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SODIUM DODECYL SULPHATE–PROTEIN COMPLEXES

CHANGES IN SIZE OR SHAPE BELOW THE CRITICAL MICELLE CONCENTRATION, AS MONITORED BY HIGH-PERFORMANCE AGAROSE GEL CHROMATOGRAPHY

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SUMMARY

We have determined the sodium dodecyl sulphate (SDS) concentration needed to complete the formation of SDS–protein complexes. A Superose-6 column was equilibrated with SDS for 7 h. A sample of a native protein or an SDS–protein complex was applied, and the elution volume, V_e, was determined. Then the SDS concentration, C_{SDS}, was changed, etc., *i.e.*, V_e was determined as a function of C_{SDS}. The critical micelle concentration of SDS (cmc_{SDS}) was 1.8 m*M* in the eluent (ionic strength 0.10 *M*). Native bovine carbonic anhydrase (BCA) formed an SDS complex above 0.2 m*M* SDS. As C_{SDS} was increased, V_e decreased gradually in two main transitions, (TI) at 0.2–1.0 m*M* and (TII) at 1.2–2.0 m*M* SDS. These concentrations are corrected for a lag in the column equilibration with SDS. SDS–BCA, pre-equilibrated at 1.6 m*M* SDS, showed transitions similar to those observed with native BCA, except that transition TII included a minor transition at 2.0–2.2 m*M* SDS. The SDS complexes of reduced and carboxamidomethylated bovine serum albumin, of N-5′-phosphoribosylanthranilate isomerase–indole-3-glycerol-phosphate synthase from *Escherichia coli* (PRAI–IGPS) and of two tryptic fragments of this enzyme behaved similarly. For SDS–PRAI–IGPS the major part of transition TII was completed at 1.6–1.7 m*M* SDS, as shown by analyses after 20-h column equilibrations with increasing as well as decreasing C_{SDS}. The SDS complex of an integral membrane protein, the glucose transporter from human red cells, was smaller or less elongated than the SDS complexes of water-soluble proteins of the same polypeptide length. The formation of all five SDS–protein complexes investigated was practically completed at cmc_{SDS}.

INTRODUCTION

One of the most powerful dissociating and denaturing detergents is sodium *n*-dodecyl sulphate (SDS). In pure water, SDS forms micelles at concentrations above 8 m*M* (refs. 1–3). These are of a spherical, fluctuating shape with an hydrophobic core

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of an average radius of 1.84 nm and they contain 74 SDS molecules each[4]. SDS is widely used in separations of proteins by gel electrophoresis[5–12] and gel chromatography on dextran gel beads[13], large-bead agarose and acrylamide gels[14], controlled-pore glass particles[15,16], silica gel particles[17–22], small- and medium-bead cross-linked agarose gels[23] and a small-bead cross-linked agarose gel[24]. The use of small agarose gel beads enhances resolution and reduces equilibration times, which was important in the present work.

The binding of SDS to proteins has been extensively studied (*cf.*, *e.g.*, refs. 12 and 25–30). Saturation is reported to occur below the critical micelle concentration (cmc) of SDS[12]. Some models for the structure of complexes between proteins and SDS have been proposed: (1) a rod-like particle model[27]; (2) the necklace model, where clusters of SDS are scattered along the free-draining polypeptide chain[30]; (3) a model in which α-helices are present, although parts of the polypeptide form a random coil[31] and (4) the flexible-helix model, in which the polypeptide chain is helically coiled around an alongated SDS micelle, attached by hydrogen bonds between sulphate group oxygens and peptide-bond nitrogen[32]. As early as in 1968–1969 some authors suggested that micellar structures form part of SDS–protein complexes[8,25].

We have studied the changes in the elution volume, V_e, of SDS–protein complexes upon binding or release of SDS by high-performance gel chromatography on a Superose-6 gel. A decrease in V_e with an increase in SDS concentration indicates an increase in size or an elongation of the complexes.

We have used five water-soluble proteins or protein fragments and one integral membrane protein to determine whether a final size and shape of the SDS–protein complex is reached at the cmc of the detergent, and we propose a model for the mechanism of complex formation.

MATERIALS AND METHODS

Materials

SDS was obtained from Merck (Darmstadt, F.R.G.; No. 13760, 'für biochemische Zwecke und Tensiduntersuchungen'). Carbonic anhydrase (No. C-7500) from bovine erythrocytes (BCA), bovine serum albumin (No. A-7030) (BSA), octyl glucoside (*n*-octyl-β-D-glucopyranoside) and dithioerythritol were obtained from Sigma (St. Louis, MO, U.S.A.). SDS complexes of N-5′-phosphoribosylanthranilate isomerase–indole-3-glycerol-phosphate synthase (PRAI–IGPS)[33,34] and of two tryptic fragments of this protein[33] were kindly provided by K. Kirschner and H. Szadkowski, Biozentrum, Basel, Switzerland (*cf.*, Discussion). Unless stated otherwise chemicals were of analytical grade. Human red cell concentrate (4–5 weeks old) was supplied by the Blood Bank of the University Hospital, Uppsala, Sweden. All solutions were passed through 0.2-μm filters (Sartorius, Göttingen, F.R.G.; SM 11107) and simultaneously degassed.

Methods

High-performance gel chromatography. High-performance gel chromatography experiments were performed with a prepacked 22-ml (28 cm × 1.0 cm) column of Superose-6 (V_o = 8.2 ml, V_t = 22.2 ml) for medium-pressure (0.5 MPa) chromatography at a flow-rate of 0.30 ml/min. This column was connected to two precision

pumps (P-500), a mixer, a sample injection valve (V-7) and an UV monitor set at 280 nm (UV-1). This system was controlled from a gradient programmer (GP-250) and an automatic injector (ACT-100). All these components were provided by Pharmacia LKB Biotechnology (Uppsala, Sweden). The same column was used for several hundreds of analyses, including those in refs. 24, 35 and 36. The samples were applied to the Superose-6 column from a 25- (Figs. 1–3, 6) or a 50-μl loop (Fig. 4). Unless otherwise stated, the column was equilibrated with SDS for 6.8 h with 122 ml of the eluent before sample application. The SDS concentration was changed in 0.2-m*M* increments at intervals of 8 h. The elution or retention volume, V_e, was determined by measuring the distance between the application mark and the peak maximum on the chart paper (speed, 2 mm/min) with a precision of ± 0.2 mm, *i.e.*, ± 0.2% or ± 30 μl at V_e = 15 ml. It was corrected for the sample volume. All column chromatography experiments were performed at 22.0–24.0°C.

Eluent. The eluent for gel chromatography was 50 m*M* sodium phosphate, pH 6.86, 1 m*M* Na_2EDTA, 0.2 m*M* dithioerythritol and 3.1 m*M* NaN_3 (solution W) with addition of SDS. Solution W was prepared by mixing equal volumes of 0.2 *M* Na_2HPO_4 and 0.2 *M* NaH_2PO_4 and then adding the other components and water, resulting in pH 6.86. In solution W (ionic strength, 0.10 *M*) the cmc of SDS is approximately 1.85 m*M* at 25°C (ref. 37), as determined by the drop number method, which corresponds to 1.80 m*M* at 23°C according to the temperature dependence graph, reported by Becker *et al.*[28]. There is a strong consensus for a cmc value of 1.76–1.80 m*M* at 22°C (dye solubilization measurements[39] and our unpublished light-scattering and electric conductivity data). The critical micelle temperature can be estimated as 20°C at the ionic strength of 0.10 *M* (*cf.*, ref. 40).

The chosen SDS concentrations in the eluent were obtained by automatic mixing of (A) solution W with appropriate fractions of (B) solution W containing 10.0 ± 0.4 m*M* SDS. The SDS concentration in (B) was checked by automated sulphur analysis[41,42] at the Department of Chemistry, Uppsala University, Uppsala. Briefly, the samples were freeze-dried and subjected to Schöniger flask combustion; the sulphite and the sulphur dioxide were converted into sulphate; the sulphate was reduced to sulphide by hydriodic and hypophosphorous acid in acetic acid solution, and the sulphide was treated with *p*-aminodimethylaniline and ferric iron to form methylene blue, which was determined photometrically.

Samples

Native carbonic anhydrase. BCA was dissolved in solution W to a concentration of 1 mg/ml and passed through a 0.2-μm Acrodisc-13 filter (Gelman Sciences, Ann Arbor, MI, U.S.A.). The BCA sample contained minor impurities of M_r 14 000–24 000, as judged by SDS-gel electrophoresis (not shown). BCA (M_r 29 000) contains Zn^{2+} but no disulphide bridge.

SDS–BCA. BCA (20 mg) was dissolved, together with 41.2 mg SDS, in 2 ml of solution W and was equilibrated with 1.6 m*M* SDS in solution W (W1.6) by passage through a 59 cm × 2.6 cm column of Sephacryl S-300 HR (Pharmacia LKB Biotechnology). This column had been equilibrated for 20 h with six column volumes of W1.6. The final BCA concentration was 1 mg/ml.

SDS–BSA. A 30-mg amount of BSA was reduced with 20 m*M* dithioerythritol for 5 min at 95°C, together with 65.8 mg SDS, in 2 ml of solution W. The protein was

then carboxamidomethylated and equilibrated with 1.6 m*M* SDS on a Sephacryl S-300 HR column as above. The product is denoted as SDS–RCAM-BSA. Native BSA (M_r 66 200) contains seventeen disulphides.

SDS–PRAI–IGPS and two fragments of this enzyme. Deuterated PRAI–IGPS (M_r 49 500, ref. 33) as well as two tryptic fragments of this protein (M_r 32 000 and 17 500, ref. 33) were dissolved with 1.6 mg SDS per mg protein in W1.6 and equilibrated, first by dialysis and then by gel chromatography on a Sephacryl S-300 column with 1.6 m*M* SDS (No. 10807 3J, AnalaR from BDH Chemicals, Poole, U.K.), otherwise as above. These preparations were made by K. Kirschner and H. Szadkowski (Biozentrum, Basel) (*cf.*, Discussion). PRAI–IGPS consists of two domains with triose-phosphate isomerase (TIM)-barrel structures[34]. The enzyme contains no disulphide[34].

Glucose transporter. Integral membrane proteins from human red cells were prepared as described earlier[43] and were solubilized at an initial protein concentration of 8 mg/ml with 75 m*M* octyl glucoside[36]. The glucose transporter (GT), as a complex with octyl glucoside, was then purified by fractionation of the solubilized integral membrane proteins on DEAE-cellulose (No. DE-52 from Whatman Bio-Systems, Maidstone, U.K.), in the presence of 75 m*M* octyl glucoside. This results in a preparation that probably contains mainly native GT monomers[35,36]. The final protein concentration was *ca.* 0.8 mg/ml (ref. 36).

The sample was kept on ice during the whole series of gel chromatography experiments. As in all other experiments, the Superose-6 column was kept at 22.0–24.0°C.

The GT is an heterogeneously glycosylated integral membrane protein with a polypeptide M_r of about 54 100 (*cf.*, ref. 44). Possibly, it contains twelve membrane-spanning α-helices and six cysteine residues (*cf.*, refs. 44 and 45). Cys 429 is exofacial[46], Cys 207 is endofacial[46] and Cys 347 is probably exposed at a binding site for glucose[46,47]. Two of the three remaining cysteine residues (Nos. 133, 201 and 421) might form a disulphide, although, to our knowledge, there is no evidence for this.

RESULTS

Accuracy and precision. In all of the gel chromatography experiments except those illustrated in Fig. 4 (below), the equilibration time before sample application was 6.8 h. The equilibration was not complete; a series of analyses with increasing SDS concentrations gave larger elution volumes than did a series with decreasing SDS concentrations (Fig. 1). The graphs in Fig. 1 indicate a lag in SDS monomer concentration of about 0.22 m*M*. This value was used to correct the SDS concentration scales in Figs. 2, 3 and 6 (below) for increasing as well as decreasing concentrations. Fig. 4 illustrates similar experiments with a much longer equilibration time, 20.5 h. In this case, the lag in SDS monomer concentration was 0.06 m*M* (see below).

The reproducibility of V_e, in different series of experiments, was ± 50 μl, similar to the precision of ± 30 μl in individual V_e values (see Methods). V_e ± 50 μl corresponds to K_d ± 0.7% (at K_d = 0.5) or M_r ± 300 (*cf.*, Fig. 5, below).

Carbonic anhydrase. Native BCA (N) bound SDS only at SDS concentrations above 0.2–0.3 m*M* (Fig. 2, *cf.* ref. 48). The graph of V_e for BCA as a function of increasing SDS concentrations showed two distinct transitions (TI, from N to CI; TII, from CI to CII, in Fig. 2).

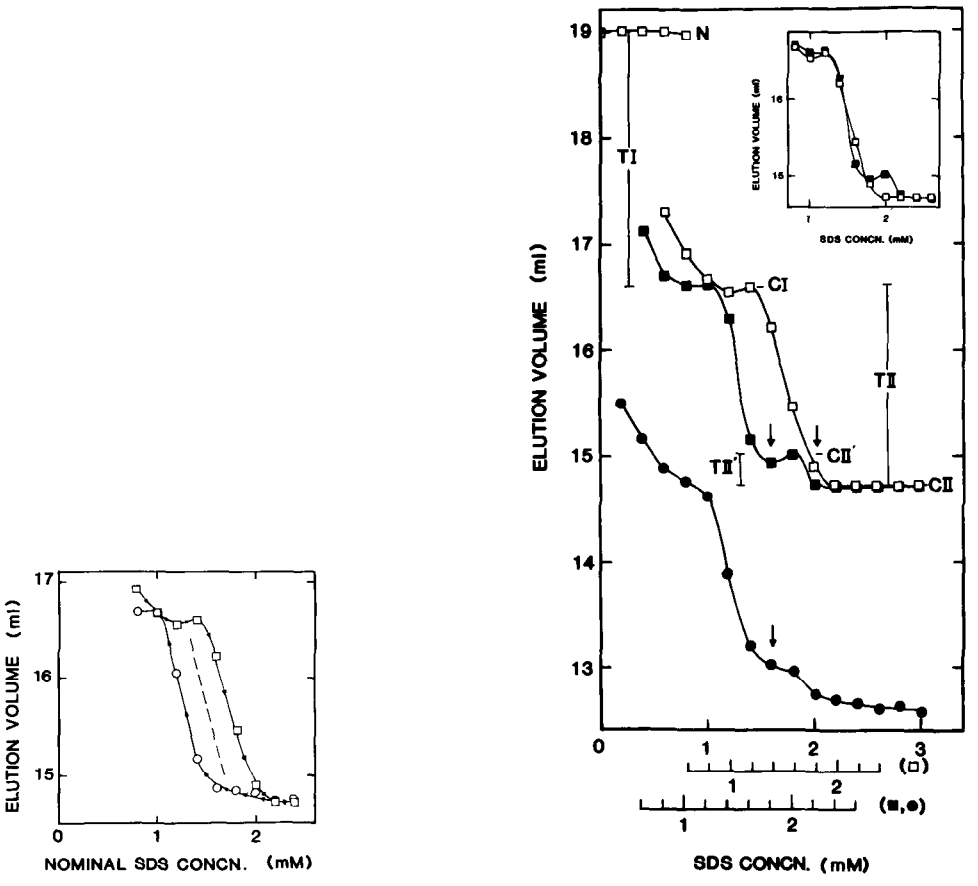


Fig. 1. Elution volumes for BCA upon high-performance gel chromatography on Superose-6 in the presence of SDS. Native enzyme was applied to the column (protein amount 12 μg). The SDS concentration in the eluent was either increased (□) or decreased (○) in steps of 0.2 m*M* SDS, at intervals of 8 h. The elution volumes observed at the same nominal SDS concentration differ, due to a lag in the column equilibration with SDS. The hatched line indicates the expected position of the graph at complete column equilibration. The displacement is ± 0.22 m*M* SDS. This value was used to correct the nominal SDS concentrations in later experiments (Figs. 2, 3 and 6).

Fig. 2. Elution volumes for native BCA (□) and SDS-denatured BCA (■) and reduced and carboxamidomethylated bovine serum albumin (RCAM-BSA) (●) upon high-performance gel chromatography on Superose-6 as a function of the SDS concentration in the column. SDS–BCA and SDS–RCAM-BSA were analyzed together. The SDS concentration was increased (□) or decreased (■, ●) as in Fig. 1. The *corresponding corrected SDS concentration scales (cf., Fig. 1) are given below the nominal scale and the* cmc for SDS is indicated (arrows). The denatured proteins were equilibrated with 1.6 m*M* SDS before application to the Superose-6 column. Sample amount: 12 μg of each protein. Elution volumes for native BCA (N) as well as for the SDS complexes of BCA (CI, CII' and CII) are indicated as are the corresponding transitions (TI, TII' and TII). Insert: the graphs for native BCA (□) and SDS–BCA (■) plotted *vs.* the corrected SDS concentrations (see above).

Two main types of SDS–BCA complexes thus exist, one (CI) at 1.0–1.2 m*M* detergent (Fig. 2, corrected scale) and another (CII) above 2 m*M* SDS. At SDS concentrations from 1.2 to 2.0 m*M* the small-size complex, CI, was gradually converted into the large-size complex, CII (Fig. 2).

When a SDS–BCA complex (in equilibrium with 1.6 m*M* SDS, see Methods) was chromatographed on the Superos-6 column (with decreasing SDS concentrations) the V_e was constant between 2.8 and 2.2 m*M* detergent (Fig. 2, corrected scale). A minor transition, TII′ (from complex CII to complex CII′) was observed between 2.2 and 2.0 m*M* SDS. Below 1.8 m*M* SDS, the V_e increased with decreasing concentrations of SDS to 1.2 m*M* SDS and also below 0.9 m*M* (Fig. 2). The BCA and SDS–BCA graphs for increasing and decreasing SDS concentrations partly coincided when the V_e values were plotted against the corrected SDS concentrations (Fig. 2, insert; *cf.*, Fig. 1). The uptake of SDS by proteins or release of SDS from SDS–protein complexes thus occurred practically instantaneously on the column for the small protein amounts that were applied. At low SDS concentrations the SDS–BCA was not eluted. Probably, the polypeptides became entangled in the agarose gel matrix during refolding upon removal of the detergent from the complex. When the SDS concentration was increased, the protein was released and eluted (not shown).

Bovine serum albumin. The SDS–RCAM-BSA complex showed a two- or three-step increase in V_e with decreasing SDS concentrations (Fig. 2), as did the SDS–BCA complex. The 'plateau' regions, corresponding to complexes CI, CII and CII′, were not as distinct as for BCA. The V_e of the SDS–RCAM-BSA complex increased somewhat from 3 m*M* SDS to the cmc (*cf.*, ref. 12). The steepest increase was between 1.6 and 1.2 m*M* SDS, similar to the case of SDS–BCA (transition TII, Fig. 2, corrected scale).

The transition TII obviously corresponds to the steep change in binding of the detergent to the RCAM-BSA between 1.2 and 1.5 m*M* SDS as well as to the dramatic decrease in distribution coefficient on Sepharose 6B between 1.1 and 1.7 m*M* SDS, reported by Takagi *et al.*[12].

At low SDS concentrations the SDS–BSA was not eluted. This was also the case for SDS–BCA (see above).

N-5′-Phosphoribosylanthranilate isomerase–indole-3-glycerol-phosphate synthase. The SDS–PRAI–IGPS complex (equilibrated with 1.6 m*M* SDS) showed a considerable decrease in V_e on the Superose-6 column from about 0.4 to 1.4 m*M* detergent, whereas the decrease in V_e above 1.6 m*M* SDS was small (Fig. 3, corrected scale). The graph for the SDS complex of the large tryptic fragment (L) of PRAI–IGPS was similar, with a 'plateau' at about 0.8 m*M* SDS. The small tryptic fragment (S) showed a more gradual decrease in V_e for its SDS complex (Fig. 3). At 0.9 m*M* SDS the SDS complexes of L and S were eluted at the same V_e but had probably reached different stages of SDS binding. SDS-polyacrylamide gel electrophoresis (not shown) confirmed that the graphs do not cross each other at this point. At a corrected SDS concentration of 0.3 m*M*, the resolution of PRAI–IGPS and the fragments was lost, maybe due to re-association of the fragments, although this does not occur when the SDS is completely removed[33]. At other SDS concentrations, the components were well separated (*cf.*, Fig. 3, insert).

In a final series of experiments with PRAI–IGPS, the SDS concentration was increased in 0.2-m*M* steps, as earlier, and the column was equilibrated for 20.5 h with

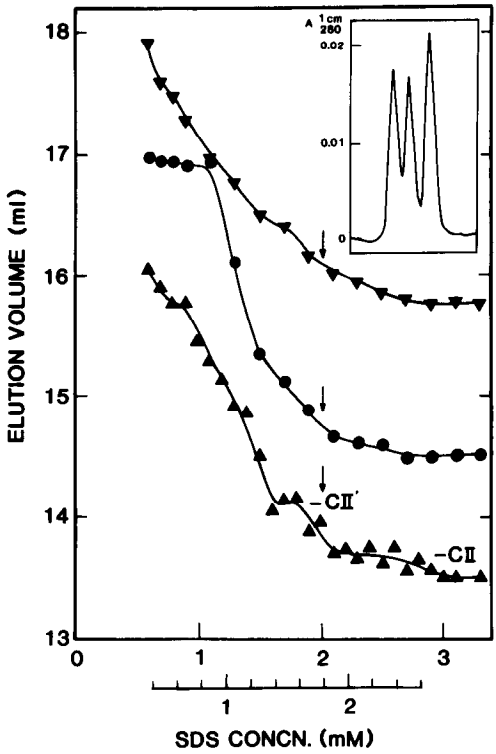
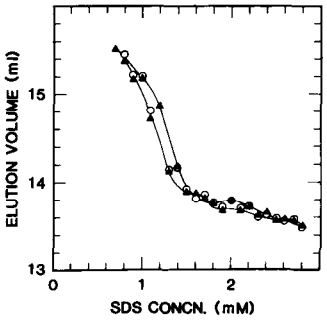


Fig. 3. Elution volumes for SDS complexes of PRAI–IGPS and of two tryptic fragments of this enzyme upon gel chromatography as in Fig. 2. (▲) PRAI–IGPS; (●) fragment of M_r 32 000 and (▼) fragment of M_r 17 500. The protein and the fragments were equilibrated with 1.6 m*M* SDS before application to the Superose-6 column. The SDS concentration was increased stepwise, and all three components were applied (8 μg of each) in a single sample at 0.7, 0.9... m*M* SDS. SDS–PRAI–IGPS alone was also applied (12 μg) in a separate series of analyses at 0.6, 0.8... m*M* SDS. A corrected SDS concentration scale is given as in Fig. 2. The elution volumes corresponding to the SDS complexes CII' and CII (*cf.* Fig. 2) for PRAI–IGPS are indicated. The cmc for SDS is indicated (arrows) using the corrected SDS concentration scale. Insert: gel chromatogram, illustrating the separation of the components at the nominal SDS concentration of 2.9 m*M*.

369 ml eluent before chromatography (Fig. 4). Also, the eluted material (SDS–PRAI–IGPS) was collected and rechromatographed, 2.5 h after the first sample application, at the same SDS concentration as used for the first chromatogram. The experiments were repeated with decreasing SDS concentrations. This time-consuming procedure resulted in an accurate determination of V_e *vs.* SDS concentration for SDS–PRAI–IGPS (Fig. 4). The lag in column equilibration with monomeric SDS was 0.06 m*M*. The equilibration with micellar SDS was slower. The results show that the SDS–PRAI–IGPS complex has nearly reached a final conformation at 1.6–1.7 m*M* SDS in our eluent. There is a minor transition at 1.7–2.4 m*M* SDS (or 1.9–2.2 m*M* SDS, as corrected for the micellar equilibration lag in Fig. 4).

A gradual slope of V_e *vs.* SDS concentration is also observed above the cmc (*cf.*, ref. 24). This might either reflect additional uptake of SDS or an interference of the



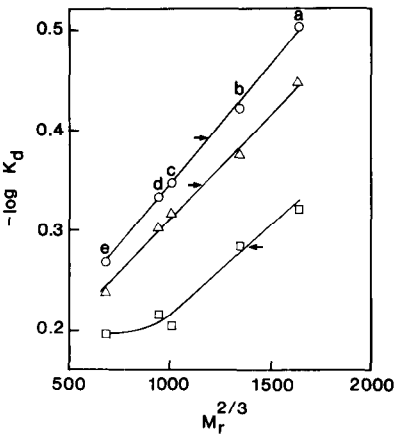
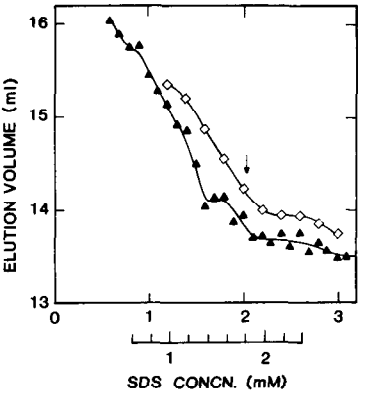


Fig. 4. Elution volumes for SDS–PRAI–IGPS (*cf.*, Fig. 3) on Superose-6 as a function of the SDS concentration. The column was equilibrated at each chosen SDS concentration for 20.5 h before sample application. The SDS concentration was changed in 0.2-m*M* steps at intervals of 24 h. (▲) SDS–PRAI–IGPS (50 μg) applied at an equilibration concentration of 1.6 m*M* SDS and collected. (○) Rechromatography of an aliquot of the collected SDS–PRAI–IGPS (2 μg). The analyses were carried out at increasing (0.8, 1.0... m*M*) as well as decreasing (2.7, 2.5... m*M*) SDS concentrations.

Fig. 5. Calibration diagram for SDS complexes of water-soluble proteins: $-\log K_d = f(M_r^{2/3})$ (see refs. 23 and 49), where $K_d = (V_e - 8.2)/14$, V_e in ml. The V_e values are taken from Figs. 2, 3 and 6. Corrected SDS concentrations: (○) 2.8; (△) 1.6; (□) 0.8 m*M*. The proteins were: (a) SDS–RCAM–BSA (M_r 66 200); (b) SDS–PRAI–IGPS (49 500); (c) SDS–PRAI–IGPS large fragment (32 000); (d) SDS–BCA (29 000); (e) SDS–PRAI–IGPS small fragment (17 500). The arrows denote the $-\log K_d$ values for SDS–GT.



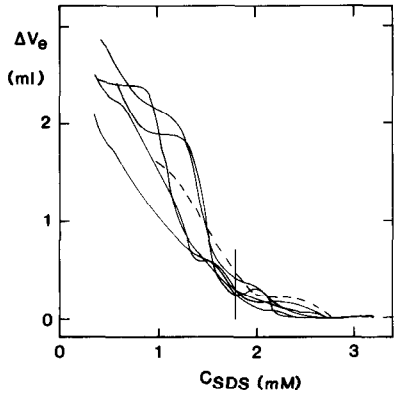


Fig. 6. Elution volumes for one amphiphilic and one water-soluble protein upon high-performance gel chromatography on Superose-6 as a function of the SDS concentration in the eluent. (◇) GT from human red cells, applied as an octyl glucoside complex (protein amount, 20 μg), and (▲) SDS–PRAI–IGPS (data from Fig. 3). The arrow denotes the cmc of SDS. Corrected SDS concentration scale as in Fig. 2.

Fig. 7. Comparison of the graphs for the SDS–protein complexes shown in Figs. 2, 3 and 6. The formation of the complexes was considered to be completed at the corrected SDS concentration, C_{SDS}, of 2.8 m*M* ($\Delta V_e = 0$). Hatched line: the glucose transporter. The vertical line indicates the cmc for SDS in the 0.10 *M* ionic strength eluent.

SDS micelles with the gel chromatography process. The equilibration of the column with SDS micelles is slow, due to the minimal micelle concentration gradients through the gel beads and the large micelle size.

The calibration diagrams, constructed from the V_e values for the above proteins at 2.8, 1.6 and 0.8 m*M* SDS, are shown in Fig. 5. The saturated complexes at 2.8 m*M* SDS as well as the nearly saturated ones at 1.6 m*M* SDS gave linear calibration graphs of $-\log K_d$ *vs.* $M_r^{2/3}$ (*cf.*, refs. 23 and 49), whereas the corresponding graph for non-saturated complexes (at 0.8 m*M* SDS) is non-linear. The calibration line for the complexes at 2.8 m*M* SDS shows the best fit to the points.

Glucose transporter. Purified human red cell glucose transporter (GT) in a complex with octyl glucoside was applied to the Superose-6 column, which had been equilibrated with given concentrations of SDS, as above. The V_e of the SDS–GT complex decreased steeply with the increase in SDS concentration between 1.0 and 2.0 m*M* SDS (Fig. 6, corrected scale). Above 2.4 m*M* SDS, a further small decrease was observed.

The amphiphilic GT shows the low apparent M_r of 40 000 (Fig. 5, 2.8 m*M* SDS) or, at high ionic strength, 37 000 (ref. 24). Its true polypeptide M_r is about 54 000 (*cf.*, ref. 43). The GT molecules also contain an oligosaccharide of heterogeneous size, representing up to 17% of the total weight[50]. The size of the SDS–GT complex is smaller (the V_e is larger) than that of the water-soluble enzyme PRAI–IGPS (M_r 49 500, ref. 33) over the range 1.0–2.8 m*M* SDS (Fig. 6).

DISCUSSION

The transition TII, in the formation of SDS–protein complexes, excluding transition TII', became complete at or somewhat below cmc = 1.8 m*M* SDS for BCA, BSA and PRAI–IGPS. For the tryptic fragments of PRAI–IGPS and for GT the 'end-points' of the transitions were less distinct, but the increase in V_e was small above the cmc (see Figs. 2, 3 and 6).

For comparison of the proteins, the graphs from Figs. 2, 3 and 6 are shown together in Fig. 7. All of the proteins or fragments show a steep decrease in V_e with increasing SDS concentration up to the cmc, and a further small and gradual decrease above the cmc (Fig. 7). We have shown earlier that further, minor transitions may occur at much higher SDS concentrations[24], possibly as a result of slightly increased monomer concentrations. In addition, SDS micelles may affect the gel chromatography process. The final SDS–protein size was approached rapidly far below the cmc, during transition TII (Fig. 7; *cf.*, Fig. 2). At this stage, the SDS binding is probably a cooperative process (*cf.*, ref. 51), and interactions other than the hydrophobic effect may contribute to the stability of the complex. Hydrogen bonding between the peptide-bond nitrogens and oxygens of the sulphate group of the SDS molecules is a candidate for a stability-enhancing interaction below cmc[32].

A complex between a water-soluble protein and SDS is formed upon binding of SDS monomers to the protein. We propose that this process takes place as described below.

(1) The dodecyl chains of the detergent penetrate the surface of the protein and come into contact with the hydrophobic interior of the protein or the protein domains. (2) Polypeptide segments from the interior of the protein or protein domain become

displaced toward the surface of the complex, since they are less hydrophobic than the dodecyl chains. Many SDS molecules become locked in an inserted position by ion-pair formation and, mainly, by hydrogen bonding (*cf.*, ref. 32). (3) The increased hydrophobicity enables more SDS molecules to become included in the complex until a spherical SDS micelle is completed, around which the polypeptide is wound. (4) Any length of the polypeptide that, for steric reasons, cannot be accommodated in direct contact with this micelle forms the core for growth of another protein-covered micelle. This process is repeated until the whole polypeptide is coiled around adjacent SDS micelles that are linked with short polypeptide segments.

The proposal that adjacent, protein-decorated, spherical micelles are formed, rather than a cylindrical structure[32], is based on preliminary small-angle neutron scattering data for deuterated PRAI–IGPS in a complex with SDS at an equilibrium concentration of 1.6 m*M* SDS[52]. A report by Mazer *et al.* indicates that very elongated SDS micells are formed at an ionic strength of 0.3–0.6 *M*, but not at 0.10 *M*, which we have used. The above preliminary neutron scattering results have been confirmed and refined[53].

The mechanism for the formation of SDS–protein complexes proposed above is consistent with our present gel chromatography data. Transition TI (Fig. 2) may correspond to the initial swelling and rearrangement of the protein (1 and 2, above), whereas transition TII may correspond to the final formation of one or more protein-covered micells (3 and 4, above).

The glycosylated GT (polypeptide M_r 54 100) was eluted at a relatively large volume. The transporter may contain a single disulphide bridge. However, the small size of the SDS–GT complex is probably ascribable to the presence of hydrophobic α-helices in the protein (*cf*, ref. 44). Several of these α-helices may well travers the SDS micelle(s) in the complex, consistent with the flexible helix model[32] and with the neutron scattering results (see above). Reduced and deglycosylated GT polypeptide migrates as a water-soluble protein of M_r 45 000 (ref. 54) or 46 000 (ref. 55) in SDS gel electrophoresis, and this also indicates a compact structure for the complex.

However, the SDS–GT complex is eluted at a smaller V_e (13.75 ml at 2.8 m*M* SDS, see Fig. 6) than is the octyl glucoside complex (16.8 ml at 50 m*M*, ref. 35) or 16.1 ml at 75 m*M*, ref. 36, octyl glucoside) and is thus larger or more elongated than the octyl glucoside–GT complex. The V_e for the SDS–GT complex corresponds to that of a native globular protein of M_r 320 000 (calibration not shown).

CONCLUSION

The time needed for equilibration of a Superose-6 column with SDS monomers to within 0.06 m*M* of the desired concentration is long: at least 20 h for 0.2-m*M* increments. Equilibration with SDS micelles is slower. The observed changes in V_e for SDS–protein complexes in gel chromatography experiments as the SDS concentration in the column is changed indicate that the complexes have nearly reached their final shape and size, corresponding to saturation with SDS, at the cmc of the detergent. We observed two major transitions and an intermediate complex. The first transition may correspond to an uptake of SDS monomers with a displacement of protein segments from the hydrophobic interior of the protein by the dodecyl chains of the detergent monomers and the second transition to the formation of one or more detergent micelle(s) surrounded by the polypeptide.

An integral membrane protein showed a more compact complex than a water-soluble one of the same molecular weight, probably due to the incorporation of hydrophobic α-helices in one or more hydrophobic core(s) of the complex.

NOTE ADDED IN PROOF

Recent data from our laboratory indicate that the slow equilibration of Superose-6 with SDS at low SDS concentrations is partly related to adsorption of SDS to the cross-linked agarose gel matrix.

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