Interaction between Dodecyltrimethylammonium Chloride and DNA

Keishiro SHIRAHAMA,* Koji TAKASHIMA, and Noboru TAKISAWA
Department of Chemistry, Faculty of Science and Engineering, Saga University, Saga 840
(Received June 12, 1986)

Binding of dodecyltrimethylammonium cation to calf thymus DNA was studied by using the surfactant-selective electrode as a function of added electrolyte concentration at various temperatures. Binding is highly cooperative and biphasic. An abruptly occurring binding attains a slight shoulder within a very narrow equilibrium concentration range followed by a further augmentation in binding, as the equilibrium concentration is increased. Temperature caused relatively small shift in binding isotherm, but a distinct inversion point was found around 27 °C. Above this temperature the isotherm shifts to the left and then to the right below it. Analogous binding studies as above were repeated with heat-denatured DNA, which binds the surfactant with higher intrinsic affinity but less cooperatively than the intact DNA. A fluorescent probe, pyrene, was employed to assess the microenvironmental polarity of pyrene in DNA before and after surfactant binding. The surfactant-DNA complex has a hydrophobic region akin to ordinary micelle.

Partition of a ligand molecule (ion) between a macromolecule and aqueous phase is very important and attractive phenomena. Actually a number of investigations on such molecular interactions have been published to cover synthetic as well as biological systems.[1-4] Binding isotherm among many others is a clear-cut evidence of interaction between ligand and macromolecule, and will allow not only qualitative but also quantitative interpretation of the phenomena by the help of statistical mechanics and other physical methods.

We also have studied some macromolecule-ligand systems in which ionic surfactants take part of the ligands.[5-12] Deoxyribonucleic acid (DNA) is viewed as a long cylinder with phosphate groups spirally arrayed on the surface, and thus has driven researchers to work on surfactant binding.[12-14] In a previous paper,[12] interaction between DNA and cationic surfactant, *N*-dodecylpyridinium halides, was reported. In the present work, dodecyltrimethylammonium chloride was used in place of the pyridinium analog to see if there is any difference in binding behavior. A fluorescence probe, pyrene was employed to assess the microenvironment of pyrene in DNA–surfactant complex. Binding studies were extended to thermally denatured DNA in the added electrolyte solutions of various concentrations at temperatures. These binding behaviors were semiquantitatively expressed in terms of one-dimensional Ising model.

Experimental

Materials. Dodecyltrimethylammonium chloride (Tokyo Kasei Co.) was recrystallized from acetone three times. The critical micelle concentration as measured by an electric conductivity method is 20.5 mM in water at 25 °C. Calf thymus DNA (Sigma, sodium salt) is the same sample as used before.[12] Thermal denaturation was carried out by heating DNA solution at 85 °C for 5 min.

Binding Isotherm. Propionyl-α-cyclodextrin electrode which had previously been used[11,12] responded also to dodecyltrimethylammonium cation as seen in Fig. 1, where a straight line has a slope of 59 mV per 10 fold concentration difference, or the Nernstian response. In the presence of DNA, however, there appeared a deviation from the linearity which was caused by a partial uptake of surfactant by DNA.

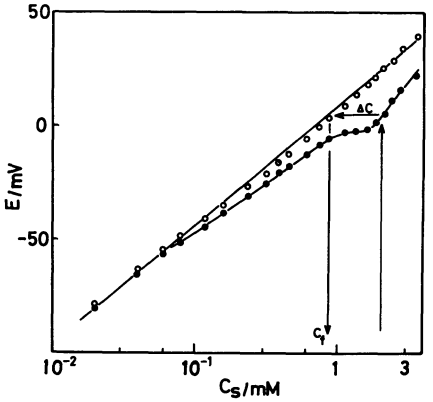

Fig. 1. Potentiometric titration of dodecyltrimethylammonium chloride in 20 mM NaCl at 25 °C. ○: without and ●: with DNA. See text for C_s, C_f, and ΔC.

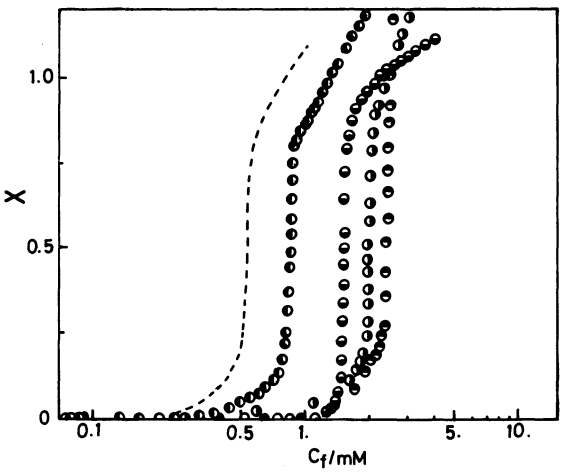

Fig. 2. Binding isotherms of dodecyltrimethylammonium chloride–DNA system at 25 °C. NaCl concentrations ◐: 20, ◒: 40, ◑: 80, and ◓: 160 mM, the dashed line for *N*-dodecylpyridinium chloride for comparison.

No hysterisis was observed when the potentiometric titration without DNA was repeated after the binding run. Following the arrows indicated in Fig. 1, an amount of binding, $\Delta C=C_s-C_f$, and an equilibrium concentration, C_f are easily obtained. A binding isotherm is built up by plotting a degree of binding, $X=\Delta C/$(total concentration of phosphate groups in DNA) vs. log C_f as shown in Fig. 2.

Fluorescent Probe Method. Emission spectrum from pyrene in the surfactant and/or DNA solution was recorded on a fluorescence spectrophotometer (Shimadzu RF-510).

We conveniently use the notation, 1 M=1 mol dm^{-3} throughout the paper.

Results and Discussion

Binding to Intact DNA. All the binding isotherms are characteristic of cooperative nature as well as biphasic. An abrupt appearance and completion of the first step binding is followed by the second step binding. It is clear that added NaCl causes the first binding shift to the right along the C_f axis as seen in Fig. 2. As for the second one, the effect of added electrolyte seems reversed. All these features are the same as observed previously with the DNA-*N*-dodecylpyridinium system and can be interpreted in the same manner. The first step binding is a process where DNA discharges and added electrolyte shields the electrostatic field attractive to surfactant cations leading to decreased binding affinity. On the other hand, in the second step binding, charge is built up on DNA on binding the surfactants with their hydrocarbon tails in contact with each other on DNA and with their charged head groups exposed toward the aqueous bulk phase. In Fig. 2, the binding isotherm of *N*-dodecylpyridinium Cl–DNA system (20 mM NaCl, 25 °C)[12] is shown by a dashed line for comparison. The equilibrium concentration at $X=0.5$ are 0.54 mM for *N*-dodecylpyridinium and 0.64 mM for dodecyltrimethylammonium, respectively. This difference, $RT\ln(0.64/0.54)=0.42$ kJ mol^{-1}, should be ascribed to the different structures of ionic head groups of the two kinds of surfactants. A positive charge delocalized throughout a flat pyridinium ring can approach a phosphate group on DNA much closer than that of trimethylammonio group gaining more electrostatic interaction energy.

Figure 3 shows binding isotherms at four temperatures. The temperature effect is small and even an inversion temperature is found around 27 °C, which is slightly higher than the one (23 °C) found with the pyridinium analog in the previous study.[12]

Binding to Thermally Denatured DNA. Figures 4 and 5 display binding isotherms for thermally denatured DNA. Binding begins at an equilibrium concentration lower than in intact DNA solution, and increases with less steep slope. This means that

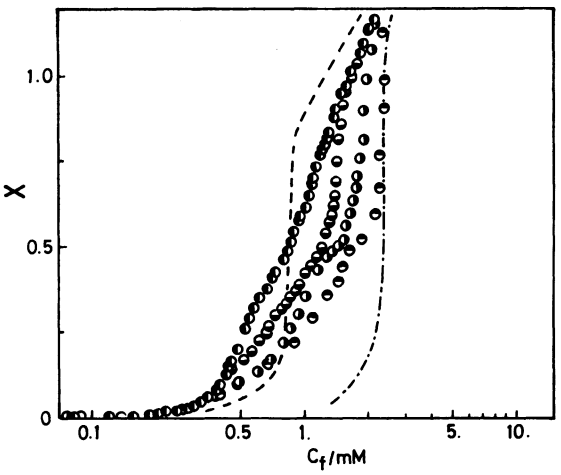

Fig. 4. Binding isotherms of dodecyltrimethylammonium chloride-heat denatured DNA system at 25 °C. NaCl concentrations: ◐: 20, ◒: 40, ◑: 80, and ◓: 160 mM. The dashed and chain lines for intact DNA in 20 and 160 mM NaCl at 25 °C, respectively.

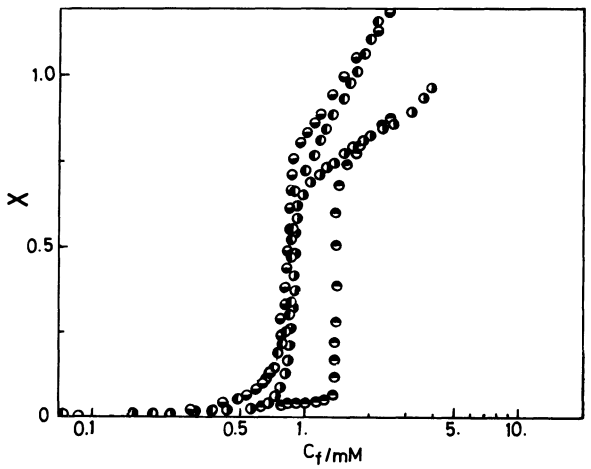

Fig. 3. Binding isotherms of dodecyltrimethylammonium chloride–DNA system in 20 mM NaCl at various temperatures(°C): ◓: 15, ◐: 25, ◒: 35, and ◑: 45.

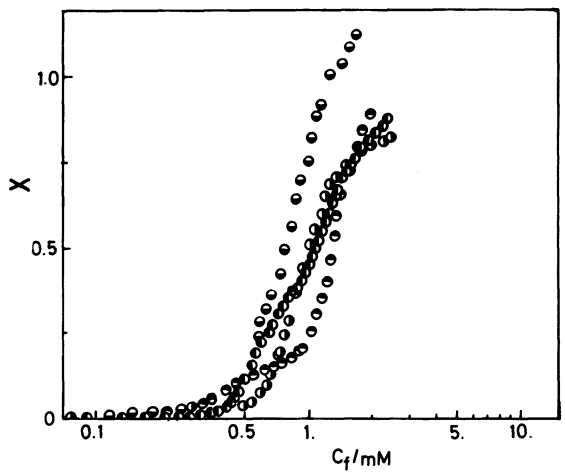

Fig. 5. Binding isotherms of dodecyltrimethylammonium chloride-heat denatured DNA system in 20 mM NaCl at various temperatures(°C); ◓: 15, ◐: 25, ◒: 35, and ◑: 45.

additional binding affinity is gained by contact between alkyl chains of bound surfactants and hydrophobic moiety of DNA which has been exposed to the aqueous phase by thermal denaturation. Randomized array of phosphate groups as a result of heat denaturation causes less effective interaction between alkyl chains of bound surfactants leading to a lower cooperativity, or a less steep growth of binding. It is to be noted that the binding isotherm overlaps that of not-heat-treated DNA (dashed and chained lines in Fig. 5) around $X=1$. This strongly indicates that the surfactant-DNA complex assumes the same dissolved state as the thermally denatured DNA does near the endpoint of surfactant titration, in other words, the cationic surfactant denaturates the DNA. Electrostatic shielding effect decreases the binding affinity in these systems, too.

In Fig. 5, the effect of temperature on the binding to the denatured DNA is shown. Here again an inversion temperature is observed around 27 °C: binding isotherm shifts to the left from 15 °C up to the inversion temperature, and moves back at 45 °C to the right. Regular structure of intact DNA may not be prerequisite for the inversion temperature phenomena, which is similar to a minimum found in a critical micelle concentration-temperature plot.[15-17]

Fluorescence from Pyrene. Fluorescence from pyrene provides significant information about microenvironment surrounding the molecule.[18] Figure 6 shows how relative fluorescence intensity at $\lambda=391$ nm, I_r, varies with the surfactant concentration. In the absence of DNA, and below cmc, I_r decreases. This means that fluorescence from pyrene molecules in aqueous environment is quenched on encounter with the cationic surfactant monomers. The pyrene molecules are, however, getting free from the quenching as soon as micelle formation begins. Pyrene molecules now find micelles where the quantum yield for fluorescence is enhanced. In the presence of DNA, I_r is much smaller than the one without DNA. When pyrene molecules are under the influence of DNA, they are probably intercalated between bases, and the quantum yield is very low.[19] However the fluorescence intensity is enhanced in parallel with the surfactant binding as seen in Fig. 6, where a dashed line shows the surfactant binding isotherm. That pyrene molecules are transferred to some hydrophobic micelle-like region in surfactant-DNA complex is suggested from comparison of curves (a) and (b).

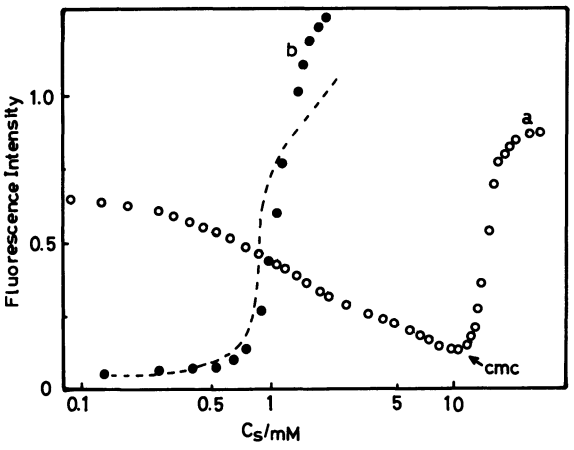

Fig. 6. Fluorescence intensity (in arbitrary unit) of pyrene ($\lambda=391$ nm) as a function of dodecyltrimethylammonium chloride concentration (in 20 mM NaCl at 25 °C). ○: Without and ●: with DNA. The dashed line shows a synchronized binding.

Ising Model. All these binding features will be semiquantitatively expressed in terms of statistical thermodynamics. Phosphate groups are taken as a one dimentional array of binding sites, on which the surfactant molecules are bound with their hydrocarbon chains in contact with each other. The partition function of the system is written as,

$$Z = aM^n a^* \tag{1}$$

with

$$M = \begin{pmatrix} 1 & 1 \\ s/u & s \end{pmatrix}, \quad a = (1, 1), \quad a^* = \begin{pmatrix} 1 \\ 0 \end{pmatrix}, \quad \text{and } s = KuC_f,$$

where K is equilibrium constant for a process that a surfactant is transferred from the aqueous bulk phase to an isolated binding site on DNA, and u an equilibrium constant of aggregation among bound surfactants, or cooperativity parameter.

When n is very large, Eq. 1 produces compact and useful expressions,[20] among which the following equations are convenient to extract thermodynamic informations,

$$X = \mathrm{d}\ln Z/\mathrm{d}\ln C_f = \{1-(1-s)/\sqrt{(1-s)^2+4s/u}\}/2 \tag{2}$$

$$K = 1/C_f(X=0.5)u, \tag{3}$$

where $C_f(X=0.5)$ is an equilibrium concentration assuring $X=0.5$. Lower halves of binding isotherms were fitted to Eq. 2 to calculate the value of u in conformity with the Manning's notion that electrostatic potential at polyelectrolyte surface remains relatively constant so long as the surface charge density is high enough.[21,22] The thermodynamic parameters thus obtained are shown in Figs. 7 and 8. It is noted in Fig. 7 that u increases, while K decreases with increasing the added electrolyte concentration. The value of u should be kept constant irrespective of added salt concentration, since it is an equilibrium constant for aggregation process of bound surfactants which are no longer charged. With increase in the amount of binding, electrostatic potential is reduced, although there may be a sort of compensation by the Manning's ion-condensation mechanism. This potential reduction brings about a negative cooperativity. The anticooperativity decreases with increase in added electrolyte concentration, as seen in Fig. 7,

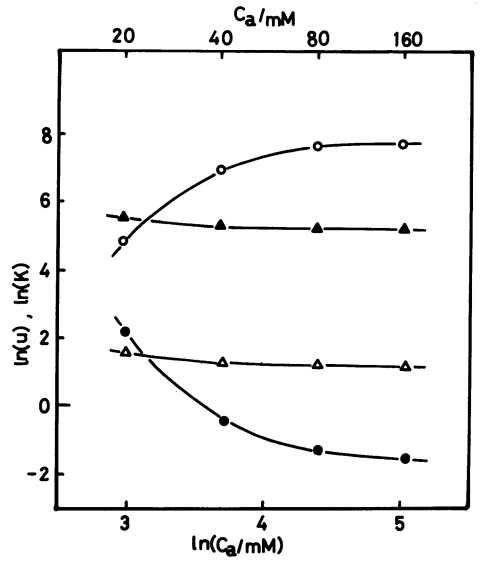

Fig. 7. ln u and ln K vs. ln C_a, where C_a is added NaCl concentration. ○: u and ●: K for intact DNA, and △: u and ▲: K for heat-denatured DNA.

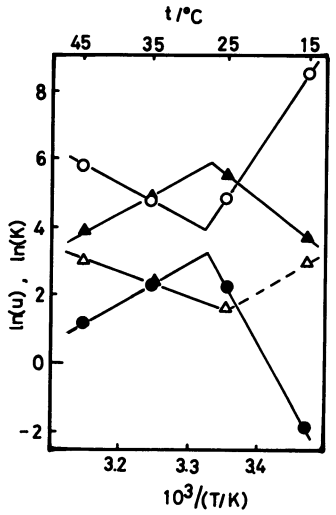

Fig. 8. ln u and ln K vs. $1/T$. ○: u and ●: K for intact DNA, and △: u and ▲: K for heat-denatured DNA.

where the effect of added electrolyte is almost saturated when C_a is as much as 160 mM. Figure 7 also shows the thermodynamic parameters for the thermally denatured DNA. Very low u and high K values may be responsible for the disordered phosphate array and exposed hydrophobic moieties of DNA as a result of heat denaturation as explained above.

Temperature dependence on the binding behavior is reflected on plots of ln u and ln K vs. $1/T$ in Fig. 8, where distinct reversal in the temperature dependence is observed around 26—27 °C, for both intact and heat-denatured DNA. Below the inversion temperature, K increases while u decreases with increasing temperature, and vice versa above it. Inversion in the temperature dependence in surfactant binding is also found for DNA[12] and dextran sulfate.[23] These phenomena are reminiscent of the similar minima found in cmc[15–17] as well as solubilities of some organic compounds in water.[24] Detailed studies on water structure and hydrophobic hydration are required before these inversion phenomena are fully understood.

Gross effect of added surfactant is to denature DNA which has ordinarily highly ordered structure. Proteins are also denatured by addition of surfactants, and the modes of action are very much complicated as well as diverse, reflecting the complexity of protein structures of primary, secondary, and higher orders.[25,26] In contrast, binding mode is simpler than that of proteins, and DNA behaves much like synthetic polyelectrolytes in binding surfactants.[8,11,23] However there is a distinct difference in DNA which undergoes an drastic order-disorder transition, while synthetic polymers will not show such a conformation change.

We thank Prof. Mitsuru Tanaka of Fukuoka University for providing the DNA sample.

References

1) A. J. Hopfinger, "Intermolecular Interactions and Biomolecular Organization," John Wiley and Sons, New York (1977).
2) J. Steinhardt and J. A. Reynolds, "Multiple Equilibria in Proteins," Academic Press, New York (1969).
3) "Microdomains in Polymer Solutions," ed by P. Dubin, Plenum, New York (1985).
4) I. D. Robb, "Anionic Surfactants, Surfactant Science Series," ed by E. H. Lucassen-Reynders, Vol. 11, (1981), p. 109.
5) K. Shirahama, *Colloid Polym. Sci.*, **252**, 978 (1974).
6) T. Takagi, K. Tsujii, and K. Shirahama, *J. Biochem. (Tokyo)*, **77**, 939 (1975).
7) K. Shirahama and N. Ide, *J. Colloid Interface Sci.*, **54**, 450 (1976).
8) K. Shirahama, H. Yuasa, and S. Sugimoto, *Bull. Chem. Soc. Jpn.*, **54**, 375 (1981).
9) K. Shirahama, M. Todoh, and M. Murahashi, *J. Colloid Interface Sci.*, **86**, 282 (1982).
10) K. Takeda, A. Iba, and K. Shirahama, *Bull. Chem. Soc. Jpn.*, **55**, 985 (1982).
11) K. Shirahama and M. Tashiro, *Bull. Chem. Soc. Jpn.*, **57**, 377 (1984).
12) K. Shirahama, T. Masaki, and K. Takashima, p. 299 in Ref. 3.
13) R. Chatterjee and D. K. Chattoraj, *Biopolymers*, **18**, 147 (1979).
14) K. Hayakawa, J. T. Santerre, and J. C. T. Kwak, *Biophys. Chem.*, **17**, 175 (1983).
15) B. D. Goddard and G. C. Benson, *Can. J. Chem.*, **35**, 986 (1957).
16) B. D. Flockhart, *J. Colloid Sci.*, **16**, 484 (1961).
17) Y. Moroi, N. Nishikido, H. Uehara, and R. Matuura,

J. Colloid Interface Sci., **50**, 254 (1975).
18) J. K. Thomas, *Chem. Rev.*, **80**, 283 (1980).
19) H. P. Nelson, Jr. and H. DeVoe, *Biopolymers*, **23**, 89 (1984).
20) G. Schwarz, *Eur. J. Biochem.*, **12**, 442 (1970).
21) G. S. Manning, *Acc. Chem. Res.*, **12**, 443 (1979).
22) G. S. Manning, *J. Phys. Chem.*, **85**, 870 (1981).
23) J. P. Santerre and J. C. T. Kwak, *Colloids Surf.*, **13**, 35 (1985).
24) A. Katayama, T. Takagishi, K. Konishi, and N. Kuroki, *Kolloid-Z. Z. Polym.*, **210**, 126 (1967).
25) W. L. Mattice, J. M. Riser, and D. S. Clark, *Biochemistry*, **15**, 4264 (1976).
26) B. Jirgensons, "Optical Activity of Proteins and Other Macromolecules," 2nd. Ed., Springer-Verlag, Berlin (1973).