The Evaluation of the Association Degree of Sodium Counterinos by Complexes between Sodium Dodecyl Sulfate and Bovine Serum Albumin, Mainly on the Basis of a Conductivity Measurement

Kunio TAKEDA

Department of Applied Chemistry, Faculty of Science, Okayama University of Science, 1-1 Ridai, Okayama 700

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Synopsis. Bovine serum albumin was titrated conductometrically with sodium dodecyl sulfate (SDS). Plots of the specific conductance *versus* the SDS concentration revealed four inflections. The association degrees of sodium counterions by the complexes were evaluated from the changes in the increment of the specific conductance of SDS with the complex growth.

The interactions of proteins with surfactants have received extensive attentions.[1] More recently, the present author and others have demonstrated the usefulness of the conductivity method for detecting protein–surfactant complex formation,[2] while this method has also been frequently used to determine the critical micelle concentration (CMC) of ionic surfactants. In the process of the complex formation between sodium dodecyl sulfate (SDS) and bovine serum albumin (BSA), four inflection points appeared in the plot of the specific conductance versus the SDS concentration at a constant BSA concentration. It is confirmed that these inflections appear with an intimate correlation with the formation of the SDS–BSA complex and the subsequent conformational changes of the protein.[2] It can generally be expected that these critical conductance changes mainly reflect the degree of association of sodium counterions.[3-6] The behavior of the counterions, despite their intrinsic interest, is still a matter of uncertainty.

The aim of this paper is the evaluation of the association degree of the sodium counterions by the complex, mainly on the basis of the conductance change with the complex formation.

Experimental

The crystalline BSA was obtained from Miles Laboratories Inc., and its concentration was determined spectrophotometrically using $E_{1cm}^{1\%}=6.8$ at 282 nm.[7] The crystalline SDS was purchased from BDH Chemicals Ltd., and recrystallized twice from 1-butanol and once from pure water. The conductivity measurements were carried out with a Universal Bridge 4265B of Yokogawa-Hewlett-Packard.[2] All the measurements were made at 25.000±0.005 °C in buffer solutions (pH 7.0; ionic strength, 0.014) containing 3.33 mM of NaH_2PO_4 and 3.56 mM of Na_2HPO_4 (1 M=1 mol/dm^3 was used as the concentration unit).

Results and Discussion

The conductance change with the complex formation was reexamined in order to evaluate the association degree of the sodium counterions by the complexes. The slope of each linear part between the inflections is presented in the second column of Table 1. The slope becomes milder, step by step, as seen in Table 1. The four linear parts below the CMC are considered to be correlated with the same number of types of complexes depending on the surfactant concentration ranges. These types of complexes are considered to be identical with the four observed previously under other experimental conditions.[8-10] When the specific conductance due to the species composing the buffer is subtracted from the total of the system, the residual conductance, κ, can be expected just for the behavior of the surfactant and the protein. Here, it is important that the contribution of the protein to the conductance is negligibly

Table 1. Association degree of sodium counterions and other parameters of SDS–BSA complexes

Type of complex (Concentration ranges of total SDS)[a]	$d\kappa/dC_{DS}^{total}$ / $10^{-5}\ \Omega^{-1}\ M^{-1}$	$\mu_{complex}$ / $10^{-4}\ cm^2\ s^{-1}\ V^{-1}$	μ_{Na} / $10^{-4}\ cm^2\ s^{-1}\ v^{-1}$	γ_{Na}	α	$(1-\alpha)$
Complex 1	5.6	1.6[b]	4.2	0.86	1.01	−0.01
(below 1.9 mM)		1.2[c]			1.06	−0.06
		1.7[d]			1.00	0
Complex 2	5.2	2.5[b]	4.1	0.86	0.82	0.18
(between 1.9 and 4.6 mM)		2.0[c]			0.88	0.12
Complex 3	4.4	2.8[b]	4.1	0.84	0.67	0.33
(between 4.6 and 6 6 mM)		2.4[c]			0.71	0.29
Complex 4	3.6	3.6[b]	4.0	0.80	0.50	0.50
(between 6.6 and 8.0 mM)						
SDS micelle	2.3	4.3[e]	4.0	0.80	0.29[f]	0.71
(above 8.0 mM)						

a) The total SDS concentrations where the inflections appeared in the plot of the specific conductance *versus* the total SDS in the presence of 1.0×10^{-5} M BSA (see Ref. 2). b), c), and d) Calculated by means of Eq. 4 on the basis of the data in Refs. 8, 9, and 10 respectively. e) This is the $\mu_{micelle}$ value obtained through the use of Eq. 4 on the basis of the reference values.[8,12] f) Calculated by substituting $\mu_{micelle}$ in place of $\mu_{complex}$ in Eq. 3.

small compared with that of the surfactant.[2] If we define the degree of the dissociation of sodium counterions from the complexes, α, the conductance, κ, may be expressed as follows:

$$\kappa = \frac{F}{1000}(\mu_{Na}\gamma_{Na}C_{Na} + \mu_{DS}^{free}\gamma_{DS}^{free}C_{DS}^{free} + \alpha\mu_{complex}C_{DS}^{bound}), \quad (1)$$

where F: Faraday's constant, μ: mobility, γ: activity coefficient, C: concentration (M), Na: sodium ion, free DS: free dodecyl sulfate ion, and bound DS: bound dodecyl sulfate ion. If we introduce the relation: $C_{DS}^{bound}=C_{DS}^{total}-C_{DS}^{free}$ where C_{DS}^{total} is the total concentration of SDS, as is often done in binding studies of these systems, we can adopt the following relation: $C_{Na}=C_{DS}^{free}+\alpha(C_{DS}^{total}-C_{DS}^{free})$. Then, Eq. 1 can be rewritten as:

$$\kappa = \frac{F}{1000}\{(\mu_{Na}\gamma_{Na}+\mu_{complex})\alpha C_{DS}^{total} + [(1-\alpha)\mu_{Na}\gamma_{Na}+\mu_{DS}^{free}\gamma_{DS}^{free}-\alpha\mu_{complex}]C_{DS}^{free}\}. \quad (2)$$

The slope, $d\kappa/dC_{DS}^{total}$, in the plot of the specific conductance *versus* the total SDS concentration is given by:

$$d\kappa/dC_{DS}^{total} = \frac{F}{1000}(\mu_{Na}\gamma_{Na}+\mu_{complex})\alpha. \quad (3)$$

If we know μ_{Na}, γ_{Na}, and $\mu_{complex}$, we can determine the α-values.

Aoki has found step-by-step changes in the complex mobilities with the complex formation between SDS and horse serum albumin and has reported four types of their mobilities.[8] Decker and Foster have also obtained three types of mobilities of the complexes between BSA and sodium dodecylbenzenesulfonate in the low concentration range of the surfactant.[9] These mobilities are 10—20% slower than those found by Aoki in a similar concentration range of SDS. Pallansch and Briggs have reported the mobility of the smallest SDS–BSA complex;[10] it is consistent with the corresponding value reported by Aoki when the difference in ionic strength is corrected by Eq. 4. These data would indicate what magnitudes of mobilities the complexes have. According to these data, the mobilities of the present SDS–BSA complexes were obtained through the correction of only the difference in ionic strength by applying the following relation:[11,12]

$$\log\mu = -A\log I_S + B, \quad (4)$$

where A and B are constants and I_s is the ionic strength. The constant, A, was determined to be 0.095 according to the dependence of the mobility of the SDS micelle on the ionic strength obtained by Stigter and Mysels;[11] it was then used in the calculation. The value of μ_{Na} was obtained from the equivalent conductivity of the sodium ion reported by Mukerjee *et al.*[4] and by the subsequent interpolation of the log-log plot of μ_{Na} *versus* the ionic strength, such as in Eq. 4. The activity coefficient, γ_{Na}, of 0.80 was adopted in the vicinity of the CMC, on the basis of the results of Botre *et al.*[3] Its value in an infinite dilution of SDS was computed to be 0.89 under the present conditions (I_s=0.014) by the application of the usual Debye-Hückel approximation.[13] The values of γ_{Na} between 0.80 and 0.89 were determined using this relationship: $-\log\gamma = S\sqrt{C_{DS}^{total}}$ (S: constant), given by Shedlovsky *et al.*[14] All the parameters used are listed in Table 1.[15] This table also shows the values of α and the association degree of counterions by each complex, $(1-\alpha)$, calculated by means of Eq. 3 using these parameters. The value of α for the SDS micelle obtained by this treatment is in good agreement with the literature values (0.22—0.29).[11,14] This would suggest the rationality of the above calculation.

The association degrees $(1-\alpha)$ of the counterions by the complexes are quite small compared with that of the SDS micelle. As may be seen in Table 1, the value of $(1-\alpha)$ increases with the complex formation. The association degree is considered to give a measure of the interactions between SDS and BSA. The increase of surfactants bound to the protein needs low electrostatic field on the surface of the complex and must lead to an increase in $(1-\alpha)$. In spite of the increase in the association degree of the counterions, the polyelectrolytic nature of the complex is expected to be strengthened because of the acquisition of a high net charge introduced by the bound dodecyl sulfate ions. It should be noted that a similarity of the complex to the micelle also appears in the behavior of the counterions, although other such similar properties between them have previously been discussed.[5,6,16]

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