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Counter-ion Association to a Protein–Surfactant Complex

SUNE BACKLUND and KENNETH RUNDT

Department of Physical Chemistry, Åbo Akademi, Porthansgatan 3–5, SF-20500 Åbo 50, Finland

Sodium ion binding to bovine serum albumin –sodium dodecyl sulfate complexes formed in a 0.0331 mol kg^{-1} NaCl solution at 298.2 K has been determined through electromotive force measurements. The degree of counter-ion association to the complex was found to be constant and equal to 0.4 over a large binding ratio interval. It was also concluded that a very large amount of surfactant anions can bind irreversibly to protein.

All proteins contain both hydrophobic and hydrophilic amino acids and the ratio between these will determine many of their physico-chemical and biological properties. Water soluble proteins may contain up to 50% amino acids with hydrophilic groups and in a water solution these groups tend to face the water while hydrophobic groups will tend to bury themselves in the interior of the folded protein. But because of the restricted flexibility of the protein molecule some hydrophilic parts will still be in a predominantly hydrophobic environment, while some hydrophobic groups will be in contact with the water. These sites may perform as binding sites for amphiphilic molecules such as surfactants. The hydrophilic side chains may attract the ionized groups of the amphiphile while hydrophobic groups may interact with the hydrocarbon chain of the amphiphile. Interaction between *e.g.* serum proteins and free fatty acids, lipids, hormones, drugs *etc.* in blood plays a significiant role in biological processes. This is one of the reasons why studies on the binding of surfactants to serum albumins can be of much value. Furthermore, the binding isotherms may give information on the structure of the proteins and on the relationship between different proteins.

One protein–surfactant system that has been extensively investigated is the bovine serum albumin (BSA)–sodium dodecyl sulfate (NaDS) system.[1–12] Sodium dodecyl sulfate is a strong disorganizer of the native structure,[2,3,4,10] but, in spite of this, low concentrations of the surfactant stabilize the protein.[4,13] The surfactant–protein complex can solubilize various molecules not soluble in water[3,9,11,15] and resembles in this way surfactant micelles. Oakes[7] has shown that at higher binding ratios the surfactant molecules are situated in a micellar-like environment, *i.e.*, they probably form a monomolecular layer with their polar head groups facing the water phase and the hydrocarbon chains adsorbed on hydrophobic parts of the protein. It is a well known fact that micelles bind a fraction of counter-ions,[16] thereby decreasing the counter-ion activity in the solution, but to date no information has been available on the binding of counter-ions to the protein–surfactant complex. This counter-ion association can be studied by using an ion selective electrode. Birch *et al.*[6] used a surfactant selective electrode to determine the binding of surfactant anion to BSA in a phosphate buffer containing approximately 0.03 mol dm^{-3} sodium ions. This excess sodium ion content prevented them from determining the counter-ion binding to the complex. We have done this measurement and report here on the results.

EXPERIMENTAL

Chemicals. Bovine serum albumin (BSA) was supplied by Nutritional Biochemicals. The molar mass of the protein was assumed to be 69 000 g mol^{-1}. Sodium dodecyl sulfate (NaDS) was obtained from BDH (Specially Pure) and sodium chloride was obtained from Merck AG (*p.a.* quality). Distilled and deionized water was used.

Apparatus. The sodium ion activity was measured with a sodium ion selective electrode, Type GEA 33, from EIL. The reference electrode was an Ag,AgCl

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electrode (Metrohm, Type EA 436) separated from the test solution by an Agar bridge containing 3 mol dm^{-3} NH_4NO_3. The emf was recorded with a Data Precision digital volt meter, Model 2520 A1, and automatically printed. The measurements required a precision of ± 0.01 mV in emf.

Method. The measurements were made as titrations at 298.2 K. A water solution of 0.0331 mol kg^{-1} NaCl was regarded as solvent. The cell was calibrated in NaCl solutions. Three principal titration series were made: (I) aliquots of a concentrated NaDS solution (in solvent) was added to the measuring cell, which contained solvent. (II) a concentrated NaDS solution and a BSA solution (0.4 weight-% in solvent) was added to the measuring cell, which now contained 0.1 weight-% BSA in solvent. By adding the 0.4 weight-% BSA solution, the protein content was kept constant and equal to 0.1 weight-%. (III) only one solution, containing both a high concentration of NaDS and 0.1 weight-% BSA, was added to the measuring cell, containing 0.1 weight-% BSA in solvent.

RESULTS AND DISCUSSION

In order to study the influence of dissolved protein on the potential of the cell, we measured the emf in a solvent solution as a function of protein content, but found that the protein did not have any effect on the Na^+ ion activity nor on the standard potential and the liquid junction potential.

Fig. 1 gives the sodium ion activities, calculated by using Nernst's equation, as a function of the total molality of sodium ions, for the systems NaCl – NaDS (I) and NaCl – NaDS – BSA (II and III). From the break in curve I a critical micellization concentration, m_c, equal to 2.7×10^{-3} mol kg^{-1} in 0.0331 mol kg^{-1} NaCl, can be determined. Curve II shows what happens when the solution contains 0.1 weight-% BSA. This curve shows two breaks, one at $m_{NaDS} = 4.2 \times 10^{-3}$ mol kg^{-1} and one at $m_{NaDS} = 7 \times 10^{-3}$ mol kg^{-1}. Before the first break, the slope of the curve is smaller than the slope of curve I, indicating sodium ion association to the BSA-NaDS complex. The fact that there is no break at 2.7×10^{-3} mol kg^{-1} is a consequence of the binding of DS^- ions to the protein.

Curve III shows what happens if only *one* solution, containing both NaDS and BSA, is added to a solution of 0.1 weight-% BSA in solvent. This curve shows no break at all and the slope of the curve is only somewhat smaller than the slope of curve I. This indicates that a large fraction of DS^- ions bound to the BSA molecules in the concentrated solution that was added, could not dissociate from the complex upon dilution in the measuring solution. In spite of this irreversible bounding of DS^- ions to the protein most of the Na^+ ions could dissociate. Reynolds and Tanford [14] have shown that as many as 325 DS^- ions can be bound to one BSA molecule.

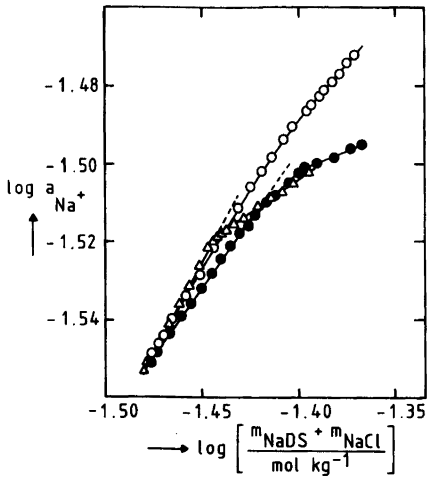

Fig. 1. The logarithm of the sodium ion activity as a function of the logarithm of the total sodium content. (△) a solution containing only NaCl and NaDS (curve I in text), (●) a solution containing also BSA (curve II), (○) a solution containing a fraction of surfactant irreversibly bound to denaturated BSA (curve III). The total protein content was 0.1 weight-%. All at 298.2 K.

By using the extended Debye-Hückel equation for the Na^+ ion activity coefficients in NaCl solutions, the amount of sodium ions bound to the protein – surfactant complex can be calculated. These results are shown in Fig. 2, where $m^P_{Na^+}$ denotes the molality of sodium ions associated to the BSA-surfactant complex. From Figs. 1 and 2 the following conclusion can be drawn: when NaDS is added to a BSA solution an equilibrium between bound and free DS^- and Na^+ ions will arise, but, if the DS^- ions have been irreversibly bound to some BSA, much less DS^- and hence, less Na^+, can associate with the initually unassociated (native) protein present in the test solution.

The degree of counter-ion association to the protein – surfactant complex can be estimated by using one of the binding isotherms published in

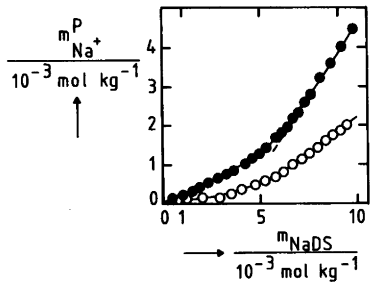

Fig. 2. The molality of sodium ions bound to protein as a function of total surfactant concentration. (●) curve II (see text for explanation), (○) curve III (see text for explanation).

literature, despite the fact that isotherms may differ from one protein batch to another.[6] Mostly binding isotherms have been determined using dialysis, but we will use the isotherm determined by Birch *et al.*[6] employing a surfactant selective electrode. This isotherm is only valid for a solution containing merely reversibly bound surfactant and only data from curve II may be used. The results are given in Fig. 3, where $m^P_{DS^-}$ denotes the molality of DS^- ions associated to protein. This curve shows that there is no association of sodium ions at low molalities, *i.e.*, before the binding ratio $\langle v \rangle$ (defined as the average number of surfactant anion molecules bound to one protein molecule) exceeds 14. The curve is linear up to $m^P_{DS^-} = 2.6 \times 10^{-3}$ mol kg^{-1} ($\langle v \rangle \simeq 175$). If we define the degree of counter-ion association β as the ratio of bound Na^+ to bound DS^-, the slope of the curve gives $\beta = 0.38$. At $m^P_{DS^-} = 2.6 \times 10^{-3}$ mol kg^{-1} the molality of bound counter-ions increases

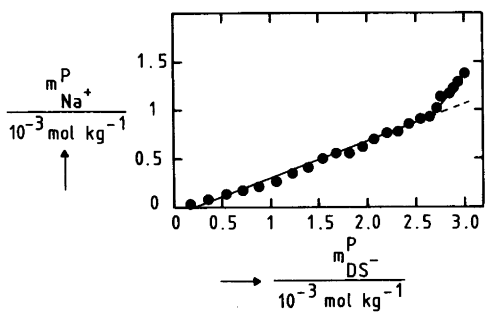

Fig. 3. The molality of sodium ions bound to protein as a function of dodecyl sulfate anions bound to protein, in a solution containing 0.0331 mol kg^{-1} NaCl and 0.1 weight-% BSA.

drastically. This is probably *not* yet a sign of micelle formation, as the concentration of free DS^- at this point is only about 1.4×10^{-3} mol kg^{-1} while m_c equals 2.7×10^{-3} mol kg^{-1} according to curve I. As a comparison, Steinhardt *et al.*[9] found indication of micelle formation at $m_{NaDS} \simeq 6.9 \times 10^{-3}$ mol kg^{-1}, and Birch *et al.*[6] at about 6.5×10^{-3} mol kg^{-1}. The break in Fig. 2 at 7×10^{-3} mol kg^{-1} must then be a consequence of micelle formation.

Several investigations[4,5,7] regarding the capability of BSA to bind NaDS, point out that there is only about ten available binding sites on the *native* protein, and that these sites are saturated at $m_{NaDS} \simeq 10^{-4}$ mol kg^{-1}. The dye solubilization power of the complex increases drastically at $\langle v \rangle \simeq 20-30$,[9] and from Fig. 3 one sees that at this point sodium ions bind to the complex.

Harrap and Schulman,[2] Reynolds *et al.*[4] and Blei,[3] found evidence for a distortion of the secondary and tertiary structure of the protein, at binding ratios higher than 40. The curve in Fig. 3 is linear up to $\langle v \rangle \simeq 175$, so no drastic changes in the surface charge density of the adsorbed layer of DS^- seem to occur before that. As a comparison, Oakes[7] found little change in the surfactant mobility for $10 < v < 180$. The fact that DS^- anions can be irreversibly bound must indicate that at some point an irreversible denaturation takes place, but because of the charged head groups, the protein will not precipitate.

CONCLUSION

Counter-ions bind reversibly to surfactant ions adsorbed on BSA molecules, indicating that the surfactant ions form a monomolecular layer with their charged groups facing the water phase. At high concentrations, the surfactant is irreversibly bound to protein, but the sodium ions are still reversibly associated to the complex.

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