On the Mode of Interaction of Surface Active Agents with Gelatin

By Toshizo ISEMURA, Fumikatsu TOKIWA* and Shoichi IKEDA

(Received May 23, 1961)

There are a number of studies on the interaction of surface active agents with high polymers and proteins. It is well known that proteins exhibit a markedly strong affinity for both cationic and anionic detergents. Among various proteins, gelatin is suitable for investigating the nature of the affinity of polypeptide chains with detergents and the mechanism of their mutual interaction. It is readily available. It has no organized conformation as globular proteins have, and it behaves as a random coil, at least, at elevated temperatures (higher than about 30°C).

Pankhurst and his coworkers[1-4] have extensively investigated the interaction of gelatin with various detergents, particularly with sodium dodecyl sulfate, by examining the precipitation of a complex as a function of mixing ratio. They have pointed out that gelatin binds with detergents to form a complex and that the binding ratio of detergent to gelatin is of importance in elucidating the mode of interaction. Tamaki and Tamamushi[5] have also obtained the same conclusion from the determination of two detergents, sodium dodecyl sulfate and dodecylamine hydrochloride, bound to precipitated gelatin on the acidic and alkaline sides of the isoionic point of gelatin respectively.

However, the mode of binding of detergents by gelatin before precipitate formation can not be known by means of such a solubility determination. Detergents should interact and combine with gelatin even without precipitation, since the formation of precipitates would arise from the enhanced hydrophobicity of the gelatin-detergent complex with an increasing amount of detergent bound to gelatin. Evidence for the combination of detergents with other proteins in a region wherein precipitation does not occur has been obtained from studies by electrophoresis[6-8], equilibrium dialysis[6,9], ultracentrifugation[10], viscosity[5,9], solubilization[11], etc.

For solutions of low detergent-gelatin ratios in which no precipitation occurred, studies by electrophoresis and equilibrium dialysis were attempted in order to find quantitatively the amount of detergent bound to gelatin. With the experiment by electophoresis, however, two peaks appeared in the electrophoretic pattern for gelatin alone. It is supposed that the pattern would be more complicated in a gelatin solution mixed with detergent and that its interpretation will be more difficult. Thus electrophoretic investigation was abandoned. In this work, therefore, the mode of binding of detergent by gelatin has been studied by means of equilibrium dialysis in the range of low detergent concentrations at various values of pH. This paper concerns the interaction of gelatin with an anionic detergent, sodium dodecylbenzene sulfonate, and two cationic detergents, dodecyl pyridinium bromide and cetyl pyridinium chloride.

Experimental

Materials.—Commercial photographic gelatin was purified according to the method of James et al.[12] A gelatin solution (2%) was passed through a column of mixed resins (5 parts of Amberlite IRA-400 to 2 parts of Amberlite IR-120) at 30~35°C. The pH of the gelatin solution thus purified was 4.9. The concentration of the stock solution was determined by dry weight.

Sodium dodecylbenzene sulfonate (SDBS), which was prepared by the sulfonation of dodecylbenzene (Oronite Chemical Co., U. S. A.), was obtained from the Japan Surfactant Association. To remove the lower homologues and inorganic salts, it was extracted with distilled water and then with ethyl alcohol. The pH of a 1% solution of the purified SDBS was 7.0. Dodecyl pyridinium bromide (DPB)

* Present address : Research Laboratory, Kao Soap Co., Ltd., Wakayama.

1) K. G. A. Pankhurst and R. C. M. Smith, *Trans. Faraday Soc.*, **41**. 630 (1945).
2) K. G. A. Pankhurst, *Discussions Faraday Soc.*, No. 6, 52 (1949).
3) K. G. A. Pankhurst and R. C. M. Smith, *Trans. Faraday Soc.*, **43**, 511 (1947).
4) K. G. A. Pankhurst and R. C. M. Smith, ibid., **40**, 565 (1944); K. G. A. Pankhurst, *Research*, "Surface Chemistry" (1949), p. 109.
5) K. Tamaki and B. Tamamushi, This Bulletin, **28**, 555 (1955).
6) J. T. Yang and J. F. Foster, *J. Am. Chem. Soc.*, **75**, 5560 (1953); **75**, 5743 (1953).
7) K. Aoki and J. Hori, This Bulletin, **29**, 104 (1956); **29**, 369 (1956).
8) T. Isemura and T. Takagi, *J. Biochem.*, **46**, 1637 (1959).
9) J. F. Foster and J. T. Yang, *J. Am. Chem. Soc.*, **76**, 1015 (1954).
10) K. Aoki and J. Hori, ibid., **81**, 1885 (1959).
11) I. Blei, *J. Colloid Sci.*, **15**, 370 (1960); M. Breuer and U. P. Strauss, *J. Phys. Chem.*, **64**, 228 (1960).
12) J. W. James, A. W. Kenlkington and A. G. Ward, *Research*, **4**, 247 (1953).

supplied by the Kao Soap Co. was recrystallized twice from acetone. Cetyl pyridinium chloride (CPC) was prepared from cetyl alcohol of high purity and recrystallized several times from acetone.

Equilibrium Dialysis. — A dialysis cell consists of two compartments of equal volume, separated by a membrane. As a membrane, Vising cellulose casings (diameter of a pore: ca. 24Å) were used after rinsing them with warm distilled water. A 10 ml. portion of a detergent solution was placed in one compartment of the cell and equilibrated against an equal volume of gelatin solution in the other compartment. Dialysis was carried out at 30 or 43°C for 3 to 4 days, with an occasional shaking of the cell. The concentration of gelatin was kept constant, 0.2%, and the pH of solution was adjusted by hydrochloric acid or sodium hydroxide. It was confirmed by a Horiba model M3 pH-meter that no change in the pH of the solutions was observed before and after dialysis, except for a slight difference of pH in the case of the binding of SDBS at the isoionic point of gelatin. After the attainment of equilibrium, the detergent concentration in the compartment containing no-gelatin was determined spectrophotometrically, because the molar extinction coefficient of detergent was greatly affected by the presence of gelatin. Since 0.1 M sodium chloride was added to all solutions to eliminate the correction for Donnan's effect, the amount of bound detergent was directly calculable from an analytical value. A control experiment with detergent alone was always carried out to minimize analytical errors. This dialysis method is applicable only to dilute solutions of detergent, since the detergent formed micelles at some high concentrations and was difficult to permeat through membrane[13].

Determination of Concentration of Detergents. —A spectrophotometric method has been developed to determine the concentration of detergents having benzene or pyridinium rings as low as 10^{-5} M[14]. Measurements of the ultraviolet absorption were made by means of a Hitachi type EPU-2A spectrophotometer at a wavelength of 230 mμ with SDBS (molar extinction coefficient, $\varepsilon_{SDBS}=8.75\times10^3$) or at a wavelength of 259 mμ with DPB and CPC ($\varepsilon_{DPB}=4.48\times10^3$ and $\varepsilon_{CPC}=4.59\times10^3$).

Results

The Binding of an Anionic Detergent, SDBS, by Gelatin.—The permeability of SDBS through dialysis membrane decreased with increasing concentration on account of the micelle formation and further decreased with the addition of neutral salts such as sodium chloride because of the lowering of the critical micelle concentration[15]. SDBS was permeable to membrane with difficulty at concentrations above 1.3×10^{-3} when 0.1 M sodium chloride was added. In addition, on the acidic side of the isoionic point of gelatin, precipitation occurred when gelatin was added to a relatively concentrated solution of SDBS. Accordingly, all the experiments were carried out at concentrations of SDBS lower than 1.0×10^{-3} M.

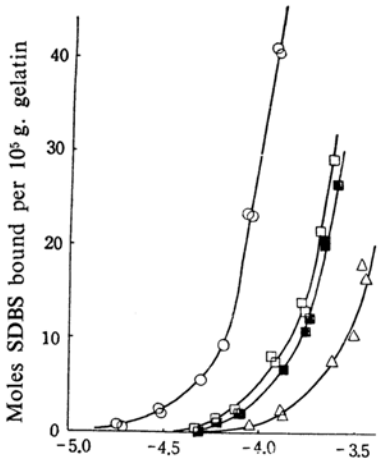

Fig. 1. Binding curves of SDBS by gelatin. ○, pH 2.7, 30°C; □, pH 5.0, 30°C; ■, pH 5.0, 43°C; △, pH 8.0, 30°C

Figure 1 illustrates the binding curves in which the number of moles bound SDBS per 10^5 g. of gelatin, r is plotted against the logarithm of the concentration of free SDBS, $\log c$. The amount of binding of SDBS increases acceleratively with increasing concentration. As the pH of the solution is lowered, the binding of SDBS is enhanced, but even on the alkaline side of the isoionic point of gelatin, SDBS combines with gelatin to a considerable extent. This indicates that gelatin has a strong affinity for SDBS, as other proteins have for long chain alkyl sulfate and alkylaryl sulfonate[4,6,7,11].

The Binding of Cationic Detergents, DPB and CPC, with Gelatin. — Since the critical micelle concentration of DPB lies in such a high concentration as 10.5×10^{-3} M[16], dialysis experiments were possible up to relatively high concentrations. However, experiments with CPC were carried out only at a low concentration region because of its low critical micelle concentration, i. e., 0.9×10^{-3} M[17].

Figure 2 shows the curves for the binding of DPB and CPC by gelatin. CPC combines with gelatin at the isoionic point as well as on the alkaline side of it, but not on the acidic side. DPB is, however, less bound with gelatin

13) B. S. Harrap and I. J. O'Donnell, *J. Phys. Chem.*, **58**, 1097 (1954).
14) J. T. Yang and J. F. Foster, ibid., **57**, 628 (1953).
15) M. L. Corrin and W. D. Harkins, *J. Am. Chem. Soc.*, **69**, 683 (1947).
16) T. Ino, T. Kondo and K. Meguro, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **76**, 200 (1955).
17) Cf. N. K. Adam and H. L. Shute, *Trans. Faraday Soc.*, **34**, 758 (1938).

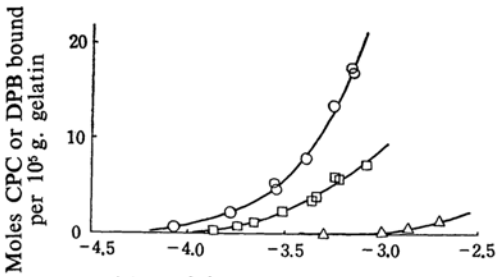

Fig. 2. Binding curves of DPB and CPC by gelatin at 30°C. ○, CPC, pH 7.0; □, CPC, pH 4.9; △, DPB, pH 7.0

even on the alkaline side. Both cationic detergents can bind with gelatin only to a less extent than the anionic detergent, SDBS. It has been observed[9] that proteins other than gelatin also have a weaker affinity for cationic detergents than for anionic detergents.

Discussion

Binding Isotherm. — If detergent ions bound with protein have no interaction with one another, the equation for the binding is written as

$$r=\frac{kmc}{1+kc} \qquad (1)$$

where k is a binding constant and m is the maximum amount of binding[18]. Eq. 1 implies that r approaches to a saturated value, m, when c is increased and that a plot of $1/r$ vs. c or r/c vs. r should be linear. It was found, however, that the binding data of detergents, SDBS and CPC, with gelatin could not fit Eq. 1. Since detergent ions can combine acceleratively with increasing concentration, detergent ions already bound seem to promote the succeeding binding; that is, the bound detergent ions interact with one another.

When the binding takes place cooperatively, Eq. 1 should be modified to[19-21]

$$r=\frac{kmc^n}{1+kc^n} \quad (n>1) \qquad (2)$$

where n is a constant characteristic of the kind of binding, and is greater than unity if the interaction is operative towards stabilization. Eq. 2 can be rewritten as

$$\frac{r}{m-r}=kc^n \qquad (3)$$

The present experiments are concerned with the low detergent-gelatin ratios at which no precipitation occurs. Since the phase separation or precipitation occurs only at relatively high detergent-gelatin ratios, the amount of bound detergent in the present experiment is expected to be far less than the maximum amount of binding. The increasing steepness of the binding curves also suggests that the saturated amount of binding or the number of the binding sites is very large, if it exists at all. Based on these experimental facts, it may be assumed that r is small compared with m. Then Eq. 3 reduces to

$$r=Kc^n \quad (K=km) \qquad (4)$$

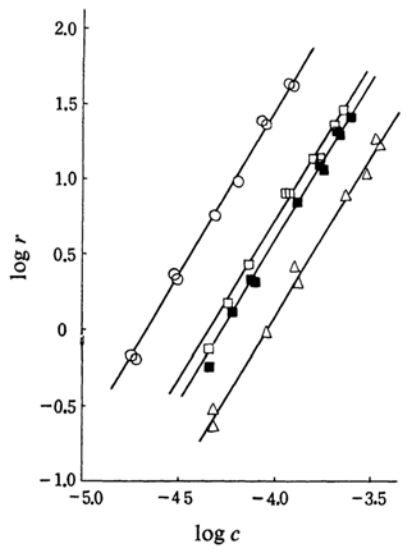

Fig. 3. The log r vs. log c diagram for the binding of SDBS by gelatin. ○, pH 2.7, 30°C; □, pH 5.0, 30°C; ■, pH 5.0, 43°C; △, pH 8.0, 30°C

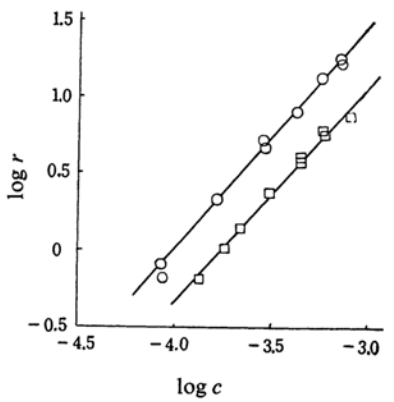

Fig. 4. The log r vs. log c diagram for the binding of CPC by gelatin at 30°C. ○, pH 7.0; □, pH 4.9

18) I. M. Klotz, F. M. Walker and R. B. Pivan, *J. Am. Chem. Soc.*, **68**, 1486 (1946).
19) I. M. Klotz, "The Chemistry of Proteins", Ed. by S. Akabori and S. Mizushima, Vol. 4, Kyoritsu Shuppan Co., Ltd., Tokyo (1956), p. 15.
20) I. M. Klotz, "The Proteins", Ed. by H. Neurath and K. Bailey, Vol. 1, Academic Press, New York (1953), p. 769.
21) M. Kubo, *Proc. Japan Acad.*, **29**, 156 (1953).

which is formally identical with the Freundlich adsorption isotherm, although n is greater than unity. Then a plot of $\log r$ vs. $\log c$ should be linear.

If the data of the binding of detergents, SDBS and CPC, with gelatin are replotted in a logarithmic scale, straight lines are obtained for all cases, as is shown in Figs. 3 and 4. Thus cooperative stabilization is effective for the binding of these detergents by gelatin. The values of the constants, K and n, which can be estimated from the intercept and slope of the straight line, are listed in Table I.

TABLE I. DEPENDENCE OF n AND K ON pH

Detergent	Temp. °C	pH	n	K	$\log K$
SDBS	30	2.7	2.13	8.71×10^{9}	9.94
	30	5.0	2.13	1.74×10^{9}	9.24
	30	8.0	2.10	2.95×10^{8}	8.47
	43	5.0	2.12	1.15×10^{9}	9.06
CPC	30	4.9	1.40	1.82×10^{5}	5.26
	30	7.0	1.44	6.03×10^{5}	5.78

Significance of the Constants, K and n. — Equation 2 can be expressed by an alternate form[21]

$$\frac{r}{m}=\frac{1}{2}\left\{1+\tanh\left(\frac{n}{2}\ln\frac{c}{c_{1/2}}\right)\right\} \qquad (5)$$

in which $c_{1/2}$ represents the concentration of free detergent ions at half saturation ($r=m/2$). Equation 5 can be identified with Eq. 2, if

$$k=c_{1/2}{}^{-n} \qquad (6)$$

Hence, in Eq. 4 for $m\gg r$,

$$K=mc_{1/2}{}^{-n} \qquad (7)$$

In this respect, K is regarded as a measure of the ability of binding or the extent of affinity.

On the other hand, the physical meaning of n is not explicit; the value of n depends on the strength of mutual interaction between bound detergent ions. If n is greater than unity, there occurs a cooperative interaction stabilizing the protein bound with detergent, so that the uptake of one ion makes another ion bind easier[20,21].

Dependence of K and n on pH and the Nature of the Binding. — As is shown in Table I, the value of K increases with the lowering of pH in the case of the binding of SDBS by gelatin. A linear relationship is found between $\log K$ and pH, as is shown in Fig. 5, which is represented by

$$\log K=10.7-0.27\,\mathrm{pH} \qquad \text{at } 30°\mathrm{C}$$

Thus the magnitude of K is related to the interaction of DBS anions with gelatin. This suggests that an electrostatic attraction between

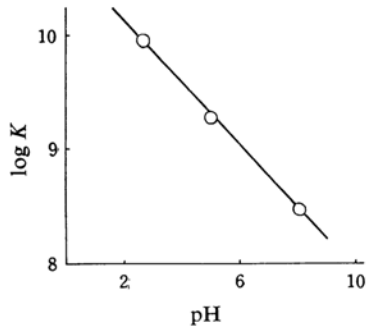

Fig. 5. Relationship between $\log K$ and pH for the binding of SDBS by gelatin.

DBS anions and positively charged groups of gelatin is partly responsible for the binding. Even on the alkaline side gelatin has positively charged sites and binds a finite number of DBS anions.

The value of n is about 2.1 and is independent of pH. This means that the uptake of DBS anions by gelatin favors further binding of other DBS anions in the vicinity of the binding site on gelatin, probably because of the hydrophobic cohesion between DBS anions. Thus, DBS anions form aggregates like micelles on a gelatin molecule. This might have some relation with the increase in the solubilizing power of sodium dodecyl sulfate in the presence of gelatin[22]. Generally, the binding of detergents on synthetic polymers is also interpreted as essentially due to the formation of this kind of micelles on a polymer molecule[23].

It may be concluded that a DBS anion combines with a positively charged group of gelatin and that its binding promotes the further binding of DBS anions around this site due to hydrophobic cohesion. Bound DBS anions form micelle-like aggregates on a gelatin molecule. The former binding will be mostly related to pH's while the latter will be influenced by the length of the alkyl chain of detergent. The effect of the length of alkyl chain has been investigated for anionic detergent-protein systems[4,7], and it is known[4] that the increase in chain length makes the detergent-gelatin ratio lower above which a precipitate separates out. This would also support the concept of the micelle formation on gelatin.

The binding isotherm of SDBS by gelatin can be expressed by

$$\log r=10.7-0.27\,\mathrm{pH}+2.13\log c \qquad \text{at } 30°\mathrm{C}$$

Temperature elevation scarcely influences the

22) S. Saito, *Kolloid-Z.*, **158**, 120 (1958).
23) S. Saito, ibid., **154**, 19 (1957).

value of n, but it does decrease the value of K by a small amount.

On the other hand, the value of K for CPC is considerably smaller than that for SDBS, and it increases with pH. Further, the value of n for CPC, 1.4, is independent of pH and smaller than that for SDBS, 2.1. These are an indication of the lower affinity of CPC for gelatin; that is, CPC and DPB are less bound to gelatin than SDBS. Generally, a cationic detergent exhibits less affinity for proteins and polymers than an anionic detergent[9,22].

The dependence of the value of K upon pH suggests that the binding of CPC by gelatin is of electrostatic nature. According to Pankhurst[2], an ion-ion binding is responsible for the combination between gelatin and CPC, but an ion-dipole binding can not occur at the peptide bonds of gelatin owing to a steric effect. However, the difference in the binding behavior of DPB from that of CPC, i. e., the insignificant binding of DPB as compared with CPC, implies that the length of the alkyl chain, i. e., the hydrophobic cohesion, is of primary importance even with the sufficiently long alkyl chain in the case of cationic detergents.

Summary

The mode of the binding of detergents, SDBS, CPC and DPB, by gelatin in the region of low detergent-gelatin ratios, where solutions are stable without precipitation, was investigated by determining the bound detergents by means of equilibrium dialysis.

Detergents bind with gelatin in accordance with the isotherm $r=Kc^n$, where K and n (>1) are constants which measure an affinity for the binding and an extent of cooperative interaction respectively. The value of K, which is dependent on pH and temperature, decreases with increasing pH for the binding of SDBS and vice versa for CPC. The value of n is characteristic of a given detergent. The value of n for SDBS is significantly greater than that for CPC. Thus the extent of the cooperative stabilization of a bound detergent is higher for SDBS than for CPC.

Both anionic SDBS and cationic CPC combine with gelatin, but cationic DPB scarcely combines with it. Further, it was inferred that a detergent, especially SDBS, binds with gelatin, forming aggregates on it in such a manner as the micelle formation.

The sign of charge of the detergent is of essential importance for the binding of the detergent by gelatin, and the length of the alkyl chain of detergent also has additional effects on the binding. The effect of chain length was examined with cationic detergents.

Institute for Protein Research
(T. I. & F. T.)
and
Faculty of Science
(T. I. & S. I.)
Osaka University, Osaka