The Binding of Sodium Dodecyl Sulphate to Bovine Serum Albumin at High Binding Ratios

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The extent of binding of sodium dodecyl sulphate to bovine serum albumin at high binding ratios was investigated by gel filtration. The weight ratio of bound sodium dodecyl sulphate to bovine serum albumin increases with the NaCl concentration, and, except at low salt concentrations, with the concentration of sodium dodecyl sulphate. In the presence of 1.0 g of sodium dodecyl sulphate/l, the binding ratio varied from 1.0 (at 0.04 M-Na⁺) to 2.2 (at 0.44 M-Na⁺). In the presence of 0.24 M-Na⁺, the binding ratio increased with sodium dodecyl sulphate concentration, from 0.9 (0.2 g of sodium dodecyl sulphate/l) to 2.0 (5 g of sodium dodecyl sulphate/l), at 26°C, in a dilute sodium phosphate buffer. No significant dependence of the binding ratio upon temperature in the range 26-45°C was observed. These results differ from those of Reynolds & Tanford (1970a) obtained by equilibrium dialysis.

The extent of binding of sodium dodecyl sulphate to proteins is an important parameter in the theoretical interpretation of the electrophoretic behaviour of protein-sodium dodecyl sulphate complexes (as in the determination of molecular weights by polyacrylamide-gel electrophoresis), of their behaviour during gel filtration, and of various physicochemical properties including sedimentation, light-scattering, diffusion and viscosity.

The binding of sodium dodecyl sulphate to proteins at relatively high concentrations of sodium dodecyl sulphate (>0.10 M) has been investigated by equilibrium dialysis (Pitt-Rivers & Impionbato, 1968; Reynolds & Tanford, 1970a), ultracentrifugation (Nelson, 1971), electrical transference and conductivity measurements (Strauss & Strauss, 1958) and solubilization of sodium dodecyl sulphate at low temperatures (Tung & Knight, 1972). The results obtained by the first two methods were in approximate agreement at low ionic strengths (approx. 0.1 M), where most proteins lacking disulphide bonds bound 1.4 g of sodium dodecyl sulphate/g at a sodium dodecyl sulphate concentration of 0.5 M, but differed markedly at high ionic strengths; the equilibrium dialysis method giving much lower values than the ultracentrifugation method.

Because of the discrepancy in these published results at high salt concentrations, the binding of sodium dodecyl sulphate to a particular protein, bovine serum albumin, has been investigated over a range of sodium dodecyl sulphate and NaCl concentrations and at different temperatures. This protein is typical of a range of denatured proteins in its interaction with sodium dodecyl sulphate (Reynolds &

Tanford, 1970a; Nelson, 1971), which has been studied in some detail (Steinhardt & Reynolds, 1969b; Reynolds *et al.*, 1967; Katz *et al.*, 1972).

Materials and Methods

Bovine serum albumin was a product of BDH Chemicals Ltd., Poole, Dorset, U.K. It gave one major band on sodium dodecyl sulphate-polyacrylamide-gel electrophoresis (10%, w/v, acrylamide), performed by the method of Weber & Osborn (1969), and only trace impurities. A single peak was observed during gel filtration on Sephadex G-100 in solutions of sodium dodecyl sulphate (1-70 mM). The presence of small amounts of fatty acids in the commercial protein (Steinhardt & Reynolds, 1969a) would not affect the results significantly, since only high binding ratios of sodium dodecyl sulphate to protein were investigated. Concentrations of bovine serum albumin were estimated spectrophotometrically, by using $E_{280}^{1\%}$ 6.67 (Reynolds *et al.*, 1967). The absorbance (280 nm) of a 0.1% (w/v) solution of bovine serum albumin was altered by less than 1% in the presence of sodium dodecyl sulphate.

[³⁵S]Sodium dodecyl sulphate (sp. activity 11.9 mCi/mmol, initially) was a product of The Radiochemical Centre, Amersham, Bucks., U.K. This material was diluted with sodium dodecyl sulphate (specially purified grade, BDH Chemicals Ltd.) to an initial specific activity of approx. 10 μ Ci/mmol, and the mixture was recrystallized from hot water and then from aq. 95% ethanol. More than 99% of the added excess of radioactivity was eluted from a Sephadex G-75 column equilibrated with a solution

of the [^{35}S]sodium dodecyl sulphate at a concentration greater than the critical micelle concentration in a single peak of material coincident with micellar sodium dodecyl sulphate, showing that the concentration of [^{35}S]sulphate was negligible. Solutions of sodium dodecyl sulphate were used within 3 days of their preparation, to minimize the effects of hydrolysis of sodium dodecyl sulphate. The concentration of sodium dodecyl sulphate was determined by liquid-scintillation counting in a Nuclear-Chicago Unilux II scintillation counter. Duplicate samples (50 μl) were transferred by means of a Hamilton microsyringe and mixed with 3.5 ml of scintillant [toluene; 2-methoxy-ethanol (3:1, v/v) containing 4.9 g of 2,5-bis-(5-*t*-butylbenzoxazol-2-yl)thiophen/l], and at least 10000 radioactive counts were accumulated for each sample. A small correction was applied for the background counts. Duplicate samples were reproducible to within 1%. It was found by a channels-ratio technique that quenching by protein in the samples was negligible.

A buffer solution was prepared, containing 40 mM- Na_2HPO_4 -2 mM-EDTA (disodium salt) with the pH adjusted to 7.0 with 12 M-HCl. Solutions for gel filtration were prepared, unless otherwise stated, from this buffer (50 ml), 1 M-NaCl (as required), 0.5 M-dithiothreitol (0.2 ml of a freshly prepared solution), water to 100 ml and [^{35}S]sodium dodecyl sulphate as required.

Columns (8 mm $\times$ 300 mm) of Sephadex G-75 and G-100 [Pharmacia (G.B.) Ltd., London W5, U.K.] were fitted with water jackets and thermostatically controlled, unless otherwise indicated, at $26 \pm 1^\circ\text{C}$. A three-way adaptor was used to allow convenient application of the samples without interruption of the flow through the column. Samples (3–6 mg) of bovine serum albumin were incubated with a slight excess of [^{35}S]sodium dodecyl sulphate (5–10 mg) in the buffer to be used (0.2 ml) with added dithiothreitol (0.01 ml of a 0.5 M solution) at 100°C for 2 min, cooled and applied to the column. Eluate fractions (approx. 1 ml) were collected at a flow-rate of 2–3 ml/h. The protein and sodium dodecyl sulphate concentrations in each fraction were determined and the weight ratios of bound sodium dodecyl sulphate to protein were calculated.

Some experiments were performed with less sodium dodecyl sulphate in the sample, and with incubation at 22°C for 1 h rather than at 100°C . Similar results were obtained.

The gel-filtration method was a modification of that of Hummel & Dreyer (1962). The concentration of sodium dodecyl sulphate was not, however, adjusted to be identical with that in the column buffer before application of the sample, and the bound sodium dodecyl sulphate was measured directly in the protein peak, rather than by measurement of the deficit in the ligand concentration following the

protein peak, as described by Hummel & Dreyer (1962).

Results

At low NaCl concentrations, the bovine serum albumin-sodium dodecyl sulphate complex and micellar sodium dodecyl sulphate were resolved by gel filtration on Sephadex G-75. However, at high salt concentrations, the effective molecular weight of sodium dodecyl sulphate micelles is greater (Elworthy *et al.*, 1968; Mysels & Princen, 1959) and the greater resolution of a column of Sephadex G-100 was required for the measurements at high salt concentration. It was assumed that the concentration of unbound sodium dodecyl sulphate in the fractions containing protein-sodium dodecyl sulphate complexes was identical with that in the buffer with which the column was equilibrated, as in the Hummel & Dreyer (1962) procedure.

The effect of variation in the concentration of NaCl in the presence of 1.0 g of free sodium dodecyl sulphate/l is shown in Fig. 1. The observations in 0.5 M-NaCl were made at 28°C since sodium dodecyl sulphate was insufficiently soluble at 26°C in this solution. Fig. 2 shows the effect of an increase in the free sodium dodecyl sulphate concentration at two constant NaCl concentrations. At low salt concentrations the results were not reproducible, and different fractions from the same run contained different ratios of bound sodium dodecyl sulphate to protein, suggesting that a partial resolution of different bovine serum albumin sodium dodecyl sulphate complexes may occur, or that effects of non-ideality under these conditions may be important. The formation of electrophoretically distinct bovine serum albumin-sodium dodecyl sulphate complexes at low NaCl concentration was reported by Katz *et al.* (1972).

Identical results obtained for particular sodium dodecyl sulphate and NaCl concentrations, except at these low salt concentrations, whether a large excess of sodium dodecyl sulphate or insufficient sodium dodecyl sulphate was included in the sample, indicated that binding equilibrium was attained during passage through the column, which took about 4 h.

There was no significant dependence of the extent of binding upon temperature from 26° to 45°C , in the 20 mM-sodium phosphate buffer, as above, containing 0.1 M-NaCl and 1.0 g of sodium dodecyl sulphate/l. In the presence of cations other than Na^+ , different weight ratios of bound sodium dodecyl sulphate to proteins were observed. For example, preliminary investigation of the binding of sodium dodecyl sulphate to denatured proteins (triose-phosphate isomerase and glyceraldehyde-3-phosphate dehydrogenase) in triethanolamine-HCl buffers showed that less detergent was bound than in

Fig. 1. Dependence upon NaCl concentration of the extent of binding of sodium dodecyl sulphate to bovine serum albumin at $26^{\circ}\pm 1^{\circ}\text{C}$

The column of Sephadex G-100 was equilibrated with 20mM- Na_2HPO_4 , adjusted to pH 7.0 with HCl, containing EDTA (1mM), dithiothreitol (1mM), sodium dodecyl sulphate (1.0g/l, 3.47mM) and NaCl (0–0.5M). ●, Samples incubated with a large excess of sodium dodecyl sulphate before gel filtration; ○, samples incubated without a large excess of sodium dodecyl sulphate; △, results obtained with Sephadex G-75; ▲, results obtained at $28^{\circ}\pm 1^{\circ}\text{C}$.

Fig. 2. The extent of binding of sodium dodecyl sulphate to bovine serum albumin as a function of sodium dodecyl sulphate concentration at $26^{\circ}\pm 1^{\circ}\text{C}$, measured by gel filtration on a column of Sephadex G-75 (superfine)

●, 20mM- Na_2HPO_4 buffer containing 0.2M-NaCl; ○, 20mM- Na_2HPO_4 buffer; △, results obtained from the rear of the bovine serum albumin–sodium dodecyl sulphate peak eluted from the column.

Na_2HPO_4 –HCl buffers containing NaCl of the same ionic strength.

Discussion

The results agree with the limited information obtained by ultracentrifugation (Nelson, 1971), but differ from the results determined by equilibrium dialysis (Reynolds & Tanford, 1970a). For example, the latter workers found that 0.36g of sodium dodecyl sulphate/g of bovine serum albumin was bound at an ionic strength 0.52M, whereas 2.4g of sodium dodecyl sulphate/g of bovine serum albumin was found in this work. The results are also consistent, when extrapolated to low ionic strength, with those of Strauss & Strauss (1958), who reported a value of 0.64g of sodium dodecyl sulphate/g of bovine serum albumin in 8mM-sodium dodecyl sulphate in the absence of buffer or other salts.

Apart from the practical difficulties (very long equilibration times) there are theoretical problems connected with the determination of the binding of associating ionic detergents to proteins by the equilibrium dialysis method. These arise from the

non-dialysable nature of detergent micelles (Abu-Hamdiyyah & Mysels, 1967), large Donnan equilibrium effects resulting from the presence of highly charged polyelectrolyte species of different concentrations on each side of the membrane, and non-ideality in even relatively dilute solutions of large polyelectrolytes (Hill, 1956). The Donnan effects are not easily eliminated by high salt concentrations since both the critical micelle concentrations [for a discussion of the concept of the critical micelle concentration, see Elworthy *et al.* (1968)] and the amount of detergent bound to proteins are functions of the NaCl concentration; reliable calculations of the Donnan correction cannot be made in the absence of a more developed theory of associating ionic detergents than is at present available (cf. Tanford, 1972).

For the interpretation of the equilibrium-dialysis results it was assumed that the activity coefficients of monomeric and micellar sodium dodecyl sulphate are not altered by the presence of protein–sodium dodecyl sulphate complexes. A small effect upon the activity coefficient of sodium dodecyl sulphate monomer could lead to large differences in the concentration

of unbound (monomeric and micellar) sodium dodecyl sulphate at equilibrium on the two sides of the membrane, since large changes in the total concentration of sodium dodecyl sulphate above the critical micelle concentration are reflected in only slight changes in the activity of the dialysable species, monomeric sodium dodecyl sulphate. If the activity coefficient of sodium dodecyl sulphate is affected by the presence of protein-sodium dodecyl sulphate complexes, some effect is to be expected upon the distribution of sodium dodecyl sulphate inside and outside the Sephadex beads, which in some ways resemble semi-permeable membranes, in the experiments described here. Indeed, a decrease in the sodium dodecyl sulphate concentration occasionally observed immediately after the elution of the bovine serum albumin-sodium dodecyl sulphate complex, even in the presence of excess of sodium dodecyl sulphate in the sample, may be due to such effects of non-ideality. Similarly anomalous elution profiles have been reported during the measurement of the binding of deoxycholate to bovine serum albumin by gel filtration (Makino *et al.*, 1973).

The effect of NaCl upon solutions of sodium dodecyl sulphate may be considered as twofold: (a) the common-ion effect, since dodecyl sulphate micelles are associated with large numbers of counterions (Elworthy *et al.*, 1968); and (b) a salting-out effect upon the hydrophobic chains of the monomers. These effects lead to lower critical micelle concentrations and larger micellar weights at higher NaCl concentrations. The structure of sodium dodecyl sulphate-protein complexes is not known in detail, although hydrodynamic data are consistent with the presence of rod-like particles of protein, which possess significant helical content, and around which the detergent is ordered in a micelle-like structure (Reynolds & Tanford, 1970b). A large number of counterions are bound to the complex. Conductance measurements showed that up to 60% of the Na⁺ ions were bound to a complex of bovine serum albumin with sodium dodecyl sulphate in the absence of other salts (Strauss & Strauss, 1958). Thus the effect of increasing salt concentration on the amount of sodium dodecyl sulphate bound is expected to be similar to the effect upon the micellar weight of sodium dodecyl sulphate. The results in Fig. 1 are consistent with such a model for sodium dodecyl sulphate-protein complexes, and inconsistent with a model in which there are a fixed number of binding sites, corresponding to 1.4g of sodium dodecyl sulphate/g of protein. The amount of sodium dodecyl sulphate bound to bovine serum albumin also increases with sodium dodecyl sulphate concentration in 0.2M-NaCl solution (Fig. 2), as is to be expected, since the activity of monomeric sodium dodecyl

sulphate increases with concentration even above the critical micelle concentration (Abu-Hamdiyyah & Mysels, 1967). There is no simple explanation for the decrease in the amount of sodium dodecyl sulphate bound to bovine serum albumin with increasing sodium dodecyl sulphate concentration in a solution of low NaCl concentration (Fig. 2).

Since the weight ratio of bound sodium dodecyl sulphate to a single denatured protein can vary widely with variation in sodium dodecyl sulphate concentration and in the electrolyte composition and concentration, care should be taken in the interpretation of physico-chemical parameters such as sedimentation, diffusion, viscosity and electrophoretic mobility to ensure that a reliable value for the amount of sodium dodecyl sulphate bound to the protein is used.

A preliminary report on this work has been presented (Allen, 1973).

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