Journal of Chromatography, 512 (1990) 3–12
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 1911

Binding of sodium dodecyl sulphate to an integral membrane protein and to a water-soluble enzyme

Determination by molecular-sieve chromatography with flow scintillation detection

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ABSTRACT

We have determined the binding of sodium dodecyl sulphate (SDS) to the human red cell glucose transporter (polypeptide, M_r 54 117) and to a water-soluble enzyme, N-5′-phosphoribosylanthranilate isomerase–indole-3-glycerol-phosphate synthase (PRAI–IGPS) from *Escherichia coli* (M_r 49 484). [^{35}S]SDS was equilibrated with each protein on molecular-sieve chromatography at a series of SDS concentrations. The binding ratios of SDS to protein were determined by flow scintillation detection and automated amino acid analyses. Unexpectedly the glucose transporter, which is a transmembrane protein, bound about the same amount of SDS per gram of protein as did the enzyme. At 1.6 m*M* SDS, slightly below the critical micelle concentration (CMC) (1.8 m*M*) in the eluent, the binding ratio was 1.6 g SDS/g protein for both the glucose transporter and PRAI–IGPS. At 2.0 m*M* SDS (above the CMC) the glucose transporter showed a binding ratio of 1.7 g SDS/g protein. The corresponding value for the enzyme was about 1.5 g/g. The SDS–glucose transporter complex seems to be more compact than the SDS–enzyme complex as judged by molecular-sieve chromatography and by SDS–polyacrylamide gel electrophoresis. Recent neutron scattering results have shown a protein-decorated triple-micelle structure for the SDS–PRAI–IGPS complex. Hypothetically, the more compact SDS–glucose transporter complex may therefore consist of a dual-micelle structure. The molecular-sieve gel beads bound considerable amounts of SDS. The SDS binding to the gel matrix and to the proteins increased with increasing SDS concentration up to at least 1.6–2.0 m*M* SDS. In the case of the water-soluble enzyme a shoulder was observed in the binding curve at 1 m*M* SDS, probably reflecting a change in the conformation of the complex.

0021-9673/90/$03.50

INTRODUCTION

Recently, a low-resolution structure of the sodium dodecyl sulphate (SDS) complex with a water-soluble enzyme, N-5′-phosphoribosylanthranilate isomerase–indole-3-glycerol-phosphate synthase (PRAI–IGPS), has been elucidated by small-angle neutron scattering analysis[1]. The complex was found to consist of a triple-micelle structure with the polypeptide mainly on the surface of the SDS micelles. The surface location of the amino acid residues is consistent with a model described earlier[2]. The experimentally found structure has been termed the "protein-decorated micelle structure". The change in elution volume of SDS–PRAI–IGPS on molecular-sieve chromatography as the concentration of free SDS is increased has also recently been monitored[3]. In this work, the binding ratio of SDS to PRAI–IGPS and to the human red cell glucose transporter has been determined at a series of SDS concentrations.

Our purpose was to compare the SDS binding to a water-soluble protein with the SDS binding to an amphipathic integral membrane protein (and to support neutron scattering measurements of the formation of SDS–PRAI–IGPS complexes). Some transmembrane proteins bind SDS more extensively than do most water-soluble proteins (*e.g.*, refs. 4–6). In the case of the glucose transporter from the human red cell, the complex with SDS seems to be relatively compact as judged by molecular-sieve chromatography[3,7]. This gives rise to the suspicion that the amount of SDS bound to the glucose transporter is not very large. This protein is thought to contain twelve hydrophobic α-helices spanning the membrane[8].

EXPERIMENTAL

Materials

Human red cell concentrate was supplied by the blood bank of the University Hospital, Uppsala, Sweden. Octyl glucoside (*n*-octyl-β-D-glucopyranoside), Tris (Trizma base) and dithioerythritol were bought from Sigma (St. Louis, MO, U.S.A.). Sodium dodecyl sulphate (AnalaR Biochemical, 99% pure) was purchased from BDH (Poole, U.K.) and sodium dodecyl [^{35}S]sulphate from Amersham International (Little Chalfont, U.K.). Sephadex G-50, Sephacryl HR S-300 HR and a Superose 6 column were obtained from Pharmacia LKB Biotechnology (Uppsala, Sweden). DEAE-cellulose (DE-52) was obtained from Whatman (Maidstone, U.K.). PRAI–IGPS was kindly provided by K. Kirschner and H. Szadkowski (Biozentrum, Basle, Switzerland). Chemicals were of analytical-reagent grade unless stated otherwise. The buffer stock solutions and the samples were filtered through 0.2-μm filters (SM 11107 Sartorius, Göttingen, F.R.G.) and Acrodisc 13 (Gelman, Ann Arbor, MI, U.S.A.), respectively, unless stated otherwise. Flow-Scint III scintillator cocktail was obtained from Radiomatic Instruments (Tampa, FL, U.S.A.).

Molecular-sieve chromatography

Chromatographic experiments were performed at 23–24°C with a 22-ml Sephacryl S-300 HR column (28 × 1 cm I.D.) connected to two precision pumps (P-500), controlled by a gradient programmer (GP-250). The eluent was 50 m*M* sodium phosphate (pH 6.86)–1 m*M* Na_2EDTA–0.2 m*M* dithioerythritol–3.1 m*M* sodium azide

(buffer A) with SDS, including [^{35}S]SDS[3]. The critical micelle concentration (CMC) of SDS is *ca.* 1.8 m*M* in this buffer[1–3]. The chosen SDS concentrations were obtained by combining buffer A and buffer A with 5.0 m*M* SDS (buffer B) from the pumps in appropriate proportions. The flow-rate was 0.4 ml/min. Before sample application, the column was equilibrated with at least 60 ml of eluent for 2.5 h or more until complete or nearly complete equilibration with SDS was achieved, as judged by the scintillation detection. For this purpose the column was by-passed by use of a three-way connection, a tubing and a two-way four-port valve. The samples were applied from 125–2000-μl loops by automatic injection (Act-100). The protein amounts were 0.2–1 mg in each run.

The eluent from the column was passed through a UV detector (UV-1) with measurement at 280 nm and then either collected by a fraction collector (Frac-100) or passed through a flow scintillation detector (Radiomatic A-300 Flo-One Beta; Radiomatic Instruments). The detector cell volume was 0.5 ml and the scintillator cocktail flow-rate was 1.6 ml/min. The protein amounts were determined, in most experiments, by automated amino acid analyses of collected fractions; the amount of bound SDS was determined in a separate run with scintillation detection of the radioactivity (the flow-split function of the detector was not used as it did not function accurately enough for the present purpose). The radioactivity in the eluent was recorded as counts per minute (cpm) for each 20-s sampling time interval. No programmed or manual background subtraction was made as the background was negligible in relation to the [^{35}S]SDS radioactivity level. To determine the amount of SDS bound to the protein, automatic peak integrations (sum of the cpm values for the sampling time intervals, per peak) were done and the values were then corrected for the contribution from the radioactivity in the buffer. The protein amounts according to the amino acid analyses, divided by the areas of the PRAI–IGPS UV peaks, were plotted against the equilibrium concentration of SDS, and from this diagram the protein amounts were determined in a few instances in which no amino acid analyses were made. For the glucose transporter, amino acid analyses were always done for calculation of binding data. All instruments except the scintillation detector were obtained from Pharmacia LKB Biotechnology.

In one experiment with the human red cell glucose transporter at 1.8 m*M* SDS and two experiments at 2.0 m*M* SDS, part of the main protein fractions were collected from two runs, combined and divided into two aliquots. One aliquot was re-run for amino acid analysis and the other was re-run with flow scintillation detection. In this way disturbances from co-purified membrane lipids and, in some instances, octyl glucoside from the transporter sample, could be eliminated or minimized.

At 0.8, 1.2 and 1.5 m*M* SDS, PRAI–IGPS samples pre-equilibrated with SDS by chromatography on Sephacryl S-300 were also applied to the columns (these samples had recently been used for neutron scattering measurements). No significant difference in SDS binding values could be found with these samples compared to native PRAI–IGPS samples. Hence it seems that all bound SDS could be displaced by [^{35}S]SDS. In the case of glucose transporter, the transporter–lipid preparation was solubilized in SDS before application. In experiments at 1.8 and 2.0 m*M* SDS, octyl glucoside-solubilized glucose transporter was also applied.

Samples

The glucose transporter. Human red cell membranes, stripped of peripheral proteins, were prepared as described earlier[9]. Integral membrane proteins were partially solubilized at 2°C at a concentration of 8 mg/ml with 75 m*M* octyl glucoside–70 m*M* Tris–HCl (pH 7.0)–1 m*M* dithioerythritol[10,11]. After centrifugation for 1 h at 160 000 *g* the protein solution was applied to a DEAE-cellulose column at 6°C in the above buffer and the material that passed through the column on isocratic elution was collected as described earlier[10] (sample A). It contained mainly the glucose transporter at a concentration of *ca.* 0.8 mg/ml and some membrane phospholipids[10,11]. In most instances the octyl glucoside was removed by chromatography at 6°C of 2–3-ml samples on a 13-ml Sephadex G-50 column (16 × 1 cm I.D.) in 50 m*M* Tris (pH 7.0, as measured at 23°C)–100 m*M* NaCl–1 m*M* Na_2EDTA at a flow-rate of 0.5 ml/min. The aggregates of lipids and transporter molecules (or proteoliposomes) that were formed were collected by centrifugation at 160 000 *g* for 90 min. The supernatant was removed and the sedimented material was solubilized in buffer A containing 30 m*M* SDS (sample B, protein concentration *ca.* 0.6 mg/ml).

PRAI–IGPS. PRAI–IGPS from *Escherichia coli* was provided as a precipitate in 0.1 *M* potassium phosphate (pH 7.0)–5 m*M* Na_2EDTA–1 m*M* DTE and 42% ammonium sulphate. The preparation had been performed as described in ref. 12. An 0.5-ml aliquot of the enzyme suspension was mixed with 2.1 ml of buffer A and dialysed at 6°C against 4 × 500 ml of buffer A for 48 h. The protein solution was then diluted with buffer A to a concentration of *ca.* 1 mg/ml as estimated by absorbance measurements ($A_{280}^{1\,cm} = 0.84$). SDS–polyacrylamide gel electrophoresis (SDS-PAGE) showed that the enzyme was nearly pure.

RESULTS

Equilibration of the molecular-sieve gel with SDS

When the SDS concentration in the Sephacryl S-300 HR column was increased in 0.2-m*M* steps up to 2 m*M* SDS, more than three column volumes of eluent were needed to equilibrate the column with SDS at each new concentration. Not until two column volumes of buffer with an increased SDS concentration had passed through the column did the SDS concentration of the eluate increase steeply (Fig. 1), but to a level lower than in the applied buffer (as controlled by flow scintillation detection after by-passing the column). At least one further column volume of buffer was needed for complete equilibration with SDS. A very similar equilibration pattern was found for each 0.2-m*M* SDS concentration increment up to at least 1.6 m*M*. In each 0.2 m*M* step *ca.* 0.22 μmol (about 63 μg) of SDS was adsorbed per millilitre of gel as illustrated in Fig. 2 (probably the binding reaches a constant value at some concentration above 2.0 m*M* SDS). Thus the gel became in principle converted to a cation exchanger. The molecular-sieve properties are retained in the fractionation of SDS–protein complexes, as these are strongly negatively charged, at least at reasonably high SDS concentrations. Superose 6 columns showed slower equilibration with SDS than did Sephacryl S-300 HR at the same flow-rate (and a slow release of SDS on lowering the SDS concentration). We conclude that Superose 6 binds even more SDS than does Sephacryl S-300 HR. For this reason we used the Sephacryl gel in all the final experiments.

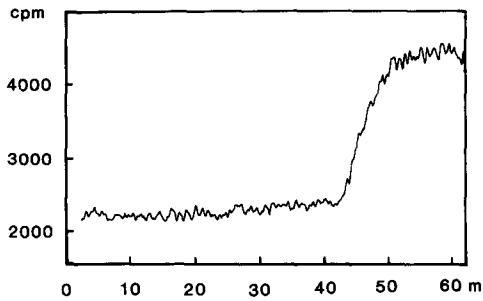

Fig. 1. Equilibration of the Sephacryl S-300 HR column with SDS as monitored by flow scintillation detection of [^{35}S]SDS. In this example the SDS concentration in the buffer applied to the column was increased from 0.2 to 0.4 m*M* at 0 ml elution volume. The SDS concentration in the buffer eluting from the column increased from 0.15 m*M* at 0 ml to 0.30 m*M* at 62 ml, as calculated from the radioactivity levels in the chromatogram. The elution volume, 43 ml, at the beginning of the steep increase in SDS concentration corresponds to 1.95 column volumes. Similar patterns were found for all equilibration steps up to 1.6 m*M* SDS, but for the next two steps up to 2.0 m*M* SDS considerably slower equilibration (greater binding) was observed (*cf.* ref. 3).

Glucose transporter

Examples of the glucose transporter flow scintillation chromatograms are shown in Fig. 3A and B. The lipids present in the SDS-solubilized glucose transporter preparation bind large amounts of SDS (the broad peak after 40 min in Fig. 3A). A re-run is illustrated in Fig. 3B. This experiment corresponds to point d in Fig. 5. In most instances the glucose transporter was applied in the form of an SDS-protein complex with a total SDS concentration of 30 m*M*. The sample included mixed SDS–phospholipid micelles, as some membrane phospholipids co-purify with the transporter[10,11]. As the SDS concentration in the column was increased from 0.4 m*M*

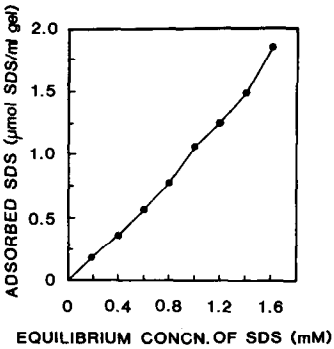

Fig. 2. Minimum amounts of SDS adsorbed to Sephacryl S-300 HR, *versus* equilibrium concentration of SDS. The SDS amounts were calculated from the type of experiments illustrated in Fig. 1. The amount of SDS applied between 22 ml elution volume (one column volume) and the end of each steep SDS concentration increase (50 ml in Fig. 1) was calculated for each 0.2-m*M* SDS concentration increment. The slow increase in SDS concentration after each steep increase (*cf.*, Fig. 1) could not be taken into account owing to lack of complete chromatograms. The values in the diagram are therefore lower limits. Still larger binding increments were observed at 1.6–2.0 m*M* SDS, but owing to the low equilibration complete chromatograms were not collected.

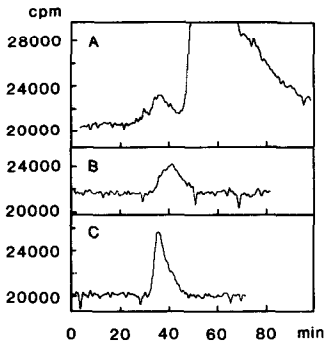

Fig. 3. Flow scintillation chromatograms. (A) SDS-solubilized glucose transporter and lipids from human red cells. Protein amount *ca.* 160 μg; sample volume, 250 μl; SDS concentration, 1.8 m*M*. (B) Re-run of an aliquot of two combined glucose transporter fractions similar to that in (A) at 35–40 min. Protein amount *ca.* 160 μg; sample volume, 2 ml; SDS concentration, 2.0 m*M*. (C), Native PRAI–IGPS. Protein amount, 250 μg; sample volume, 250 μl; SDS concentration, 1.8 m*M*.

SDS, the elution volume of the glucose transporter increased through one main transition between 0.8 and 1.4 m*M* SDS (Fig. 4B). This can be attributed to aggregation of the SDS–transporter complex into multimeric forms of the transporter when the concentration of free SDS, which initially was far above the CMC, is decreased to 0.4–1.2 m*M* as the sample passes into the column. Probably also some lipids aggregate with the transporter multimers. At low SDS concentrations the elution volume was therefore low. At higher SDS concentrations, enough SDS was present to keep

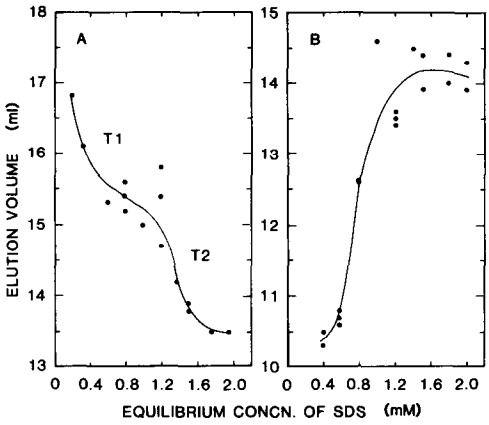

Fig. 4. Elution volume *versus* equilibrium concentration of SDS on molecular-sieve chromatography on Sephacryl S-300 HR of (A) the water-soluble enzyme PRAI–IGPS, applied in native form, and (B) the glucose transporter from human red cells, applied in the form of an SDS complex. For details, see Experimental. The sample volume was 250 μl, except at 2.0 m*M* SDS, where 500 μl of the glucose transporter were applied. The elution volume for PRAI–IGPS (A) decreases with increasing SDS concentration as the SDS binding increases. The elution volume for the glucose transporter (B) increases with increasing SDS concentration, as aggregation occurs at low but not at high SDS concentrations. The experiments were done identically in A and B, so the elution volumes can be compared. Note the difference in elution volume scales.

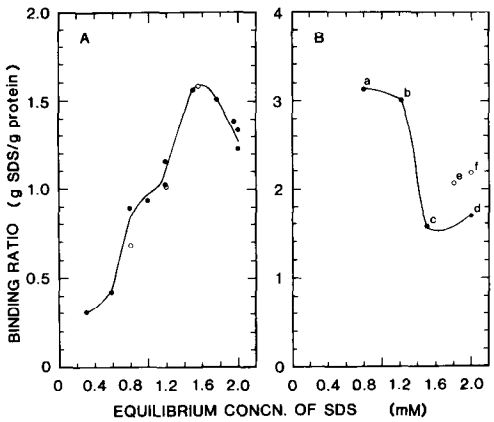

Fig. 5. Binding of SDS to (A) PRAI–IGPS and (B) the human red cell glucose transporter *versus* equilibrium concentration of SDS. For details, see Experimental. The ionic strength of the eluent is 0.1 *M* and the CMC of SDS is 1.8 m*M*. (A) ○ = PRAI–IGPS pre-equilibrated with SDS was applied to the Sephacryl S-300 HR column with [^{35}S]SDS; ● = native PRAI–IGPS was applied. (B) The eluted glucose transporter fraction contained (a) 12%, (b) 9%, (c) 6%, (d) 0% and (f) 0.7% of phosphatidylserine. ● = SDS–glucose transporter complex was applied to the column; ○ = octyl glucoside–glucose transporter was applied. Values d, e and f represent experiments in which eluted material was re-run on the Sephacryl column to remove lipids more efficiently (*cf.*, Fig. 3). The phosphatidylserine values were estimated by use of the known yields of serine from protein (90%) and the experimentally determined yield from phosphatidylserine (79%).

the transporter soluble as a monomer. The elution volume thus reached a large and nearly constant value above 1.4 m*M* SDS.

The binding ratios were very high at 0.8 and 1.2 m*M* SDS (3.1 and 3.0 g SDS/g transporter, respectively). This may be due to the formation of an SDS–transporter–lipid complex. The binding may also be overestimated by overlapping between the SDS–transporter and the SDS–lipids fractions (see Fig. 3A). It decreased to 1.52 g SDS/g transporter at 1.6 m*M* SDS but increased to 1.70 g SDS/g transporter at 2.0 m*M* SDS (Fig. 5B). This is a reliable value as it is obtained from a re-run of SDS-solubilized material that resulted in a transporter fraction free from phosphatidylserine. The value of 2.18 g/g at the same SDS concentration is probably too high as it is based on a re-run of octyl glucoside-solubilized material, and the final fraction was not entirely free from phosphatidylserine. Except at 2.0 mM SDS (point d in Fig. 5B), amino acid analyses showed the presence of an excess amount of serine, probably derived from phosphatidylserine (see the legend to Fig. 5). The protein amounts were corrected for this excess of serine. Phosphatidylserine is negatively charged and may bind by a combination of hydrophobic and electrostatic interactions with the glucose transporter. Presumably other lipids are also present in bound or non-bound form in the transporter fractions in the experiments illustrated in Fig. 5B. As no lipids can be expected to bind more strongly than phosphatidylserine, probably no lipids are present after the re-run at 2.0 m*M* SDS corresponding to point d.

PRAI–IGPS

Native PRAI–IGPS was applied to the Sephacryl S-300 HR column, which had

been equilibrated with buffer A containing SDS. The experiments were done with a series of increasing detergent concentrations in steps of 0.2 m*M* from 0.2 to 2.0 m*M* SDS. PRAI–IGPS is converted into an SDS–polypeptide complex at the top of the column[3]. When the SDS concentration was increased, the elution volume of PRAI–IGPS decreased through two main transitions, the first at 0.2–0.5 m*M* (T1) and the second at 1.2–1.6 m*M* SDS (T2) (Fig. 4A)[3]. The binding ratio of SDS increased with increasing SDS concentration from 0.3 up to 1.6 m*M* SDS (Fig. 5A), with a shoulder around 1.0 m*M* SDS. The SDS–PRAI–IGPS complex thus showed a maximum in SDS binding at 1.6 m*M* SDS, slightly below the CMC. The complex at 1.6 m*M* SDS was found to contain 1.58 g SDS/g PRAI–IGPS. The accuracy can be estimated to be *ca.* ±0.15 g/g (relative error in amino acid analysis 3% and in SDS determination 6%). Earlier determinations[1] with neutron scattering, with methylene blue binding and with a procedure similar to the present one resulted in values of 1.26–1.7 g/g. However, a transition into a complex with a slightly lower SDS-to-protein ratio is indicated by the graph in Fig. 5A at 1.6–2.0 m*M* SDS. The nature of this change in terms of the structure of the complex is not yet known.

Comparison of SDS binding to PRAI–IGPS and to the glucose transporter

Around the CMC of SDS, the maximum SDS binding was found to be about the same for the glucose transporter as for the water-soluble enzyme PRAI–IGPS. However, the binding increased slightly for the glucose transporter as the SDS concentration was increased from 1.6 to 2.0 m*M*, whereas the opposite was true for PRAI–IGPS.

At 2 m*M* SDS the membrane protein bound more SDS than did PRAI–IGPS. In addition, the glucose transporter has a higher molecular mass, M_r (54 117, ref. 8) than PRAI–IGPS (M_r 49 484, ref. 1). Further, the glucose transporter polypeptide also binds a branched oligosaccharide of average M_r *ca.* 9200 (17% of the polypeptide mass[13]). The total molecular mass of the SDS–glycosylated transporter complex (M_r *ca.* 155 000) is thus considerably larger than that of the SDS–PRAI–IGPS complex (M_r *ca.* 127 000) at 2.0 m*M* SDS. However, the glucose transporter was eluted later than PRAI–IGPS on molecular-sieve chromatography in SDS (Fig. 4 in this work, Fig. 6 in ref. 3 and Table II in ref. 7). Either the SDS–glucose transporter complex was retarded on the columns or its SDS complex is more compact (smaller or less elongated) than the SDS–PRAI–IGPS complex. It cannot be excluded that some retardation is caused by the oligosaccharide or by hydrophobic segments of the transporter, but no evidence known to us supports this hypothesis. It seems very likely that the SDS–glucose transporter complex really is more compact (less elongated) compared with SDS complexes of water-soluble proteins, as in SDS-PAGE the deglycosylated glucose transporter migrates with an apparent M_r of *ca.* 44 000–46 000 (refs. 14–16), whereas the true M_r is *ca.* 54 117. The water-soluble protein PRAI–IGPS migrates at a normal rate on SDS-PAGE[17].

DISCUSSION

Sephacryl and Superose

The column materials (Sephacryl S-300 HR and Superose 6) that we used bind SDS. The equilibration thus becomes slow and shows a special behaviour (Fig. 1; *cf.*,

ref. 3). The binding can be explained by hydrophobic and electrostatic interactions in addition to possibly, hydrogen bonding and other weak interactions. It has been shown that SDS also binds to polyoxyethylene[18]. The SDS binding to Superose is probably so high that an electrostatic exclusion effect may explain the lowering of the elution volumes for several proteins with increasing SDS concentration, which was previously observed in the range 5–100 m*M* SDS (Fig. 2 in ref. 7). The special SDS–gel matrix binding properties also caused a considerable retardation of the valleys in SDS concentration after each run with protein or lipid or octyl glucoside. Flow scintillation detection is a valuable tool in monitoring these effects.

SDS–PRAI–IGPS

The SDS binding to PRAI–IGPS at 1.6 m*M* SDS (slightly below the CMC) was found to be 1.58 ± 0.15 g/g. This is higher than the value of 1.26 g/g reported on the basis of neutron scattering measurements[1]. The "normal" binding ratio of two SDS molecules per amino acid residue[2] corresponds to a binding of 1.43 g SDS/g PRAI–IGPS (492 amino acid residues, M_r 49 484). Surprisingly, the SDS binding seems to decrease just above 1.6 m*M* SDS. A similar decrease in the binding above the CMC can be inferred from data for a membrane protein from a *Rhodospirillum rubrum* chromatophore (Fig. 4 in ref. 6). In that case the binding increased again at 3 m*M* SDS[6]. A similar tendency was found for bovine serum albumin, bovine carbonic anhydrase and PRAI–IGPS and the glucose transporter in a preliminary series of experiments on Superose 6 (not shown).

SDS–glucose transporter

Our results show the importance of removing all lipids from membrane protein preparations before attempts to determine SDS binding (or binding of other detergents). Re-runs may be needed in chromatographic procedures. The free lipids and free octyl glucoside (when present) also bind large amounts of SDS in mixed micelles (*cf.*, Fig. 3A). The same certainly applies to other detergents.

PRAI–IGPS forms a protein-decorated triple-micelle structure with SDS just below the CMC[1]. The probably more compact SDS–glucose transporter complex may hypothetically be envisaged as a dual-micelle structure or, possibly, as a short cylinder or ellipsoid. Speculatively, the apparent reduction in size on heating of the SDS–glucose transporter complex[7] may correspond to a transition from a dual-micelle structure to a still more compact cylindrical or ellipsoidal structure. It seems plausible that some or all of the hydrophobic amino acid residue stretches[8] in the glucose transporter traverse the SDS micelle core(s) in the SDS complex[2]. This is not known, however, and these amino acid residues can alternatively be inserted in the complex near its surface with the hydrophobic side-groups pointing inwards. The hydrophilic amino acid residues are probably positioned on the surface of the complex, as is the case for most of the PRAI–IGPS amino acid residues[1].

CONCLUSIONS

Careful equilibration of molecular-sieve columns is needed for the formation of SDS–protein complexes at accurately known detergent concentrations and for accurate determinations of SDS binding. Sephacryl gel beads bind SDS in amounts that

increase with increasing SDS concentration, as probably do Superose gel beads, which makes the equilibration slow. Also, re-equilibration after each chromatographic run needs to be carefully controlled. The use of a radioactive detergent and a flow scintillation counter is thus advantageous for binding studies. Our results indicate that at the CMC the partly hydrophobic glucose transporter protein from human red cells binds SDS in about the same amount per amino acid residue as does the water-soluble enzyme PRAI–IGPS when the membrane protein is properly freed from lipids. The SDS binding to the glucose transporter seemed to increase when the SDS concentration was increased above the CMC.

ACKNOWLEDGEMENTS

We are grateful to Kasper Kirschner and Halina Szadkowski for providing PRAI–IGPS. We thank Eva Greijer for general assistance and help with membrane preparations and manuscript editing, Robert Wallstén for mathematical advice on the scintillation detection-based binding calculations, Erik Mascher for comments on the manuscript, Konrad Ibel and Roland P. May for discussions and neutron scattering measurements (work in progress) and David Eaker for help with phosphatidylserine determinations. We appreciate the loan of an automatic sample applicator system from Hans Lindblom and Kurt Andersson (Pharmacia LKB Biotechnology). This work was supported by the Swedish Natural Science Research Council, the Swedish National Board for Technical Development (89-00519P) and the O.E. and Edla Johansson Science Foundation.

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