recrystallization from acetone. Alkylbromides used for the preparation of the cationic detergents were gas chromatographically pure. All the detergents prepared gave correct elemental analyses.

A solution of BSA (Armour, Lot. CK5010 and PK4471), after storage at 2—5 °C and pH 2 for 2 days was centrifuged and filtered to remove lipid contaminants.[4] The solution was dialyzed against distilled water and finally against the buffer solution of desired pH.

Equilibrium Dialysis. Detergent binding measurements were carried out by the method of equilibrium dialysis at 8(±0.01) and 25(±0.01) °C in phosphate buffer of ionic strength 0.1 and pH 6.8 and also at 25 °C in citrate buffer of ionic strength 0.1 and pH 3.0. Visking tubing for dialysis was heated for 3 hr in the saturated solution of $NaHCO_3$ near boiling point and rinsed thoroughly with distilled water to remove impurities. The tubing treated was then stored in distilled water at 2—5 °C. Bags were filled with 5 ml of *ca.* 1% protein solution in buffer and suspended in 10 ml of the same buffer for 3 days (at 25 °C) or 4 days (at 8 °C) with occasional shaking. The detergent was placed outside the dialysis bag. The amount bound to BSA was calculated by determining concentrations of detergent in the outside solution before and after the dialysis. A blank correction was made for the adsorption of detergent to the bag when no protein was present. The concentration of cationic detergent was determined by a spectrophotometric dye method.[11] The complex of orange II-detergent cation was extracted by chloroform and the optical density of the chloroform solution was determined at 485 mμ using a Hitachi 101 spectrophotometer. The protein concentration was determined from optical density measurements at 279 mμ; $E_{1\%}^{1cm}$ was assumed to be 6.67 for BSA at this wavelength.[4]

Critical Micelle Concentration (CMC). The CMC's of cationic detergents were determined by the surface tension method using the stalagmometer at 25(±0.01) °C. The values of CMC in aqueous solution of DeTAB, DTAB, and TTAB were 64.45, 15.54, and 4.01 mmol/kg-H_2O, respectively.

Results and Discussion

Binding Isotherms. The binding isotherms at

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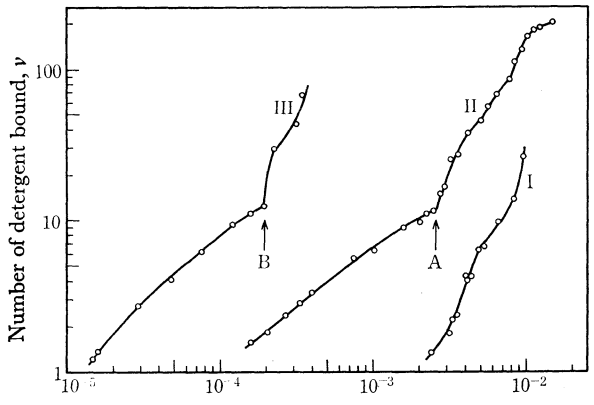

Fig. 1. Binding isotherms for cationic detergents and BSA at 25 °C and pH 6.8 (ionic strength 0.1). I: DeTAB, II: DTAB, and III: TTAB.

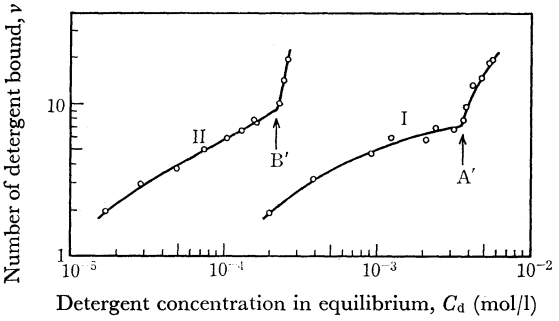

Fig. 2. Binding isotherms for cationic detergents and BSA at 8 °C and pH 6.8 (ionic strength 0.1). I: DTAB and II: TTAB.

pH 6.8 and 25 °C for three detergents are shown in Fig. 1. The shape of isotherm for DeTAB differs from that for others. The binding isotherms at 8 °C and pH 6.8 for DTAB and TTAB are shown in Fig. 2. At each temperature, the longer the hydrocarbon chain of detergents, the lower the concentration of free detergent in equilibrium. The slope of binding isotherm for DTAB and TTAB changes at 25 °C at points A and B (in Fig. 1) at which the number of bound detergent $\bar{\nu}$ is 12 and 13 for DTAB and TTAB, respectively. The slope of isotherm changes at 8 °C also at A′ and B′ (in Fig. 2) at which $\bar{\nu}=8$ and $\bar{\nu}=10$ for DTAB and TTAB, respectively. The concentration at A is the same as that at which several zones appear suddenly in the pattern of acrylamide-gel electrophoresis for this system.[12]

It is known that a cationic detergent, dodecylpyridinium bromide, as well as anionic detergents, forms the AD_m type complex with horse serum albumin.[3] It is seen in Figs. 1 and 2 that such a complex is formed at points A, B, A′, and B′, and that changes in conformation of the protein occurs in the region where $\bar{\nu}$ is larger than that at A or B, and the steepening of the isotherm begins. It was observed by using acrylamide-gel electrophoresis that the complex AD_{11} of sodium dodecylsulfate (SDS) prevented heat denaturation but the complex formed at point A did not prevent denaturation but rather promoted it.[13] Markus *et al.*[14] stated that the protective effect by SDS against urea denaturation of BSA is due to cross-links formed by the detergent anions between groups of nonpolar residues (presumably located in crevices formed by the folding of the peptide chain) and positively charged lysine residues. The mechanism of binding by Markus *et al.* is not applicable to the present system since no protection against denaturation occurs.[13] Thus, it is expected that the site on BSA for DTAB binding differs from that for SDS binding.

Thermodynamic Parameters. If the binding sites on protein are equivalent and non-interacting, the binding of detergents to protein is known to follow the equation[15]

$$\frac{1}{\bar{\nu}} = \frac{1}{Kn}\frac{1}{C_d} + \frac{1}{n} \qquad (1)$$

where $\bar{\nu}$ is the average number of moles of detergent cation bound per mole of protein, K the intrinsic association constant of each site for the detergent, n the total number of sites having an association constant K, and C_d the free detergent concentration in equilibrium. Plots of $1/\bar{\nu}$ *vs.* $1/C_d$ lie on a straight line for DTAB and TTAB in the region $\bar{\nu}<ca.$ 10. The intercept and slope of these lines for DTAB and TTAB give $1/n$ and $1/Kn$, respectively. The reciprocal plot for DeTAB, however, is not satisfactorily linear. The values of n and K thus obtained for DTAB and TTAB are given in Table 1 together with the standard free energy change calculated by the relation $\Delta G^\circ=-RT\ln K$. The values for anionic detergents by other workers are also included in Table 1 for comparison. The association constant K for cationic detergents increases with the increase in length of hydrocarbon tail and is lower than that for the corresponding alkylsulfates. The value of n increases with the increase in length of hydrocarbon tail at a constant temperature.

TABLE 1. ASSOCIATION CONSTANTS K, AFFINITIES $-\Delta G^\circ$, AND NUMBERS OF BINDING SITES FOR DETERGENTS ON BSAn

Detergent	K	$-\Delta G^\circ$ (kcal/mol)	n	Temp. °C	Ref.
$C_{12}H_{25}N(CH_3)_3Br$	1.6×10^3	4.1	8	8	
	4.5×10^2	3.6	22	25	
$C_{14}H_{29}N(CH_3)_3Br$	1.2×10^4	5.3	10	8	
	3.1×10^3	4.8	33	25	
$C_8H_{17}SO_4Na$	0.6×10^6	7.3	4—5	2	5
$C_{10}H_{21}SO_4Na$	1.4×10^6	7.8	5—6	2	5
$C_{12}H_{25}SO_4Na$	1.2×10^6	7.7	8—9	2	5
$C_{14}H_{29}SO_4Na$	1.0×10^6	7.5	10—11	2	5
$C_8H_{17}SO_4Na$	0.4×10^4	4.5	6	2	2
$C_{10}H_{21}SO_4Na$	3.4×10^4	5.7	8	2	2
$C_{12}H_{25}SO_4Na$	1.8×10^5	6.6	10	2	2

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15) C. Tanford, "Physical Chemistry of Macromolecules," Wiley, New York, N. Y., (1963), p. 526.

The n value at 8 °C is in fair agreement with the number of detergents bound at points A′ and B′ in Fig. 2. The value of n at 25 °C, however, is far larger than $\bar{\nu}$ at A and B in Fig. 1.

The difference in values of $-\Delta G°$ for DTAB and TTAB can be attributed to the difference in the length of hydrocarbon tail. The difference in the standard free energy change thus calculated gives 1.0 kT per methylene group at two temperatures. The values for alkylsulfate, calculated from the data by Karush and Sonenberg,[2] was found to be 0.96 kT. These values are comparable with the cohesive energy change 1.0—1.2 kT[16] when one methylene group is transferred from the aqueous medium to the hydrocarbon environment on micellization of various detergents.

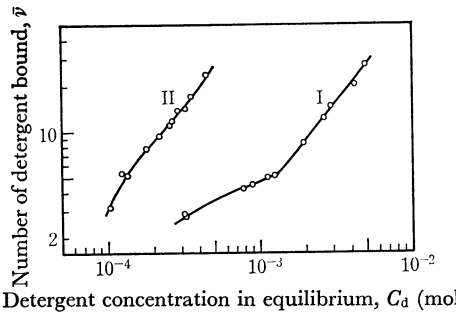

Fig. 3. Binding isotherms for cationic detergents and BSA at 25 °C and pH 3.0 (ionic strength 0.1). I: DTAB and II: TTAB.

Effect of pH. Equilibrium dialysis was carried out at more alkaline pH than the isoelectric point of BSA. If the pH of solution turns more acidic than that of isoelectric point, the net charge of BSA changes from negative to positive. Thus, if the binding is assumed to be electrostatic, the number of detergent cations bound should decrease remarkably at the acid pH. Isotherms of BSA with DTAB and TTAB at pH 3.0 are shown in Fig. 3. Comparing Fig. 3 with Fig. 1, we see that the difference in $\bar{\nu}$ between isotherms at pH 6.8 and 3.0 is not remarkable. The reversible expansion of BSA which occurs at below pH 3.5[17] would expose both sites for electrostatic binding and hydrophobic binding. However, the number of bound detergent cations did not change remarkably with pH. Thus, the interaction of BSA with detergent cations appears to be hydrophobic rather than electrostatic.

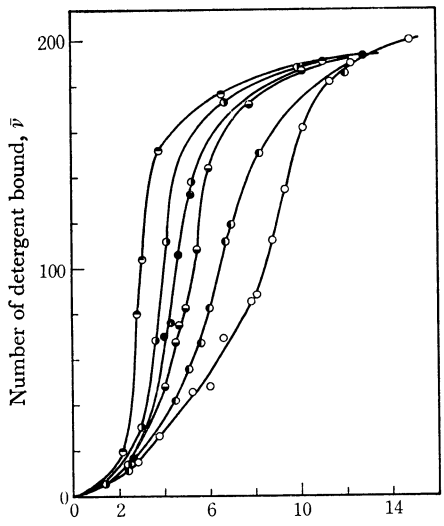

Fig. 4. Effect of KBr on the binding of DTAB to BSA at 25 °C and pH 6.8 (ionic strength 0.1). Concentrations of KBr; 0 (○), 0.02 (◐), 0.04 (◒), 0.06 (●), 0.10 (◑), and 0.15 (◓) mol/l.

Table 2. Effect of KBr on the CMC of DTAB in phosphate buffer (ionic strength 0.1, pH 6.8) at 25 °C

Concn of KBr (mol/l)	CMC $\times 10^3$ (mol/l)
0	12.2
0.02	8.6
0.04	6.6
0.06	5.3
0.10	4.0
0.15	3.2

Effect of Added Salt. The interaction of BSA with detergents at higher concentration is as follows. The binding isotherms of BSA with DTAB, obtained at various concentrations of added KBr, are shown in Fig. 4. In the range 0.02—0.15 mol/l, the increase in concentration of KBr shifts the isotherm toward the lower concentration of DTAB in equilibrium, *viz.*, the concentration of detergent at which a particular number of bound detergent cations lowers as the concentration of salt increases. This tendency is in line with the fact

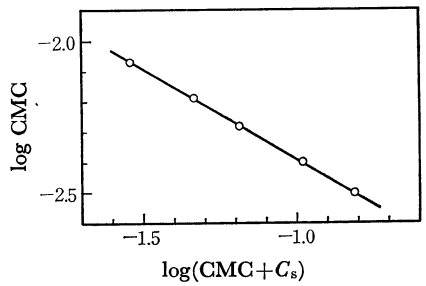

Fig. 5. Effect of KBr concentration on the CMC of DTAB in phosphate buffer (pH 6.8, ionic strength 0.1) at 25 °C.

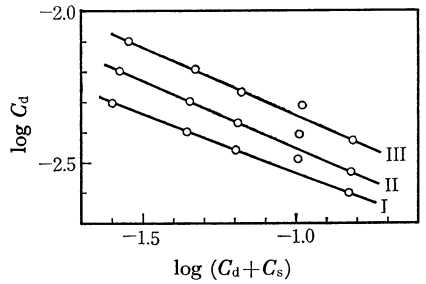

Fig. 6. Plots of log C_d *vs.* log (C_d+C_s) at constant $\bar{\nu}$. I: $\bar{\nu}$= 50, II: $\bar{\nu}$=100, and III: $\bar{\nu}$=150.

16) J. N. Phillips, *Trans. Faraday Soc.*, **51**, 561 (1955). K. Shinoda, T. Nakagawa, B. Tamamushi, and T. Isemura, "Colloidal Surfactants" Academic Press, New York, N. Y., (1963), p. 42.

17) C. Tanford, *Proc. Iowa Acad. Sci.*, **59**, 206 (1952). G. Scatchard, *Am. Scientist*, **40**, 61 (1952).

that the added salt lowers the CMC of detergents. The effect of added KBr on the CMC of DTAB in the phosphate buffer solution is presented in Table 2. The CMC of detergent is related to the concentration of added salt C_s by the relation[18]

$$\log \mathrm{CMC} = a - b \log (\mathrm{CMC} + C_s) \qquad (2)$$

where a and b are constants. Data in Table 2 satisfy Eq. (2) as is shown in Fig. 5. If we assume that the binding of DTAB to BSA is governed by the same force as in the micelle formation, CMC in Eq. (2) can be replaced by the concentration of detergent in equilibrium C_d at a constant $\bar{\nu}$. The plot of $\log C_d$ *vs.* $\log (C_d + C_s)$ at a constant $\bar{\nu}$, for example $\bar{\nu}=50$, 100, and 150, falls on a straight line (Fig. 6).

In conclusion, the nature of the binding of alkyltrimethylammonium bromides with BSA is very similar to that of micelle formation of detergent. In the micelle formation of ionic detergent, hydrophobic interaction plays a predominant role. From the analogy to micelle formation, the cationic detergent-BSA interaction is hydrophobic in nature in the ranges of $\bar{\nu}$ and concentration of detergent in this experiment.

18) K. Shinoda, T. Nakagawa, B. Tamamushi, and T. Isemura, "Colloidal Surfactants" Academic Press, New York, N. Y., (1963), p. 58.