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THE BINDING OF CATIONIC SURFACTANTS BY DNA

Katumitu HAYAKAWA *, J. Paul SANTERRE and Jan C.T. KWAK

Department of Chemistry, Dalhousie University Halifax, Nova Scotia, Canada B3H 4J3

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Isotherms for the binding of dodecyltrimethylammonium (DTA^+) and tetradecyltrimethylammonium (TTA^+) ions by DNA in aqueous solution at 30°C are reported. The binding isotherms were determined using a potentiometric technique with cationic surfactant-selective electrodes. The DNA concentrations used are 5×10^{-4} and 10^{-3} equiv./kg, surfactant concentrations varying from 3×10^{-6} M to the critical micelle concentration. The influence of added NaCl (0.01 M) on the binding process is studied. The binding process is shown to be highly cooperative. Applying the binding theory of Schwarz and of Satake and Yang, binding constants and cooperativity parameters can be calculated. The binding constant K is found to be $1.2kT$ larger for TTA^+ than for DTA^+ in salt-free solution, and $1.4kT$ larger for TTA^+ than for DTA^+ in 0.01 M NaCl. The cooperativity parameter u is about $1.1kT$ larger for TTA^+ in salt-free solution and $1.2kT$ larger in 0.01 M NaCl. It is concluded that the hydrophobic part of the bound surfactant is not completely immersed in the hydrophobic DNA core, but also interacts with other surfactant molecules. This situation is compared to the case of micelle formation.

1. Introduction

The binding of organic ions by biological polymers is of importance in many biological processes. Only a few physicochemical studies have been reported on the particular case of the interaction of cationic surfactants with biological tissues [1–4], even though the germicidal action of cationic surfactants against viruses, bacteria, fungi, bacterial spores, protozoa and invertebrates has been studied widely [5]. In this paper we will investigate the binding of two cationic surfactants, dodecyltrimethylammonium bromide (DTABr) and tetradecyltrimethylammonium bromide (TTABr) to DNA, at very low surfactant concentrations. Apparently, very few reports on the interaction between cationic surfactants and DNA exist [6]. Through the use of recently developed surfactant ion-selective electrodes, which have been applied successfully in investigations on the binding of ionic surfactants by polymers in aqueous solution [7–12], accurate data on the binding of DTA^+ and TTA^+ to DNA could be obtained at surfactant concentrations several orders of magnitude below the critical micelle concentration of these surfactants.

2. Experimental

2.1. Materials

Highly polymerized DNA (salmon sperm, sodium form, nitrogen/phosphorus ratio stated by the supplier as 1.75) was obtained from ICN Pharmaceuticals, Cleveland, OH. A concentrated solution of DNA was dialyzed twice against a mixture of Tris buffer, Na_2EDTA and NaCl, and then dialyzed repeatedly at room temperature

* Permanent address: Department of Chemistry, Faculty of Science, Kagoshima University, Kagoshima, Japan 890.

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against distilled and deionized water [13]. No chloride could be detected in the final rinse solutions. The phosphate concentration was determined spectrophotometrically by using $\epsilon(P) = 6500$ l mol^{-1} cm^{-1} at 269 nm [14]. DTABr and TTABr were obtained from Sigma Chemical Co., St. Louis, MO, and purified by repeated recrystallization from acetone. Analytical grade NaCl was used without further purification.

2.2. Potentiometry

Free surfactant concentrations were determined by means of a surfactant cation-selective plastic membrane electrode which has been reported to have an excellent surfactant cation selectivity in the presence of excess simple salt, and a Nernstian response [11,15]. The electrode assembly is as follows:

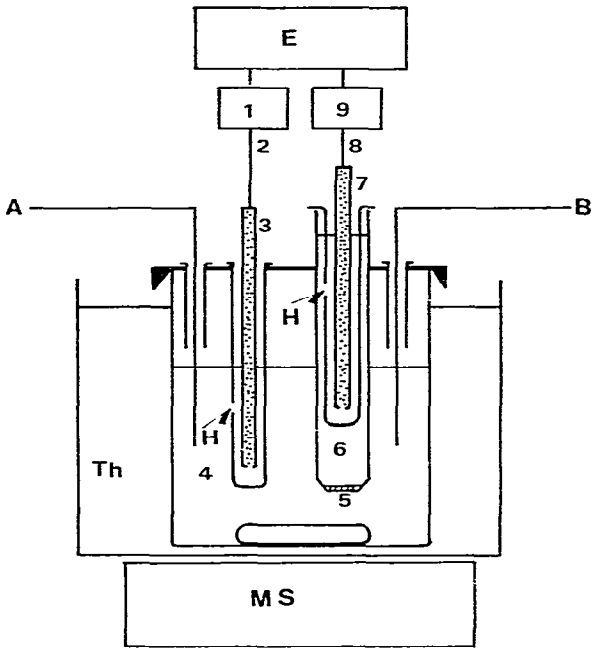

Fig. 1. Schematic diagram of potentiometric cell assembly. Numbers 1–9 correspond to numbers in eq. 1 (see text). E, digital electrometer; A and B, autoburets; H, pinhole; Th, thermostat; MS, magnetic stirrer.

Calomel electrode 1	Satd. KCl solution 2	Agar NH_4Cl 3	Test solution 4	Membrane 5				
Reference solution 6	Agar NH_4Cl 7		Satd. KCl solution 8		Calomel electrode 9			(1)

The membrane is made of PVC plasticized with bis(2-ethylhexyl)phthalate, and contains an ion carrier in the form of the complex of the cationic surfactant with dodecyl sulfate. A titration method was used for surfactant additions. The cell assembly is depicted in fig. 1, where the numbers correspond to the numbers in electrochemical cell 1, above, and A is an autoburet filled with surfactant stock solution, B an autoburet filled with DNA or a mixed solution of DNA and NaCl, added to keep the DNA and NaCl concentrations constant in the test solution. E is a Keithley 616 digital electrometer connected to a potentiometric recorder. H is a pinhole to prevent the test solution from being contaminated by agar salt. The pinhole junction method is necessary in the case of salt-free systems, because surfactant binding is fairly sensitive to the added salt concentration [12]. In order to keep the concentrations of DNA and NaCl constant in a test solution every time surfactant solution is added, the same volume of a mixed solution of DNA and NaCl both with double the concentration of the test solution is added by autoburet B.

The electromotive force (emf) reaches a stable value immediately after each addition of surfactant. A very slow time dependence (e.g., less than 2 mV/h in the TTABr/DNA system), however, was found in the salt-free systems. This drift was followed for a few hours and still lasted with a constant rate. This very slow time dependence was not found in DTA binding by dextran sulfate or polystyrenesulfonate with added salt [12]. Since the mechanism of this slow mode may be considered to be different from simple surfactant binding, emf values were taken about 20 min after each addition of surfactant.

All measurements were carried out at 30°C in the absence of a pH buffer. The pH was measured after the completion of each titration and was found to be between 5 and 7, dependent on the

added salt concentration. The stability of DNA is relatively insensitive to pH in the pH range 5–9 [16,17].

In order to check for denaturation of the DNA after dilution, the absorbance at 259 nm was measured for diluted, salt-free DNA samples (1×10^{-3} and 5×10^{-4} equiv./kg H_2O) kept at 30°C for the time it takes to complete a binding experiment (titration method). No significant change in absorbance was observed. In addition, no change in absorbance was observed after the addition of NaCl to this solution to a total ionic strength of 0.1 M, indicating that there is no appreciable denaturation of the DNA. On the other hand, it is quite possible that the slow time dependence of the emf in salt-free surfactant/DNA solutions is due to denaturation or other macromolecular conformational changes. At this moment, we are not in a position to comment on the nature of this slow process, however, the measurements as reported should be seen as binding data very shortly after surfactant addition.

3. Results and discussion

Results of the potentiometric experiments are given in fig. 2. The calibration curves clearly show the excellent performance of the plastic membrane electrode even in the presence of 0.01 M NaCl. In the presence of DNA, the deviation from the calibration curve in the log m_D axis, where m_D is the added surfactant concentration, allows us to calculate the amount of bound surfactant. The constant deviation from the calibration curve (log m_D axis) (fig. 2a) at very low surfactant concentration indicates that at low concentration the amount of bound surfactant is proportional to the free surfactant concentration. From the potentiometry curves in fig. 2, the binding isotherms are constructed in fig. 3, where the binding degree β, defines as the amount of bound surfactant for each ionic site on DNA, is plotted against the concentration of free surfactant. Inspection of the results presented clearly shows the excellent reproducibility of the data obtained with surfactant

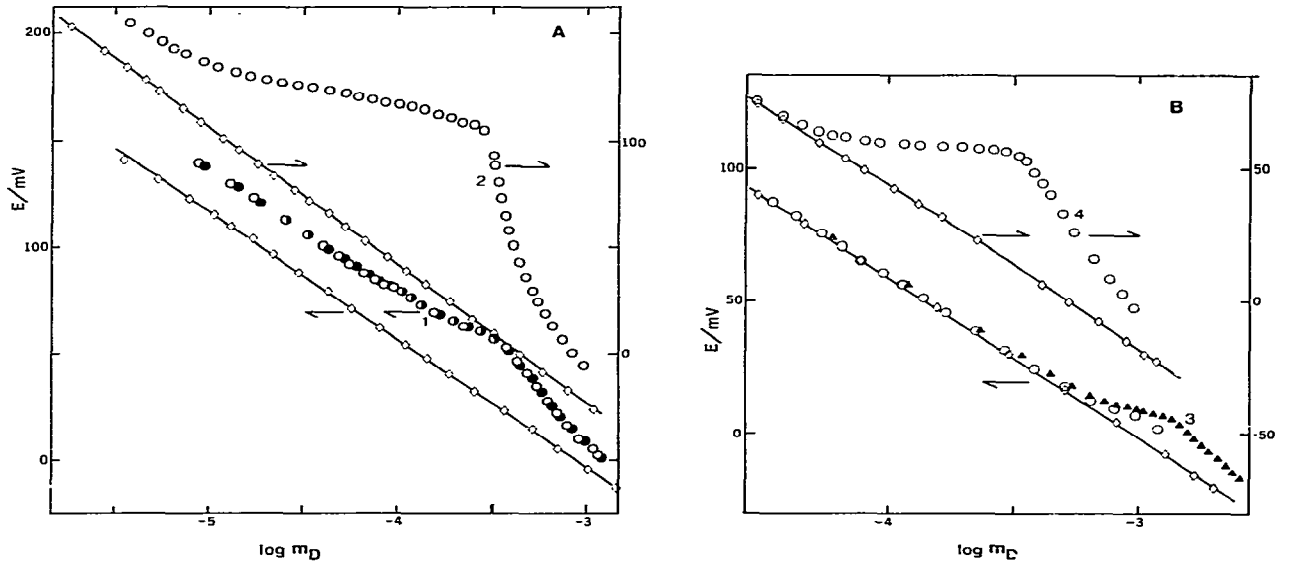

Fig. 2. Plot of emf against m_D, total DTA^+ or TTA^+ concentration, at 30°C. (A) Salt-free systems, $m_{DNA} = 5 \times 10^{-4}$ equiv. kg^{-1}. (◇) Calibration curves: (1) DTABr/DNA (open and solid circles: duplicate measurements); (2) TTABr/DNA. (B) 0.01 M NaCl, $m_{DNA} = 5 \times 10^{-4}$ equiv. kg^{-1}. (◇) Calibration curves: (3) DTABr/DNA (▲: $m_{DNA} = 1 \times 10^{-3}$ equiv. kg^{-1}); (4) TTABr/DNA.

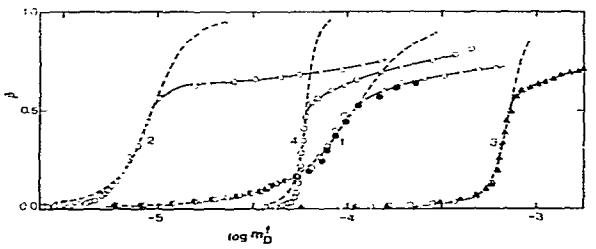

Fig. 3. Plot of binding degree β vs. free surfactant concentration m_D^f at 30°C. Series 1–4 correspond to those in fig. 2. Broken lines: eq. 4; Ku and u as given in table 1.

selective electrodes (compare, e.g., the results of independent duplicate series represented by the open and solid circles in fig. 3). Accurate and reproducible results are generally obtained much more easily with the emf method described here than with equilibrium dialysis methods [18–21].

DTA and TTA surfactant cations bind to DNA at very low equilibrium concentration, far below the surfactant's critical micelle concentration even in the presence of excess salt. On the other hand, dodecyl sulfate anions exhibit no binding by DNA [21] This observation shows the importance of an electrostatic term in surfactant binding by DNA. The longer chain surfactant TTA^+ is more easily bound by DNA, indicating that a hydrophobic interaction between surfactant and DNA is also important. The binding isotherm does not depend on the DNA concentration at least at concentrations below 10^{-3} equiv./kg DNA as shown by curve 3 in fig. 3 (open circles and solid triangles).

Fig. 3 also shows the cooperative nature of surfactant binding by DNA. The following equilibrium reactions can be separately taken into account for a cooperative binding reaction:

$$(OO)+D^+ \overset{K}{\rightleftarrows} (OD) \quad (2)$$

$$(DO)+D^+ \overset{Ku}{\rightleftarrows} (DD) \quad (3)$$

where K and Ku are corresponding apparent equilibrium constants. OO are two neighboring binding sites on DNA, D^+ represents a free surfactant ion, and D an occupied site on the DNA polyion. Reaction 2 indicates the binding of surfactant to an isolated site on the polymer. K may be considered to be a function of the electrical potential around DNA and/or the concentration of competitive Na^+. Reaction 3 indicates the binding of surfactant to a site adjacent to a site already occupied by bound surfactant. u gives a criterion by which the cooperativity can be estimated: $u > 1$ for cooperative binding, $u = 1$ for noncooperative binding, and $u < 1$ for anticooperative binding. Hydrophobic interaction between the bound surfactants causes the cooperative nature in surfactant binding by polymers [7–12,18–23]. Clearly, a nearest-neighbor-type description, and the equations below derived from it, may not be sufficiently accurate to represent the complex binding phenomena we encounter here, especially considering the fact that conformational changes of the polymer may be induced by the surfactant-binding process. Nevertheless, such a description provides us with a convenient formalism for a first approximation of the binding process. In particular, we will show that the contribution of the hydrophobic interaction between the alkyl chains of the bound surfactants derived from the model employed here yields data which can reasonably be compared to the case of micelle formation in surfactant solutions.

Table 1

Cooperative binding constant, Ku, cooperativity, u, and intrinsic binding constant, K, of DTABr and TTABr with DNA

Binding constant	Salt-free		0.01 M NaCl	
	DTABr	TTABr	DTABr	TTABr
Ku (mol^{-1} kg) (±10%)	9.3×10^3	1.07×10^5	1.38×10^3	1.66×10^4
u (±10%)	6	20	70	200
K (mol^{-1} kg) (±10%)	1600	5400	20	80

Applying the Zimm-Bragg theory for helix–coil transitions of biopolymers [24] to cooperative binding by polymers, Schwarz [25] and later Satake and Yang [8] derived the following expression for the binding degree β:

$$\beta = \tfrac{1}{2}\left[1-(1-s)/\left[(1-s)^2+4s/u\right]^{1/2}\right] \quad (4)$$

where s is defined by the product of Ku and equilibrium surfactant concentration m_D^f and is equivalent to s in the Zimm-Bragg theory. At the half-bound point ($\beta = \frac{1}{2}$ or $s = 1$), one can obtain the following relationships:

$$(m_D^f)_{\beta=1/2} = 1/Ku \quad (5)$$

$$(\mathrm{d}\beta/\mathrm{d}\ln m_D^f)_{\beta=1/2} = \sqrt{u}/4 \quad (6)$$

From fig. 3, one can easily estimate precise values of Ku and less accurate values of u at the half-bound point. These values are given in table 1.

The emf vs. $\log m_D$ plot for the salt-free DNA/DTA$^+$ system in fig. 2 also allows us to estimate the amount of bound surfactant at low surfactant concentrations, i.e., well below the region of cooperative binding of DTA$^+$ by DNA. In this region one can neglect the cooperative binding reaction 2 and derive the following expression:

$$\beta/(1-\beta) = Km_D^f \quad (7)$$

The left-hand term is plotted against the corresponding equilibrium concentration of surfactant in fig. 4. From the linear part of the curve at small binding degree, the value of K is estimated to be 2450 kg mol^{-1} (to be compared to the value of 1600 ± 10% as found from the parameters Ku and u shown in table 1).

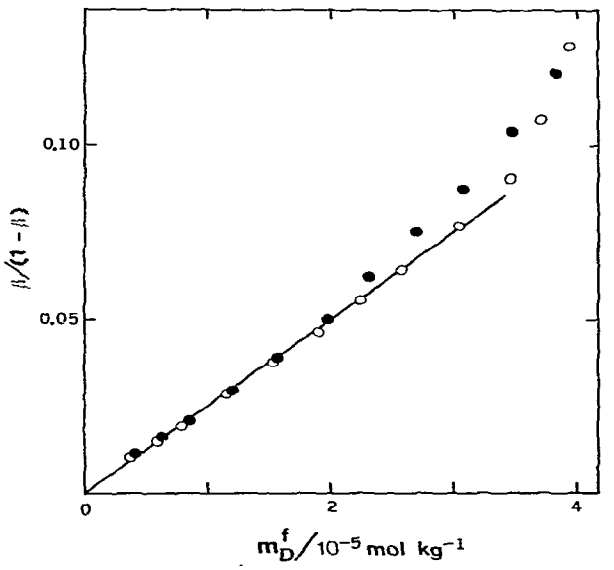

Fig. 4. $\beta/(1-\beta)$ vs. free surfactant concentration m_D^f at low degree of binding. DTABr/DNA system, salt-free (○, ●) Duplicate measurements. Note that the difference between the curves at higher m_D^f is within the limits of error of a ±0.5 mV reproducibility in measured emg values.

According to the condensation model of polyelectrolyte solutions [26,27], a constant fraction of counterions is trapped in the close vicinity of the polyelectrolyte backbone when the linear charge density parameter ξ exceeds $1/z$, where z is the counterion charge and ξ is defined by $e^2/\epsilon kTb$, where b is the linear charge spacing on the polyion, e the elementary charge, ϵ the dielectric constant, k Boltzmann's constant, and T the temperature. Because of counterion condensation the effective charge density parameter is reduced to $1/z$. Since the value of ξ of a double-stranded DNA is estimated to be 4.2 [28] and only monovalent counterions are included in the present system, 76% of the polyion charges are considered to be compensated by the presence of condensed counterions. This means that the effective charge density of DNA is kept constant upon surfactant binding until the binding degree of surfactant reaches 0.76, binding being accompanied by an exchange reaction with Na$^+$. Since the ionic strength of the solution and the concentration of competitive Na$^+$ are kept constant in the presence of excess NaCl, K (eq. 2) may be expected to be independent of the binding degree until β reaches 0.76. On the other hand, for the salt-free system K may be expected to depend on the added surfactant concentration through the ionic strength effect and the concentration change of Na$^+$ in bulk solution. The lower value of K (1600) at the half-bound point compared to its value (2450) at very low binding degree may be due in part to this dependence of K on the added surfactant concentration.

Fig. 3 indicates that the binding isotherms level

off at a binding degree of about 0.6, i.e., less than the value 0.76 where the condensation model predicts the electrical potential at the polyion to decrease with a further increase in the binding reaction. The linear charge density parameter $\xi = 4.2$ is equivalent to 1.7 Å of linear charge spacing on DNA. However, the real nearest charge-charge distance is estimated to be about 5 Å for the β-form of DNA [29]. The head size of the alkyltrimethylammonium ion is estimated to be 6.9 Å diameter from a molecular model, or 4.1 Å from the limiting mobility by Stokes's law applied to $(CH_3)_4N^+$ [30]. Apparently, the sizes of the ionic head groups of both DTA^+ and TTA^+ are too large to allow attachment to a site adjacent to a site already occupied by surfactant. This steric hindrance may contribute to the saturation at a low binding degree and second layer adsorption may begin before complete coverage of the first layer. Although second-layer adsorption may be less preferable because of the ionic environment around DNA, the solubilization of a precipitate of surfactant-polyelectrolyte complex in the presence of excess surfactant [31–33] does suggest the ionization of the complex by further adsorption of ionic surfactant through second or further layer adsorption.

From the difference in Ku for DTA^+ and TTA^+ (table 1), the difference in the free energy of surfactant binding between DTA^+ and TTA^+ can be estimated to be $2.49kT$. $1.1kT$ is contributed by the difference in the cooperativity parameter u, and the remaining $1.4kT$ from the difference in K, all in the presence of 0.01 M NaCl. The corresponding values are $2.44kT$ from Ku, $1.2kT$ from u, $1.2kT$ from K for the salt-free system. Since the cooperativity in surfactant binding by DNA may be caused by hydrophobic interaction between bound surfactants, the difference of $1.1kT$ or $1.2kT$ as calculated from u is equivalent to the difference in hydrophobic interaction energy between bound DTA^+ and between bound TTA^+ on DNA. The values are about half of Shinoda's estimate for micelle formation ($1.08kT$ per methylene group or $2.2kT$ between DTA^+ (C_{12}) and TTA^+ (C_{14})) [34]. In micelle formation, methylene groups except those near the hydrophilic head are transferred from an aqueous environment to a hydrocarbon environment. The value of $1.08kT$ per methylene group, therefore, is comparable to the free energy of transfer of a methylene group from water to hydrocarbon liquid. The lower value of the hydrophobic interaction energy between bound surfactants on DNA ($0.55–0.6kT$ per methylene group) suggests that the interaction amoung bound surfactants is lower in surfactant binding to polyions than in micelle formation, possibly because a bound surfactant interacts also with the hydrophobic DNA core.

Since the Coulombic term in reaction 2 is assumed to be the same for both DTA^+ and TTA^+ binding by DNA in the presence of 0.01 M NaCl, the difference of $1.4kT$ in K between DTA^+ and TTA^+ binding may be attributed to the difference in hydrophobic and/or specific interaction between these surfactants and DNA. Again this value is less than $1.1kT$ per methylene group. It suggests that the tail of an isolated bound surfactant is not completely immersed in the hydrophobic core of DNA and therefore is still partly in contact with an aqueous enviroment.

Table 1 indicates that the presence of 0.01 M NaCl affects both u and K. No dependence of u on added NaCl concentration, however, was found in the presence of excess NaCl in the DTABr/dextran sulfate system [12] and in the interaction of sodium decyl sulfate with a copolymer of dimethylammonium chloride and sulfur oxide [10]. The difference in u between salt-free and salt-added systems is difficult to understand by a model along the lines of the treatment by Schwarz [25] and Satake and Yang [8] proposed in the present paper. A plot of log Ku against the total concentration of counterions gives slopes of -0.66 for DTABr/DBA and -0.62 for TTABr/DNA. These slopes are similar to the dependence of log Ku on added NaCl concentration in DTABr/dextran sulfate [12] and of the first transition point of polyvinylpyrrolidone-sodium dodecyl sulfate interactions [35]. They are also comparable to the dependence of the critical micelle concentration of DTABr on the added NaCl concentration [36]. This observation points at the similarity between the dependence of micelle formation and the DNA-surfactant cooperative binding process on the added salt concentration, presumably because both processes

involve the transfer of a surfactant ion to a highly charged and hydrophobic polyion or micelle [33,34].

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