Journal of Chromatography, 604 (1992) 95–102
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 069

High-performance hydroxyapatite chromatography of integral membrane proteins and water-soluble proteins in complex with sodium dodecyl sulphate

Per Lundahl

Department of Biochemistry, Biomedical Centre, Uppsala University, Box 576, S-751 23 Uppsala (Sweden)

Yasushi Watanabe and Toshio Takagi

Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565 (Japan)

ABSTRACT

Integral membrane proteins from human erythrocytes were fractionated in the presence of sodium dodecyl sulphate (SDS) on four types of high-performance hydroxyapatite columns. A column of 2-μm sintered hydroxyapatite beads from Asahi Optical (Tokyo, Japan) gave the best resolution. With this column, glycophorin was eluted early in a gradient of increasing sodium phosphate buffer concentration, the glucose transporter was eluted later in two zones, one of which contained this protein alone, and the anion transporter was eluted last. Water-soluble proteins applied in complex with SDS also separated reasonably well upon elution. The water-soluble proteins and the membrane proteins were all eluted mainly in the order of increasing polypeptide length, but with considerable individual variation. SDS–polypeptide complexes are probably adsorbed onto hydroxyapatite by the interaction of positively charged amino acid side groups with phosphate ions (at P-sites) and of negatively charged amino acid side groups and polypeptide-bound dodecyl sulphate anions with calcium ions (at C-sites). As a rule, the number of charged side groups and dodecyl sulphate anions, and thus the number of binding sites, increases with the polypeptide chain length, which explains the general order of release of the polypeptides.

INTRODUCTION

Hydroxyapatite chromatography of water-soluble proteins in the presence of sodium dodecyl sulphate (SDS) was introduced by Moss and Rosenblum in 1972 [1]. Later applications failed to contribute any persuasively efficient example of such separations and often suffered from unsatisfactory reproducibility. Sintered hydroxyapatite and other new preparations afford improved reproducibility between runs and between different batches of hydroxyapatite. A significant reduction in the run time was achieved upon development of the high-performance liquid chromatography (HPLC) type hydroxyapatite packing materials that are now commercially available from several sources. Recently, Horigome *et al.* [2] efficiently fractionated rat erythrocyte membrane proteins solubilized in SDS on HPLC columns of ceramic (sintered) or coral-shaped hydroxyapatite and could resolve several components. They identified one transmembrane protein, the anion transporter. This might be the first successful application of this technique for separation of membrane proteins in the presence of SDS.

It seemed important to examine the performance of the HPLC-type hydroxyapatite chromatography in the presence of SDS for integral (intrinsic) membrane proteins only, the major ones in erythrocytes being the anion transporter, the glucose transporter

Correspondence to: Dr. P. Lundahl, Department of Biochemistry, Biomedical Centre, Uppsala University, Box 576, S-751 23 Uppsala, Sweden.

0021-9673/92/$05.00

and glycophorin, rather than a more complicated mixture containing also several other proteins. Mascher and co-workers [3,4] have previously fractionated integral membrane proteins from human erythrocytes by molecular sieve chromatography on Superose 6 in the presence of SDS after thorough removal of cytosolic proteins, cytoskeletal proteins and other peripheral membrane proteins. We have now fractionated such integral membrane proteins by HPLC on sintered and coral-shaped hydroxyapatite in the presence of SDS. Several water-soluble globular proteins were also complexed with SDS and the behaviour of the corresponding SDS-polypeptide complexes was investigated to ascertain whether there was something special about the behaviour of intrinsic membrane proteins.

Horigome *et al.* [2] found a positive correlation between the retention time of SDS complexes of 24 water-soluble proteins and the logarithm of their molecular weight upon phosphate buffer gradient elution from hydroxyapatite. The proteins were not specified. The authors discuss this in terms of the binding of SDS to water-soluble proteins in proportion to the polypeptide length and imply that SDS-polypeptide complexes bind to hydroxyapatite mainly as a result of the negative charge derived from the dodecyl sulphate (DS) anions in the complexes. This is probably correct, and the purpose of our present work was to verify or disprove that polypeptides are released from hydroxyapatite in the presence of SDS essentially in the order of increasing polypeptide chain length, for membrane proteins as well as for water-soluble proteins, as the phosphate buffer concentration is increased. However, we want to emphasize that charged amino acid side groups can contribute to the binding and cause individual variations in the elution of polypeptides of similar lengths. A tentative model is thus presented for the equilibrium state of binding of SDS–polypeptide complexes to hydroxyapatite: we propose that positively charged amino acid side groups interact with the "phosphate sites" (P-sites) of the hydroxyapatite, whereas negatively charged side groups and dodecyl sulphate anions interact with the "calcium sites" (C-sites) [5–8].

The structure of free SDS–polypeptide complexes may be described by the "necklace model" [9–11] or by the "protein-decorated micelle modell" [12], but the complexes probably rearrange upon binding. As discussed below, it is important that SDS is released upon binding of the complexes to hydroxyapatite, as shown by Watanabe *et al.* [13].

EXPERIMENTAL

Materials

SDS (AnalaR No. 10807) was obtained from BDH Chemicals (Poole, UK). Low-molecular-weight calibration proteins for electrophoresis were purchased from Pharmacia-LKB Biotechnology (Uppsala, Sweden). Carbonic anhydrase (C-7500), cytochrome *c* (C-7752) and chicken egg ovalbumin (A-7641) were bought from Sigma (St. Louis, MO, USA). β-Lactoglobulin was obtained from Miles (UK) and β-galactosidase was a gift from Daiichi Chemicals (Tokyo, Japan). Rabbit IgG was prepared and purified on DEAE–cellulose by T. Takagi. Bovine serum albumin (No. 002, "reagent grade") was bought from Chiba Chikusan Kogyo (Chiba, Japan). Chemicals were of reagent grade and high-quality deionized water was used. All solutions were filtered through 0.3-μm filters (PHWP 04700, Nihon Millipore Kogyo, Yonezawa, Japan).

Four hydroxyapatite columns were used: (a) Tonen Taps-020810, 2-μm beads (Toa Nenryo Kogyo K. K., Tokyo, Japan); (b) PENTAX SH-0710F, 2-μm beads (Asahi Optical); (c) A-7610, 3-μm beads, with guard column C-3201 (Koken, Tokyo, Japan) and (d) TSKgel HA-1000, 5-μm beads, with TSK guard HA-1000 column (Tosoh, Tokyo, Japan). Columns a and b were used without guard columns. The dimensions of columns a, b and d were 100 mm × 7.5 mm I.D., and those of column c were 100 mm × 7.6 mm I.D.

The chromatographic equipment consisted of an ERC-3510 degasser from Erma Optical Work (Kawaguchi, Japan), a CCPM HPLC pump with a controller for gradient programming and an injector, a CM-8000 conductometer, a UV-8 Model II spectrophotometer set at 280 nm and a TSK two-channel recorder, all from Tosoh.

Sample preparation

(1) Integral membrane proteins together with membrane lipids from human erythrocytes (*i.e.* membranes stripped of peripheral proteins and free from water-soluble proteins) were prepared and finally adjusted to 10 mg of protein per ml of 50 m*M*

Tris–HCl (pH 6.7 at 25°C), frozen in liquid nitrogen and stored at −70°C as described previously [4]. A 2.5-ml aliquot of the preparation was mixed with 1 *M* sodium phosphate buffer (pH 6.56), 350 m*M* SDS and other components to a final composition of 2 mg of protein per ml, 10 m*M* Tris–HCl, 10 m*M* sodium phosphate buffer (final pH 6.8), 0.5 m*M* dithioerythritol (DTE), 100 m*M* SDS and 2 m*M* sodium azide. The solution was stirred for 5 min at 25°C and centrifuged at 160 000 *g* for 40 min at 25°C. A very small pellet was formed. The supernatant was collected, frozen immediately in 350-μl aliquots and kept at −70°C.

(2) Low-molecular-weight calibration proteins for electrophoresis (60–130 μg of each of bovine milk α-lactalbumin, soybean trypsin inhibitor, bovine erythrocyte carbonic anhydrase, egg white ovalbumin, bovine serum albumin and rabbit muscle phosphorylase *b*) were mixed with 1.2 ml of solution S (10 m*M* sodium phosphate buffer, pH 6.8, 0.1 m*M* calcium chloride, 100 m*M* SDS and 3 m*M* sodium azide. DTE (3.0 mg) was added to give a final concentration of 16 m*M*. The solution was heated to 80°C over 2 min, kept at that temperature for 6 min and then cooled to 25°C and centrifuged at that temperature at 100 000 *g* for 30 min. No pellet was seen. The solution was divided into aliquots, frozen immediately and kept at −70°C.

(3) Horse heart cytochrome *c* (1.4 mg), β-lactoglobulin (1.5 mg), bovine erythrocyte carbonic anhydrase (1.5 mg), rabbit IgG (2.4 mg) and β-galactosidase (1.4 mg) were mixed and dissolved in 5 ml of solution S. DTE (16.3 mg) was added and the solution was treated as described in (2) above except that it was filtered through a 0.22-μm filter (type SLGV025LS, Nihon Millipore Kogyo, Yonezawa, Japan) before freezing.

(4) Cytochrome *c* (1.1 mg), β-lactoglobulin (0.8 mg), carbonic anhydrase (0.7 mg), ovalbumin (0.7 mg), rabbit IgG (1.1 mg), bovine serum albumin (0.6 mg) and β-galactosidase (0.7 mg) were dissolved separately, each in 2 ml of solution S. DTE (6 mg) was added to each sample. The samples were treated as in (3) above.

Hydroxyapatite chromatography

Before each experiment the column was equilibrated with at least 120 ml of solution A 10 m*M* sodium phosphate buffer (10 m*M* phosphorus), pH 6.82, 0.1 m*M* calcium chloride, 3.5 m*M* (0.1%) SDS and 3 m*M* sodium azide] at 0.8 ml/min, except for the PENTAX column (see below). The ionic strength of this solution is 27 m*M* and the corresponding critical micelle concentration (CMC) of SDS is about 3.1 m*M* according to Fig. 7-2 in ref. 14. During this equilibration the column became saturated with SDS [13] and the conductivity of the eluted buffer stabilized at 2.10 ± 0.02 mS/cm. The end solution, solution B, was 585 m*M* sodium phosphate buffer (585 m*M* phosphorus), pH 6.56, 5 μ*M* calcium chloride, 3.5 m*M* SDS and 3.5 m*M* sodium azide. This solution was stable at and above 25°C; at lower temperatures SDS precipitated. The experiments were performed at 25–27°C. Solutions A and B were both prepared from stock solutions of (a) 1.00 *M* sodium phosphate buffer containing 6 m*M* sodium azide, pH 6.51 at 25°C, (b) 10 m*M* calcium chloride and (c) 350 m*M* SDS containing 6 m*M* sodium azide. The given final pH values were those obtained after dilution of the stock buffer; no adjustment was done. The SDS solutions were stored in glass bottles as plastic bottles containing SDS solutions can release components which become adsorbed to hydroxyapatite.

After column equilibration, 250 μl of protein sample were applied at a flow-rate of 0.8 ml/min. At 5 min after the start of sample application the percentage of solution B was linearly increased to 50% (298 m*M* phosphate buffer) over 1 min and was then increased linearly to 100% (585 m*M* phosphate buffer) over another 38 min (slope 9.4 m*M*/ml). The elution with solution B was continued until the UV absorption became constant. The flow-rate was 0.8 ml/min throughout the elution. With the PENTAX column, which tolerated high pressure, re-equilibration was performed at 1.5–1.8 ml/min, but the flow-rate was lowered to 0.8 ml/min about 30 min before each run, since lowering the flow-rate resulted in the elution of a broad zone of slightly increased conductivity.

Electrophoresis. SDS polyacrylamide gel electrophoresis (PAGE) was done essentially as described by Laemmli [15] with a 70-mm-long and 0.5-mm-thick separation gel of 12.2% acrylamide and 0.34% *N,N′* methylenebisacrylamide. The stacking gel contained 4.4% acrylamide and 0.12% of the cross-linker. A 30-μl sample was mixed with 10 μl of

water containing 40% (v/v) glycerol and 8% (w/v) SDS and with 2 μl of water containing 0.6 μg of bromophenol blue. A 10-μl volume of the mixture was applied in 4-mm-wide wells. The gels were stained with the silver-staining kit 2D-Silver Stain II DAIICHI (Daiichi Pure Chemicals).

RESULTS

Integral erythrocyte membrane proteins

Fig. 1 shows the elution patterns obtained for the SDS complexes of the human erythrocyte integral (intrinsic) membrane proteins using the four HPLC-type hydroxyapatite columns. As is clear from the comparison, the PENTAX column gave the best overall resolution (Fig. 1B). Electrophoretic analysis of fractions from this column, as illustrated in Fig. 2, showed good separation between the major proteins glycophorin (Figs. 1B and 2, fraction a), the glucose transporter (broad zones [3,4], in fractions d and e) and the anion transporter (fractions f–h). However, only the peak corresponding to fraction e in Fig. 1B contained a single essentially pure component, namely the glucose transporter. For details, see the legend to Fig. 2. With the other three columns the elution profiles were compressed (Fig. 1A, C and D) and the resolution was lower.

Nine runs were made on the PENTAX column with essentially the same result (Fig. 1B) in the last eight. Four to six runs were made on the other columns, also with consistent results.

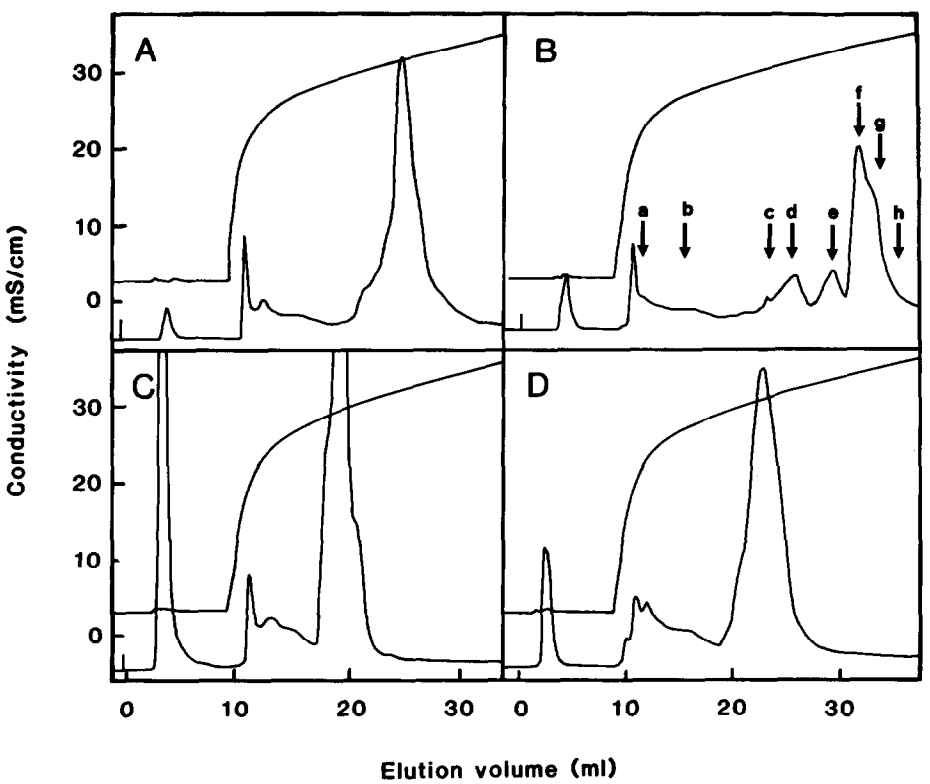

Fig. 1. Elution patterns (absorbance at 280 nm) obtained for solubilized human erythrocyte integral (intrinsic) membrane proteins (see Experimental) in complex with SDS using the HPLC-type hydroxyapatite columns: (A) Tonen Taps-020810, (B) PENTAX SH-0710F, (C) A-7610 and (D) TSKgel HA-1000. Protein amount: 0.5 mg. The SDS–polypeptide complexes were eluted with a gradient in phosphate buffer concentration, as described in the Experimental section. Flow-rate: 0.8 ml/min. The height of the panels corresponds to an absorbance of 0.12. The arrows indicate fractions analysed by electrophoresis (see Fig. 2). A conductivity of 10, 20 and 30 mS/cm at 25°C corresponds to a phosphate concentration of 85, 205 and 370 m*M*, respectively, in the eluent.

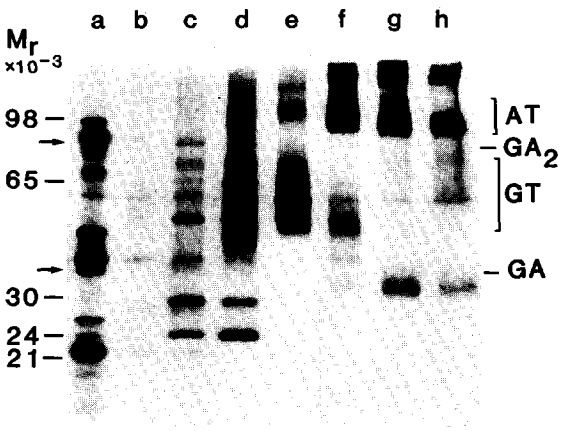

Fig. 2. Electrophoretic analysis of fractions a–h in the chromatogram in Fig. 1B. (a) Glycophorin A monomers and dimers (arrows, denotations GA and GA_2, respectively) and other components; (b) mainly lipids, appearing as a clear zone near the front in the original gel; (c) unidentified protein zones; (d) broad zones probably corresponding to the glucose transporter monomer and dimer, and sharper zones corresponding to other components similarly as in (c); (e) pure glucose transporter monomer, dimer and trimer; and (f–h) mainly the anion transporter and different proportions of unidentified slowly migrating components. The broad zones in (d) have migrated slightly faster than the corresponding zones in (e), possibly because of differences in the glucose transporter oligosaccharide.

Electrophoretic analyses (not illustrated) confirmed that the order of elution of the components was the same with all four columns, although the resolution differed. No attempt was made to optimize the resolution by changing the chromatographic conditions.

Water-soluble proteins

Only the PENTAX column was used for water-soluble proteins. The resolution of low-molecular-weight electrophoresis calibration proteins was reasonably good, as illustrated in Fig. 3A (fourth run with these proteins). The resolution was slightly better in three earlier runs, possibly because of a small batch-to-batch change in the composition of starting buffer before the fourth run. α-Lactalbumin (j), carbonic anhydrase (k), ovalbumin (l) and phosphorylase *b* (m) were identified by electrophoresis. The mixture of proteins that was prepared as described in the Experimental section (Sample preparation 3) was resolved as shown in Fig. 3B. Electrophoresis revealed the order of elution: cytochrome *c* (n), H-chain of IgG (o), carbonic anhydrase (k) and β-galactosidase (p). To confirm the identification of the zones, several of the water-soluble proteins were converted to SDS protein complexes as described in the Experimental section (Sample preparation 4) and were run separately. The results were as expected (not illustrated).

Elution order

The buffer concentrations corresponding to the positions of the SDS-polypeptide peaks in the chromatograms were plotted against the relative molecular weights (M_r) of the polypeptides. This was done for several water-soluble proteins and for two integral membrane proteins, the glucose transporter (polypeptide M_r 54 117) [16] and the anion transporter (polypeptide M_r 101 791) [17] (Fig. 4). Glycophorin is small (131 amino acid residues, M_r about 31 000, including oligosaccharides) [18] and was eluted early (see Figs. 1B and 2). This is not shown in Fig. 4 as the elution of glycophorin preceded the main gradient elution with the slope 9.4 m*M*/ml.

Glycophorin contains only one transmembrane α-helix, which is perhaps inserted into a single SDS micelle, and the extracellular part of the polypeptide is long and heavily glycosylated. This explains the special behaviour of glycophorin. The diagram in Fig. 4 shows that the longer polypeptides were in general eluted at higher buffer concentration than the shorter ones. For proteins with similar polypeptide lengths the elution positions varied considerably. The two large, major integral membrane proteins, the glucose and anion transporters, which are thought to contain twelve and fourteen hydrophobic transmembrane α-helices, respectively [16,17], were eluted at about the same positions as water-soluble proteins of similar polypeptide chain lengths.

DISCUSSION

The performances of the four hydroxyapatite columns that we have studied differed considerably under the single set of chromatographic conditions used. Changes in the conditions can perhaps improve the result for each individual column. All of them gave reproducible elution profiles and the same order of elution of integral (intrinsic) erythro-

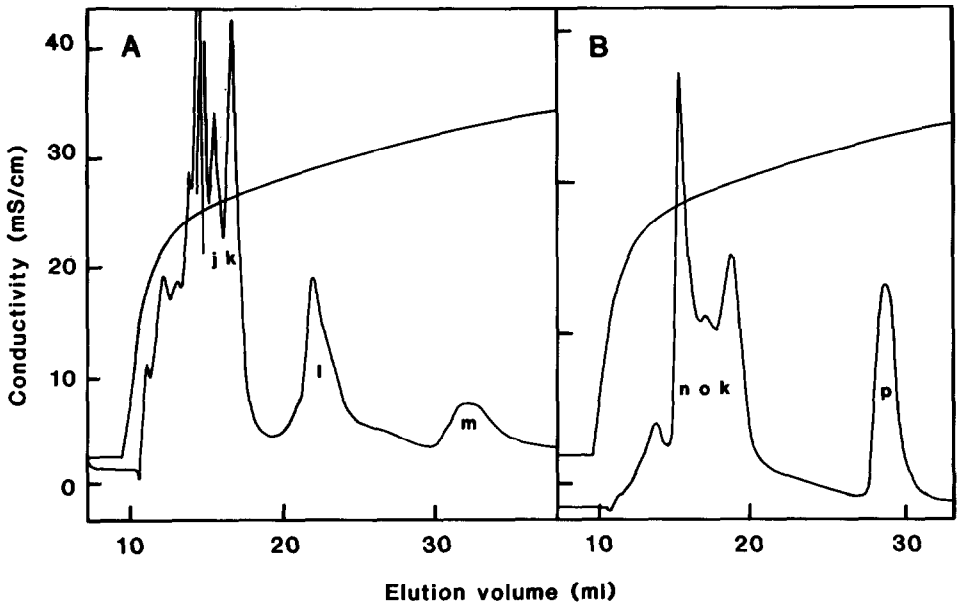

Fig. 3. Elution patterns (absorbance at 280 nm) of water-soluble proteins on the PENTAX column. Mixtures of proteins were prepared as described in the Experimental section (A, *Sample preparation* 1, B, *Sample preparation* 2) and the order of elution was identified by electrophoresis (not shown). (A) Low-molecular-weight electrophoresis calibration proteins: j = α-lactalbumin; k = carbonic anhydrase; l = ovalbumin; and m = phosphorylase *b*. (B) Protein mixture: n = Cytochrome *c*; o = H-chain of IgG; k = carbonic anhydrase; and p = β-galactosidase. Protein amount: 13–27 μg of each protein in (A) and about 70 μg of each protein (polypeptide) in (B). Flow-rate: 0.8 ml/min. The height of (A) corresponds to an absorbance of 0.03 and that of (B) to 0.13. A conductivity of 40 mS/cm at 25°C corresponds to a phosphate concentration of approximately 540 m*M* in the eluent.

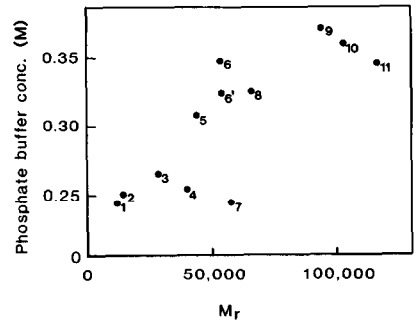

Fig. 4. SDS–polypeptide complexes eluted from the ceramic PENTAX hydroxyapatite column. The phosphate buffer concentration corresponding to each polypeptide peak was estimated from the recorded conductivity and a calibration curve and was plotted against the polypeptide relative molecular weight (M_r). This was done for nine water-soluble proteins and for two integral membrane proteins from human erythrocytes, the glucose transporter and the anion transporter. Data are from experiments illustrated in Figs. 1–3 and an experiment with β-lactoglobulin (not illustrated). The proteins were: 1 = cytochrome *c*; 2 = α-lactalbumin; 3 = carbonic anhydrase; 4 = β-lactoglobulin; 5 = ovalbumin; 6 = the glucose transporter (e in Figs. 1B and 2); 6′ = the glucose transporter (d in Figs. 1B and 2); 7 = H-chain of IgG; 8 = bovine serum albumin; 9 = phosphorylase b, 10 = the anion transporter (f in Figs. 1B and 2); and 11 = β-galactosidase.

cyte membrane proteins, but the resolution differed. The PENTAX column SH-0710F showed the best separation of the membrane proteins, as illustrated in Fig. 1, and allowed a high flow-rate. However, even with this excellent column only one fraction contained a pure protein, the glucose transporter. This transporter also appeared in another fraction. The reason for this dual elution is not known, but the tendency of integral membrane proteins to form dimers and to appear in both monomeric and dimeric forms in the presence of SDS (as in the presence of non-ionic detergents) may be of importance. Monomers may be released earlier than dimers, and dimers may dissociate upon electrophoresis. Most fractions contained several components, and the result of hydroxyapatite chromatography of membrane proteins in the presence of SDS therefore usually has to be analysed by electrophoresis.

The chromatographic experiments presented here were all done with 3.5 m*M* SDS in the eluent, which is above the CMC of SDS even at the low ionic strength (27 m*M*) of the starting buffer, as men-

tioned above. As is well known, the CMC for ionic detergents decreases with increasing ionic strength. Later experiments on the PENTAX column showed that the resolution of the intrinsic membrane proteins decreased, whereas the resolution of water-soluble proteins increased, when the SDS concentration was increased to 35 m*M* (1%). We do not know why this was so. The SDS/protein binding ratio for a membrane protein, at an equilibrium concentration of free SDS near the CMC, can be similar to that for water-soluble proteins [19]. Nevertheless, differences in the SDS/protein binding ratios and in the homogenity of binding may occur upon adsorption to hydroxyapatite and may depend on the SDS concentration. However, although the glucose transporter and the anion transporter contain several hydrophobic α-helices which may bind especially large amounts of dodecyl sulphate, they did not bind exceptionally strongly to hydroxyapatite in the presence of SDS in the present experiments. The contribution of dodecyl sulphate to the adsorption of the complex to hydroxyapatite is thus probably similar for integral membrane proteins and water-soluble proteins at a concentration of free SDS near the CMC, although the distribution of hydrophobic amino acid side groups along the polypeptide chains differs.

The structure of SDS–protein complexes has recently been studied by small-angle neutron scattering in Brookhaven [10,11] and in Grenoble [12]. The recent result of Guo *et al.* [11] indicated, for example, that eight SDS micelles of an average aggregation number of 43 were associated with the bovine serum albumin (BSA) polypeptide (M_r 66 267) [20] in the presence of 1.5 g of SDS per g of BSA at an ionic strength of 0.2 *M*. The structure of the complex was described as being of the "necklace" type. The normal binding level of 1.5 g of SDS per g of BSA (344 = 8 × 43 SDS molecules per BSA molecule) at the CMC of SDS is consistent with the data of Guo *et al.* [11]. However, a certain concentration of free SDS is required for equilibrium to prevail. This was not explicitly taken into account in the paper of Guo *et al.* [11]. The results of Ibel *et al.* [12] indicated that three SDS micelles of about 42, 101 and 73 SDS molecules (from the polypeptide N-terminus to the C-terminus) are part of the SDS complex with a water-soluble enzyme of M_r 49 484, *i.e.* smaller than BSA, near the saturation level and at an ionic strength of 0.1 *M*. The structural model is denoted "the protein-decorated micelle model". The number of SDS molecules in these three micelles, 216, corresponds to 1.26 g of SDS per g of the enzyme. The limit of error in the SDS aggregation numbers observed by Ibel *et al.* [12] is about ± 5%, and it cannot be excluded that an additional small SDS micelle has escaped detection. The discrepancy between the reported numbers of SDS micelles in the complexes [11,12] is thus reasonably small when the differences in polypeptide lengths and experimental conditions are taken into consideration.

For the binding of SDS–polypeptide complexes to hydroxyapatite it is probably important to realize that ionic amino acid side chains can participate in the equilibrium state of binding of the polypeptides to the hydroxyapatite. As recently shown by Watanabe *et al.* [13] the binding process is accompanied by release of a substantial amount of SDS from the hydroxyapatite or the SDS–polypeptide complex, probably from both. Release of an amount of SDS corresponding to about 80% of the SDS initially bound to the polypeptide was observed. It is likely that the SDS–polypeptide complex changes its structure upon binding to hydroxyapatite and that the binding involves ionic bonds between negatively and positively charged amino acid side groups and the C-sites and P-sites of the hydroxyapatite, respectively. This will lead to different binding strengths depending on the number of charges and the distribution of charges along the polypeptide chain, in agreement with the spread of the elution positions for polypeptides of similar sizes in earlier chromatographic experiments [2] and in our present experiments. It will also lead to the strongest binding of a polypeptide to hydroxyapatite near its isoelectric point (p*I*), where the net charge is low but where the number of charged amino acid side groups usually has a maximum. This is consistent with the results illustrated in Fig. 6, left panel, in ref. 2. However, as a rule, at a given pH value and for polypeptides of similar p*I* values the overall number of charged groups and therefore of potential binding sites increases with the length of the polypeptide. The number of polypeptide-bound dodecyl sulphate anions increases with the polypeptide length in free complexes and probably also in the hydroxyapatite-adsorbed state. These two circumstances explain the results summarized in our

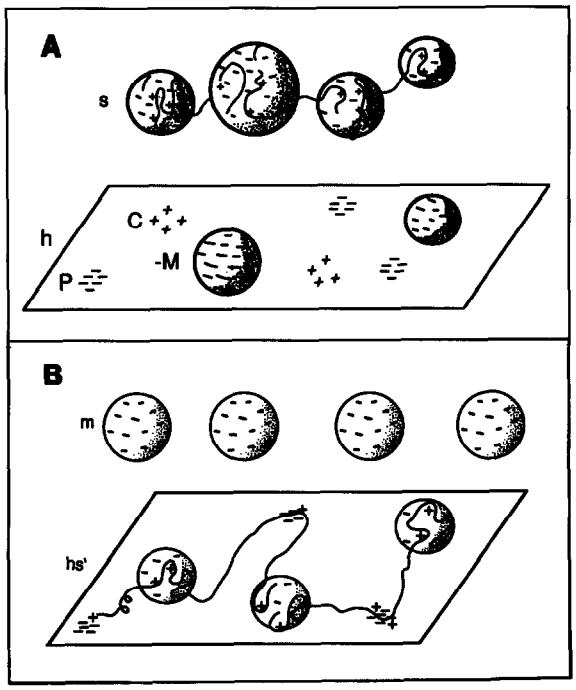

Fig. 5. Schematic illustration of the binding of an SDS–polypeptide complex to hydroxyapatite. (A) An SDS–protein complex (s) depicted according to the "protein-decorated micelle model" [12] approaches a hydroxyapatite surface (h) with P- and C-sites [6] where SDS micelles (M) are bound. (B) Hypothetical model of an equilibrium state with bound polypeptide (hs'). Part of the SDS has been released (micelles m) [13] and charges on the rearranged polypeptide bind electrostatically to P- and C-sites, whereas residual dodecyl sulphate anions interact electrostatically with the C-sites and by hydrophobic interaction and possibly also by hydrogen bonding [21] with the polypeptide.

Fig. 4 and the results illustrated in Fig. 6, middle panel, of ref. 2.

Taking the above into consideration, and using the "protein-decorated micelle structure" [12] for SDS–protein complexes as a starting point, since this is the most detailed model yet available, we envisage that the binding of an SDS–polypeptide complex to hydroxyapatite, schematically and hypothetically, is as illustrated in Fig. 5.

Molecular sieve chromatography separates SDS–protein complexes mainly according to size and shape, as exemplified in ref. 3, and hydroxyapatite chromatography is probably based on polypeptide chain length and the number of charged amino acid side groups, as discussed above. These two methods, in combination, can therefore be expected to give good results in many cases, and require little time with the modern HPLC materials.

ACKNOWLEDGEMENTS

This work was carried out during the stay of P. L. as a "visiting professor" at the Institute for Protein Research, Osaka University. It was supported by the Osaka University, the Swedish Natural Science Research Council and the O.E. and Edla Johansson Science Foundation. We are grateful to E. Greijer for help with the writing of the manuscript, to L. Andersson for useful criticism and to D. Eaker for linguistic improvements.

REFERENCES

1 B. Moss and E. N. Rosenblum, *J. Biol. Chem.*, 247 (1972) 5194.
2 T. Horigome, T. Hiranuma and H. Sugano, *Eur. J. Biochem.*, 186 (1989) 63.
3 E. Mascher and P. Lundahl, *Biochim. Biophys. Acta*, 856 (1986) 505.
4 P. Lundahl, E. Greijer, S. Cardell, E. Mascher and L. Andersson, *Biochim. Biophys. Acta*, 855 (1986) 345.
5 G. Bernardi, *Methods Enzymol.*, 27 (1973) 471.
6 T. Kawasaki, S. Takahashi and K. Ikeda, *Eur. J. Biochem.*, 152 (1985) 361.
7 S. Shimabayashi, H. Tanaka and M. Nakagaki, *Chem. Pharm. Bull.*, 35 (1987) 3539.
8 H. Tanaka, K. Miyajima, M. Nakagaki and S. Shimabayashi, *Chem. Pharm. Bull.*, 37 (1989) 2897.
9 K. Shirahama, K. Tsujii and T. Takagi, *J. Biochem. (Tokyo)*, 75 (1974) 309.
10 S. H. Chen and J. Teixeira, *Phys. Rev. Lett.*, 57 (1986) 2583.
11 X. H. Guo, N. M. Zhao, S. H. Chen and J. Teixeira, *Biopolymers*, 29 (1990) 335.
12 K. Ibel, R. P. May, K. Kirschner, H. Szadkowski, E. Mascher and P. Lundahl, *Eur. J. Biochem.*, 190 (1990) 311.
13 Y. Watanabe, T. Okuno, K. Ishigaki and T. Takagi, *Anal. Biochem.*, (1992) in press.
14 C. Tanford, *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, Wiley, New York, 2nd ed., 1980.
15 U. K. Laemmli, *Nature*, 227 (1970) 680.
16 M. Mueckler, C. Caruso, S. A. Baldwin, M. Panico, I. Blench, H. R. Morris, W. J. Allard, G. E. Lienhard and H. F. Lodish, *Science*, 229 (1985) 941.
17 S. E. Lux, K. M. John, R. R. Kopito and H. F. Lodish, *Proc. Natl. Acad. Sci. U.S.A.*, 86 (1989) 9089.
18 M. Tomita and V. T. Marchesi, *Proc. Natl. Acad. Sci.*, U.S.A., 72 (1975) 2964.
19 M. Wallstén and P. Lundahl, *J. Chromatogr.*, 512 (1990) 3.
20 R. G. Reed, F. W. Putnam and T. Peeters, Jr., *Biochem. J.*, 191 (1980) 867.
21 P. Lundahl, E. Greijer, M. Sandberg, S. Cardell and K.-O. Eriksson, *Biochim. Biophys. Acta*, 873 (1986) 20.