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High performance agarose gel chromatography in sodium dodecyl sulfate of integral membrane proteins from human red cells, with special reference to the glucose transporter

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Integral membrane proteins from human red cells were fractionated in sodium dodecyl sulfate solutions by high performance gel filtration on the small-bead cross-linked agarose gel Superose 6™. The components were identified by acrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The combination of Superose chromatography with electrophoresis afforded high resolution. As expected the gel filtration elution volumes depended essentially on the molecular mass, but the elution volumes decreased stepwise as the detergent concentration was increased from 0.6 to 100 mM, with the largest decrease for the glucose transporter. The resolution increased as the flow rate was decreased from 60 to 1 ml · cm^{-2} · h^{-1}. The M_r values for the anion and glucose transporters as estimated by Superose 6-chromatography at 50 mM detergent were 75–80% of the corresponding M_r values obtained by electrophoresis. At 50 mM dodecyl sulfate the proteins were resolved into four fractions (a–d) which mainly contained: (a) dimer and (b) monomer of the anion transporter, (c) the glucose transporter and (d) components of M_r below 40 000. Monoclonal antibodies that possibly are directed against the glucose transporter (Lundahl, P., Greijer, E., Cardell, S., Mascher, E. and Andersson, L. (1986) Biochim. Biophys. Acta 855, 345–356) interacted only with part of the 4.5-material in fraction c in immunoblotting (Western blotting). Superose 6-chromatography of red cell glucose transporter that had been partially purified on DEAE-cellulose and Mono Q resolved one major and two minor fractions. Electrophoretic analysis showed that components of M_r 90 000, 50 000, and 25 000 had been separated from the major M_r-55 000-4.5-material and revealed size heterogeneity within the major chromatographic fraction. Heating of the glucose transporter in the presence of dodecyl sulfate caused an unexpected retardation of monomeric transporter on Superose 6. The apparent M_r decreased from 44 000 to 29 000.

Introduction

Membrane proteins can be solubilized in detergents for the purpose of fractionation. Among the most powerful dissociating detergents is sodium dodecyl sulfate, which denatures water-soluble proteins by a special stoichiometric binding, possibly by hydrogen bonding between the oxygens in the sulfate group and peptide bond nitrogens (Lundahl, P., Greijer, E. and Cardell, S., unpublished hypothesis). Furthermore, this detergent solubilizes most membrane proteins into mainly di- and monomeric forms [1–3].

Membrane proteins can, for chemical investigation, be separated in sodium dodecyl sulfate for instance by gel electrophoresis [4], hydroxyapatite

chromatography [5] or gel filtration [6–10]. Improved resolution can be expected in molecular-sieve chromatography by the use of the recently introduced small-bead Superose 6™ gel. Gel electrophoresis gives unsurpassed resolution, but gel filtration affords simpler recovery of the separated proteins and is easier to scale up. Chromatography of water-soluble proteins in sodium dodecyl sulfate on another type of small-bead cross-linked agarose gel has recently been described by Eriksson [11].

We have here investigated fractionation of integral membrane proteins from human red cells. Due to heterogenous glycosylation, some of these proteins elute in relatively broad peaks and the separation of the major components might be incomplete. The aim of our work has been to analyze the resolution of integral membrane proteins upon chromatography on a small-bead agarose gel in sodium dodecyl sulfate, with special emphasis on the purification of the glucose transporter from human red cell membranes.

Materials and Methods

Materials

Human red cell concentrate (4–5 weeks old) was supplied by the Blood Bank at the Academic Hospital, Uppsala, Sweden. Sodium dodecyl sulfate for chromatography was Merck-Schuchardt (F.R.G.) No. 13760 ('für biochemische Zwecke') and No. 822050 (90% detergent and 10% inorganic salts) for electrophoresis. Dithioerythritol, No. 8255 was purchased from Sigma (U.S.A.). Calibration proteins for chromatography and electrophoresis (high- and low-molecular weight markers) were from Pharmacia (Sweden).

All solutions were filtered through 0.2 μm filters (Sartorius, SM11107) and degassed. Other chemicals were pro analysi unless otherwise stated.

Methods

Preparation of integral membrane proteins. Human red cell membranes were prepared and stripped of peripheral proteins by a tandem-column procedure combined with centrifugation [12]. The integral membrane proteins and membrane lipids were immediately frozen in liquid nitrogen and stored at −70°C.

Solubilization. All procedures, except storage, were done at 21–24°C. Membranes were stirred for 15 min with 0.1 M sodium dodecyl sulfate in solution P containing 0.1 M phosphate, pH 7.4, 1 mM EDTA and 1 mM dithioerythritol. The membrane protein concentration was 2 mg/ml. A minimal insoluble residue was sedimented down during 60 min at $160\,000 \times g$. The supernatant was collected in small aliquots and stored at −20°C.

Gel filtration. Gel filtration (molecular-sieve chromatography) was done at 21–24°C on a Superose 6™ gel consisting of 13 ± 2 μm beads of cross-linked agarose. A prepacked 22 ml Superose 6-column, 28×1.0 cm (column no. 5045120) was connected to a pump, a sample injection valve and a detector for light absorbance at 280 nm; P-500, V-7 and UV-1 respectively, from Pharmacia (Sweden).

The routine chromatographic experiments were done at a flow rate of 9 ml/h (11 $ml \cdot cm^2 \cdot h^{-1}$). Studies of the effect of flow rate on resolution were done at flow rates from 45 to 1 ml/h. When the concentration of dodecyl sulfate was to be changed, the column was equilibrated for at least 10 h at 3 ml/h. Before each day's experiments one column volume of fresh buffer was pumped through the system. At room temperature, sodium dodecyl sulfate concentrations up to at least 100 mM can be used. A sample volume of 100 μl was applied to the column, unless otherwise stated. The column was calibrated at 5 mM and 50 mM detergent with: Blue dextran 2000, β-galactosidase (M_r 116 000), serum albumin (66 200), ovalbumin (43 800), aldolase (39 500), chymotrypsinogen A (25 000), RNAs (13 700) and NaCl. All calibration proteins were reduced at 95°C and *S*-carboxymethylated. Calibration diagrams were plotted as $-\log K_d$ versus $M_r^{2/3}$ (Refs. 11 and 13) and gave straight lines.

After several experiments adsorbed material was removed by passing 400 μl of 70% formic acid through the column. This caused some retardation and partial adsorption of the integral membrane proteins and the yield decreased to 35% (Table I). Chromatography of another 100 μl aliquot of integral membrane proteins (200 μg protein with about 550 μg membrane lipids) or one 500 μl aliquot of egg yolk phospholipids (1 mg) restored the yield (Table I), but two additional 100 μl

TABLE I

YIELD OF INTEGRAL MEMBRANE PROTEINS FROM HUMAN RED CELLS, UPON SUPEROSE 6-CHROMATOGRAPHY

Before chromatography of sample 1, the column was washed with (A), 70% formic acid or (B), 70% formic acid followed by egg yolk phospholipids (see Methods). Eluent: solution P (see Methods) with 50 mM sodium dodecyl sulfate. Protein amount: 200 μg per experiment.

Sample No.	Yield (%) [a]	
	(A)	(B)
1	35	92
2	100	100

[a] Estimated by the peak areas of eluted material.

aliquots of the membrane protein-lipid mixture were needed to restore the normal elution patterns.

Electrophoresis. Electrophoretic analyses were done as described in Ref. 12 (cf. Ref. 14) at 65–300 V, 18 mA, for 24 h. The separation gel had a linear gradient in acrylamide concentration of 8–25%. The gel slabs were silver-stained as in Ref. 14 (cf. Ref. 15).

Fractionation of a glucose transporter preparation. Purified glucose transporter (preparation OG-P in Ref. 12) was dissolved with 0.1 M sodium dodecyl sulfate in solution P (see 'Solubilization' in this section) to give a final protein concentration of 0.8 mg/ml. A 200 μl sample was applied to the Superose 6-column which was equilibrated in 5 mM or 50 mM sodium dodecyl sulfate.

Immunoblotting. Monoclonal antibodies were prepared after immunizing Balb/c mice with octyl glucoside solubilized 4.5-material (preparation OG-A in Ref. 12). Chromatographically separated integral membrane proteins were fractionated by gel electrophoresis and transferred to a nitrocellulose paper. The nitrocellulose paper was then incubated with the monoclonal antibodies and subjected to an enzyme-linked immunosorbent assay (ELISA). A detailed account of the production of the monoclonal antibodies and the immunoblotting procedures is given in Ref. 12.

Results

A. Chromatographic fractionation of integral membrane proteins

Integral membrane proteins from human red cells were fractionated by molecular-sieve chromatography on a Superose 6-column in sodium dodecyl sulfate. Superose 6 afforded higher resolution than the similar, but denser gel Superose 12.

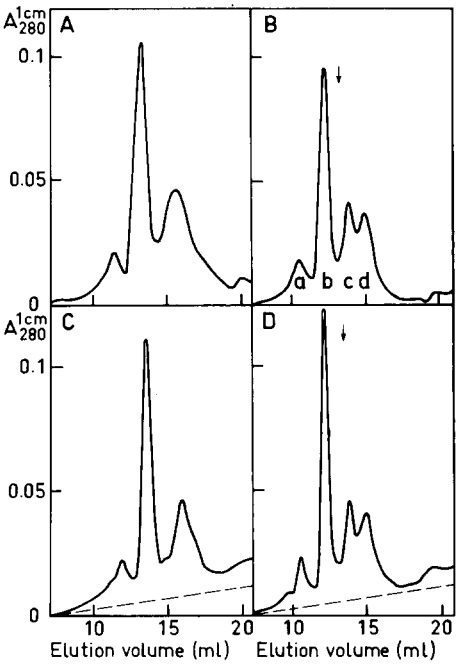

Fig. 1. Chromatography of integral membrane proteins from human red cells prepared as in Ref. 12, on Superose 6™. Eluent: solution P (see Methods) with 2.5 mM (A and C) or 50 mM sodium dodecyl sulfate (B and D). Flow rate: 9 ml/h (A and B) or 1 ml/h (C and D). The protein concentration was 2 mg/ml and 100 μl samples were applied. The arrows in B and D indicate the elution volume of the monomer of the anion transporter at 2.5 mM sodium dodecyl sulfate (A and C). The fractions a–d, panel B, were analyzed by gel electrophoresis (Fig. 5, below). The dashed lines in C and D indicate the baseline slope caused by oxidation of dithioerythritol. Elution time in A and B was about 2.5 h and in C and D around 23 h. Each panel comprises elution volumes from $V_0 = 8.2$ ml to $V_t = 22.2$ ml.

A.1. Effect of sodium dodecyl sulfate concentration on elution pattern. At 2.5 mM sodium dodecyl sulfate the integral membrane proteins separated into two main fractions on Superose 6, whereas chromatography in 50 mM detergent achieved separation of three major fractions, b–d (Fig. 1, panels A and B). The elution volumes for the integral membrane proteins as well as water-soluble proteins decreased stepwise when the concentration of sodium dodecyl sulfate increased

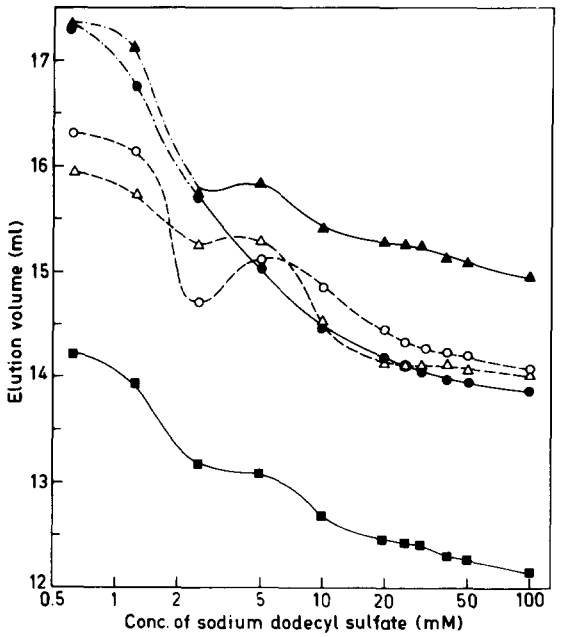

Fig. 2. Elution volumes for the major fractions of human red cell integral membrane proteins on Superose 6 as a function of the concentration of sodium dodecyl sulfate. (▲———▲), low-molecular-weight components, (●———●), the glucose transporter and (■———■), the anion transporter (d, c and b, respectively, in Fig. 1B). For comparison, the curves for aldolase (△- - -△) and ovalbumin (○- - -○) are shown. Except for the varying detergent concentration the conditions were as in Figs. 1A and B. At low detergent concentration fractions c and d (see Fig. 1B) were not resolved and the elution volumes were estimated by electrophoresis (·-·-·).

(Fig. 2). The decrease was most pronounced with the glucose transporter (Fig. 2, ●). The largest step occurred between 1 and 2.5 mM dodecyl sulfate and might correspond to the conversion from binding of 0.4 g to binding of 1.4 g detergent per g protein, that has been reported for some reduced proteins, including red blood cell membrane proteins [16,17]. Integral membrane proteins may bind still more detergent. Our results indicate that a second smaller transition might take place between 5 mM and 10 mM dodecyl sulfate (Fig. 2).

Partially purified glucose transporter (see Methods) was eluted in one major fraction at a volume corresponding to fraction c in Fig. 1B at 50 mM and 0.75 ml later at 5 mM sodium dodecyl sulfate (Fig. 3).

A.2. Chromatographic resolution as a function of flow rate. A decrease in flow rate increased the

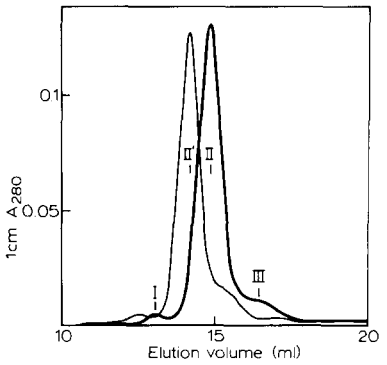

Fig. 3. Chromatography on Superose 6 of partially purified glucose transporter from human red cell membranes. The material applied was the transporter preparations OG-P described in Ref. 12, (cf. Methods). Eluent: solution P (see Methods) and 5 mM (thick line) or 50 mM (thin line) sodium dodecyl sulfate. Sample volume: 200 μl. Amount of protein: 170 μg. Fractions I, II and III were analyzed by gel electrophoresis (Fig. 7) and corresponded to apparent chromatographic M_r values of 71500, 37000 and 20500, respectively. The total protein yield in the chromatographic experiment at 5 mM detergent was about 100% as determined by quantitative amino acid analysis. Fraction II corresponds to about 80% or 135 μg of protein.

resolution of the integral membrane proteins and sharpened the peaks (Fig. 1, panels B and D). However, at low concentration of sodium dodecyl

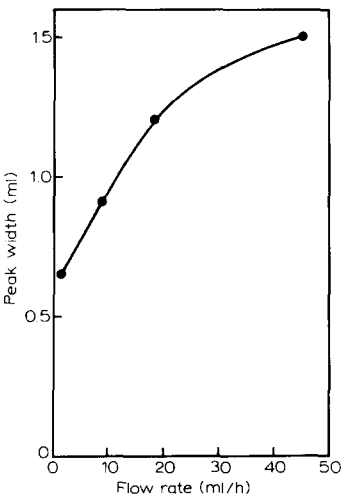

Fig. 4. The peak width in Superose 6-chromatography for the anion transporter as a function of flow rate. Conditions as in Fig. 1, except that the detergent concentration was 5 mM and that the flow rate was varied. The widths were measured at half the height of the peaks.

sulfate fractions c and d were not resolved even at 1 ml/h (Fig. 1, panels A and C). The peak width increased linearly with increasing flow rate in the interval 1–18 ml/h (Fig. 4). At 9 ml/h the resolution was reasonably good and fractionation was completed in approx. 2.5 h. The large sizes of the protein-detergent complexes give slow mass transfer which obviously lowers the resolution at high flow rates.

B. Analyses of chromatographic fractions

B.1. Integral membrane proteins. Fractions a and b in Fig. 1B contained relatively pure anion transporter (Fig. 5), probably as dimer and monomer, respectively. Fractions c and d were found to contain several components each, although the glucose transporter dominated in fraction c (Fig. 5). The electrophoretic pattern showed in all about 30 bands. The anion and glucose transporters formed the well-known broad bands 3 and 4.5, respectively (denotations according to Steck, Ref. 18).

The immunoblotting experiments showed that the monoclonal antibodies interacted only with material, mainly the glucose transporter, in the band 4.5-region and not with other proteins (Fig. 6A). Furthermore, the monoclonal antibodies bound only with the upper two-thirds of this region (Fig. 6B), in the M_r interval 52000–65000 (compare with Table II). Since the glucose trans-

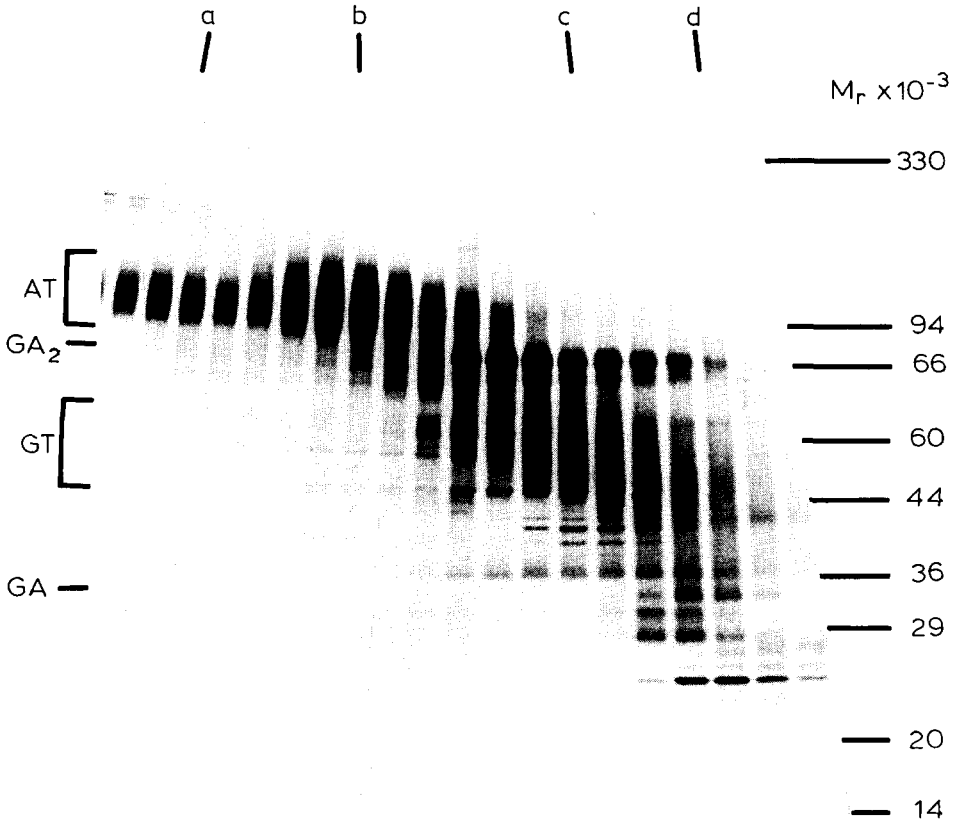

Fig. 5. Sodium dodecyl sulfate gel electrophoresis of human red cell integral membrane proteins fractionated on Superose 6. All conditions as in Fig. 1B. About 5 μg of protein was applied in a well of cross-section 1.0×6 mm. The proteins were silver-stained. Calibration proteins: thyroglobulin (M_r 330000), phosphorylase B (94000), serum albumin (66200), catalase (60000), ovalbumin (43800), lactate dehydrogenase (36000), carbonic anhydrase (29000), soybean trypsin inhibitor (20100) and α-lactalbumin (14400). Positions a, b, c and d correspond to the fractions in Fig. 1B. For electrophoretic conditions and sample preparation see Methods. The positions of the anion transporter (AT), the glucose transporter (GT) and the dimer (GA_2) and monomer (GA) of glycophorin A are indicated in the figure.

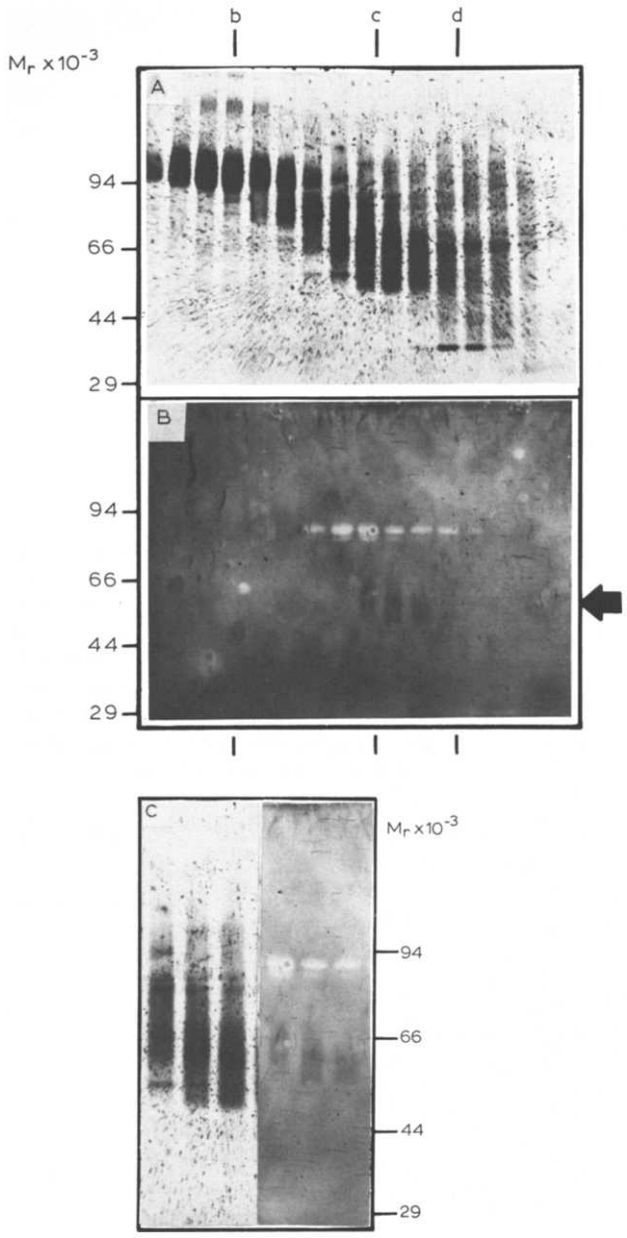

Fig. 6. Identification of immunoreactive 4.5-material in electrophoretic analysis of Superose 6-fractions of integral membrane proteins from human red cells. The Superose 6-chromatography was done as in Fig. 1B. The chromatographically separated proteins were transferred to a nitrocellulose paper and were incubated with monoclonal antibodies [12] and the immunoreactive material was detected by use of an enzyme-linked immunosorbent assay. (A) The silver-stained gel, after transfer; (B) the immunoblotting pattern; (C) detail of A and B. Interaction is seen only with proteins in the band 4.5-region (Fig. A and B), arrow. Fractions b, c and d correspond to fractions b, c and d in Figs. 1B and 5. The white bands above the 4.5 zone in B and C correspond to glycophorin A dimers.

porter is the major 4.5 component the antibodies probably are directed against this protein.

B.2. Glucose transporter preparation. The electrophoretic analysis of the fractions I–III in Fig. 3 showed that the glucose transporter (II in Fig. 7) was separated from a minor amount of an unknown component, I, with an approximate M_r of 90000. The origin of component III will be discussed in section D, below.

At the front of fraction II (Fig. 3) some material of higher apparent M_r is eluted (Fig. 7). This, together with the broad shape of the bands is probably due to heterogeneity in glycosylation of the glucose transporter [19,20]. The amino acid composition of the purified glucose transporter was essentially the same as reported by Baldwin and co-workers [21] and was also similar to the composition of the glucose transporter in human HepG2 hepatoma cells [22].

C. Effect of heating

C.1. Integral membrane proteins. The proteins were heated for 5 min with 0.1 M sodium dodecyl sulfate in solution P (see Methods), except that dithioerythritol was excluded, and were fractionated on Superose 6 in 50 mM sodium dodecyl sulfate. Contrary to the case with non-heated proteins (Fig. 1B) the heated components separated only into two major fractions (Fig. 8). The 4.5-material corresponding to fraction c (Fig. 1B) was unexpectedly retarded and eluted at an apparent M_r of 29000 (elution volume 15.0 ml, Fig. 2), whereas the anion transporter was slightly retarded (Table II). Reduction and *S*-carboxymethylation in combination with heating gave the same result.

C.2. Glucose transporter preparation. Purified glucose transporter was heated and chromatographed in the same way as the integral membrane proteins in section C.1, above. Normally molecular-sieve chromatography of the glucose transporter gives one major fraction (Fig. 9, thin line, cf. Fig. 3), but chromatography of heated transporter resulted in two fractions (Fig. 9, thick line). The broad peak α in Fig. 9 (thick line) was found to contain tetra-, tri-, and dimers of the glucose transporter whereas the fraction β that eluted afterwards contained mainly the monomer (Fig. 10A). In agreement with the results in the section

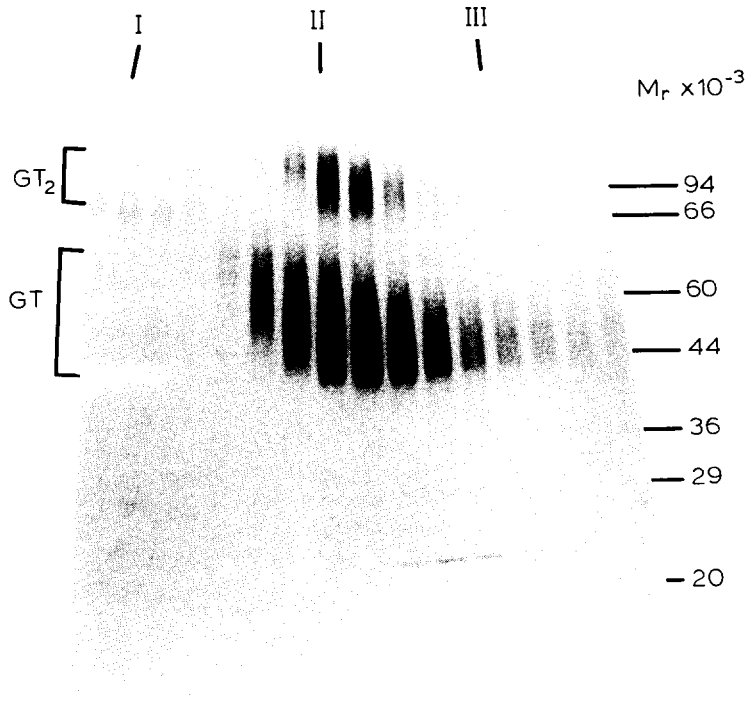

Fig. 7. Sodium dodecyl sulfate gel electrophoresis of partially purified glucose transporter that had been fractionated on Superose 6, as illustrated in Fig. 3. Electrophoresis and calibration were done as in Fig. 5. I, II and III indicate the positions of the corresponding fractions in Fig. 3. The positions of the dimer (GT_2) and the monomer (GT) of the glucose transporter are given in the figure.

above, the fraction β-monomer was strongly retarded compared to the non-heated transporter. Even here reduction and *S*-carboxymethylation combined with heating did not alter the result (compare section C.1).

When the glucose transporter was incubated at

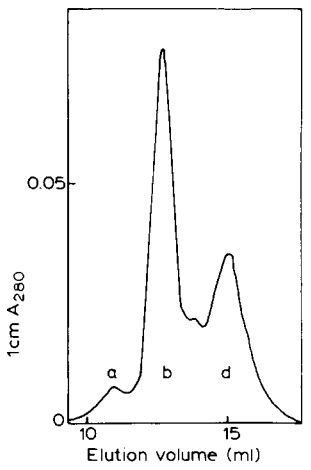

Fig. 8. Chromatography of heated integral membrane proteins from human red cells on Superose 6. The material was heated to 95°C and kept at this temperature for 5 min in solution P (see Methods) with 0.1 M sodium dodecyl sulfate. Chromatographic conditions as in Fig. 1B. Elution volumes a, b and d correspond to those of the fractions in Fig. 1B.

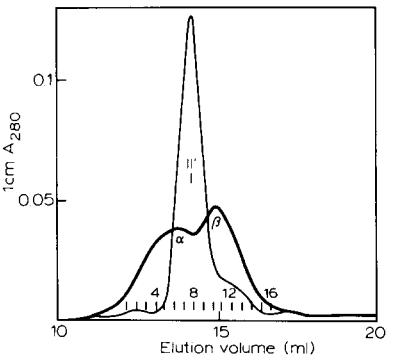

Fig. 9. Chromatography on Superose 6 of partially purified and heated glucose transporter from human red cell membranes. Transporter preparation OG-P (see Fig. 3) was heated to 95°C for 5 min at a protein concentration of 0.8 mg/ml in solution P (see Methods) with 0.1 M dodecyl sulfate (cf. Fig. 8). Thick line, heated transporter; thin line, non-heated transporter, fractionation identical with that in Fig. 3. Chromatography was done at 50 mM detergent as in Fig. 1B. Electrophoretic analyses of fractions 1-16 of heated transporter are shown in Fig. 10. α and β symbolizes the two main fractions of heated transporter.

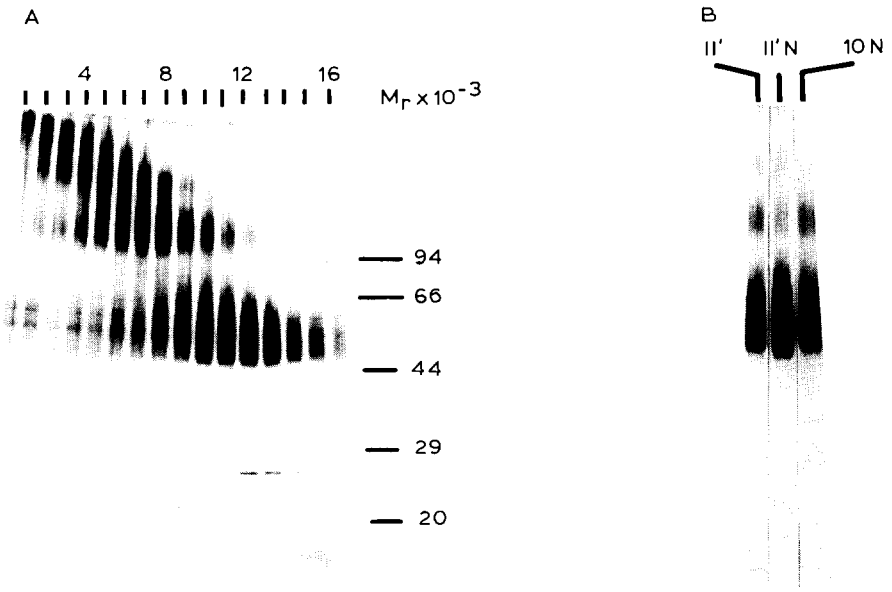

Fig. 10. Sodium dodecyl sulfate gel electrophoresis of Superose 6-fractions of partially purified, heated and non-heated, glucose transporter. (A) Fractions 1–16 of heated transporter (Fig. 9, thick line); (B) II′, fraction II′ (Fig. 9, thin line); II′N, fraction II′ (Fig. 9, thin line), not heated before electrophoresis; 10N, fraction 10 (fig. 9, thick line), not heated before electrophoresis. In each well was a sample volume of 20 μl applied.

95°C, either before chromatography or before electrophoresis, the electrophoretic migration distance was the same as for non-heated transporter (Fig. 10B).

D. Review of apparent molecular weights

The apparent M_r values at which the integral membrane proteins eluted were found to be about 17% higher in 50 mM sodium dodecyl sulfate than

TABLE II

APPARENT M_r VALUES ON SUPEROSE 6 OF MAJOR HUMAN RED CELL INTEGRAL MEMBRANE PROTEINS

According to calibrations with water-soluble proteins (see "Methods") at sodium dodecyl sulfate concentrations of 5 mM and 50 mM and by sodium dodecyl sulfate gel electrophoresis.

	SDS (mM)	Apparent M_r value ($\times 10^{-3}$)			
		Anion transporter		Glucose transporter	Components of low M_r
		Dimer	Monomer		
	Fraction [o]:	a	b	c	d
Chromatography	5	120	68	36.5, 37 [p]	–
	50	145	78, 70 [q]	44[s], 29 [q,t]	29, 29 [q,t]
Electrophoresis		230	105	55 (45–65), (50–70) [r]	20–40

[o] Notation as in Figs. 1B, 5 and 8.
[p] Purified glucose transporter (Fig. 3).
[q] Heated proteins (Fig. 8).
[r] Purified glucose transporter (Fig. 7).
[s] Peak c in Fig. 1B and purified glucose transporter (Figs. 3 and 9).
[t] Heated purified glucose transporter (Fig. 9).

in 5 mM detergent (Table II) using calibration curves for chromatography of water-soluble proteins at 5 mM and 50 mM detergent, respectively (cf. Fig. 2). For instance, the M_r values of the anion and the glucose transporter were 78 000 and 44 000 in 50 mM sodium dodecyl sulfate whereas the values obtained in 5 mM detergent were 68 000 and 36 500, respectively (Table II).

Heating of the purified glucose transporter had a striking effect (Fig. 9, thick line). After incubation for 5 min at 95°C the transporter eluted at 29 000 instead of 44 000, in 50 mM sodium dodecyl sulfate (Table II). In the analysis (Fig. 7) of fractions collected from the experiment shown in Fig. 3 (thick line) the glucose transporter was found to be separated from two minor components. The component III eluted after the glucose transporter in 5 mM sodium dodecyl sulfate molecular-sieve chromatography (Fig. 3, thick line), but seems to have almost the same apparent M_r as the glucose transporter (component II) in electrophoresis (Fig. 7). Possibly small amounts of the glucose transporter are converted to a form resembling that of the heated transporter even at room temperature (cf. Fig. 9). The material in fraction III (Fig. 3) would thus be the glucose transporter altered as if by heating.

The M_r values differed markedly in determinations by chromatography as compared to determinations by electrophoresis (Table II). For example, the apparent M_r values corresponding to fractions b and c (Fig. 1B) were estimated at 78 000 and 44 000, respectively, as mentioned above. In electrophoresis (Fig. 5) the same fractions were estimated to 105 000 and 55 000, respectively (see Table II).

Discussion

Gel filtration (molecular-sieve chromatography) as well as acrylamide gel electrophoresis separate complexes between proteins and sodium dodecyl sulfate essentially according to shape and size. However, in the two-dimensional pattern provided by combination of these two methods integral membrane proteins did not fall strictly on a diagonal line and at least 30 components from the stripped red cell membrane were resolved. In fact, the glycosylated anion and glucose transporter were separated better by the molecular-sieve chromatography on the small-bead agarose gel Superose 6 in combination with multi-well acrylamide electrophoresis, both in the presence of sodium dodecyl sulfate (Figs. 1 and 5), than by two-dimensional electrophoresis with electrofocusing in the first dimension [12]. In the latter methods the glucose transporter focuses at varying pH values depending on the sample treatment [12]. Similarly, Superose chromatography gave a considerably increased elution volume for the glucose transporter after heating of the protein sample. This indicates a drastic change in the conformation of the transporter-dodecyl sulfate-complex upon heating. The nature of this change is not yet known, but it seems that heating induces irreversible release of some detergent. A similar, but smaller, effect in electrophoresis has been reported by Allard and Lienhard [23]. However, our gradient gel electrophoresis showed no difference between heated and non-heated samples (Fig. 10B).

The chromatographic resolution of protein-dodecyl sulfate complexes on the cross-linked small-bead agarose gel was high at moderately high flow rates. The elution volumes for all proteins that were chromatographed decreased with increasing dodecyl sulfate concentration. The stepwise pattern for the elution volume as a function of detergent concentration for the anion transporter, aldolase and ovalbumin indicates that the dodecyl sulfate might bind to the proteins causing stepwise conversion from one conformation to another (cf. Ref. 16). It is also possible that dodecyl sulfate micelles compete with dodecyl sulfate-protein complexes for interaction with certain groups on the cross-linked agarose gel. The integral membrane proteins were somewhat retarded compared to water-soluble proteins, probably by virtue of a different structure of the protein-detergent complex. It has been reported that dodecyl sulfate binds to water-soluble proteins in the ratio 1.4 g detergent per g protein [16,17] whereas integral membrane proteins may bind detergent in a ratio that varies from protein to protein [16,24–27]. A model for stoichiometric binding of dodecyl sulfate to water-soluble proteins was mentioned in Introduction. Some integral membrane proteins, for instance the human red cell anion and glucose transporters, may have

several hydrophobic helices as basic structural elements, for example 12 as is the case for the murine red cell anion and the human hepatocyte glucose transporters [28,22]. It is tempting to speculate that these helices might be included in a detergent micelle. The deviant detergent binding and the presence of sizeable carbohydrate moieties in integral membrane proteins affect gel filtration and electrophoresis behaviour in different ways. In fact, the electrophoretically estimated M_r values for the red cell anion and glucose transporters appear to be nearly correct [12,28,22], whereas the corresponding values from Superose 6-chromatography are lower.

Washing of the Superose gel with 70% formic acid (see Methods) seemed to expose some groups with fairly high affinity for integral membrane proteins and lipids. These groups were easily saturable and presented no major problem.

Immunoblotting after fractionation of the integral membrane proteins (Figs. 6A and B) showed that the silver-stained 4.5-region was heterogeneous; only the two upper thirds reacted with the monoclonal antibodies which had been prepared after immunization with a preparation of glucose transporter. Reaction with other components in the electrophoretic pattern could not be detected (cf. Refs. 29 and 30), and the two sharp bands in the lower part of the 4.5-material that were present before chromatographic fractionation [12] were not observed. The lower third of the 4.5-material that did not react with our monoclonal antibodies might correspond to part of the nucleoside transporter.

Superose 6-chromatography proved to be efficient for a final purification of the red cell glucose transporter and revealed heterogeneity of the dimer as well as the monomer zones seen in one-dimensional electrophoresis (Fig. 7, cf. Ref. 12).

Acknowledgements

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References

1 Guidotti, G. (1972) Annu. Rev. Biochem. 41, 731–752
2 Steck, T.L. and Fox, C.F. (1972) in Membrane Molecular Biology (Fox, C.F. and Keith, A.D., eds.), p. 27, Sinauer Press Associaties, Stanford, CT
3 Steck, T.L. and Yu, J. (1973) J. Supramol. Struct. 1, 220–232
4 Maizel, J.V., Jr. (1969) in Fundamental Techniques in Virology (Habel, K. and Salzman, N.P., eds.) pp. 334–362, Academic Press, New York
5 Moss, B. and Rosenblum, E. (1972) J. Biol. Chem. 247, 5194–5198
6 Konishi, T. (1982) Methods Enzymol. 88, 202–207
7 Konishi, T. and Sasaki, K. (1982) Chem. Pharm. Bull. 30, 4208–4212
8 Fish, W.W., Reynolds, J.A. and Tanford, C. (1970) J. Biol. Chem. 245, 5166–5168
9 Welling, G.W., Nijmeijer, J.R.J., Van der Zee, R., Groen, G., Wilterdink, J.B. and Welling-Wester, S. (1984) J. Chromatogr. 297, 101–109
10 Lüdi, H. and Hasselbach, W. (1984) J. Chromatogr. 297, 111–117
11 Eriksson, K.-O. (1985) J. Biochem. Biophys. Methods 11, 145–152
12 Lundahl, P., Greijer, E., Cardell, S., Mascher, E. and Andersson, L. (1986) Biochim. Biophys. Acta 855, 345–356
13 Hjertén, S. (1970) J. Chromatogr. 50, 189–208
14 Lundahl, P., Greijer, E., Lindblom, H. and Fägerstam, L.G. (1984) J. Chromatogr. 297, 129–137
15 Tunón, P. and Johansson, K.-E. (1984) J. Biochem. Biophys. Methods 9, 171–179
16 Reynolds, J.A. and Tanford, C. (1970) Proc. Natl. Acad. Sci. USA 66, 1002–1007
17 Reynolds, J.A. and Tanford, C. (1970) J. Biol. Chem. 245, 5161–5165
18 Steck, T.L. (1974) J. Cell Biol. 62, 1–19
19 Gorga, F.R., Baldwin, S.A. and Lienhard, G.E. (1979) Biochem. Biophys. Res. Commun. 91, 955–961
20 Lienhard, G.E., Crabb, J.H. and Ransome, K.J. (1984) Biochim. Biophys. Acta 769, 404–410
21 Baldwin, S.A., Baldwin, J.M. and Lienhard, G.E. (1982) Biochemistry 21, 3836–3842
22 Mueckler, M., Caruso, C., Baldwin, S.A., Panico, M., Blench, I., Morris, H.R., Allard, W.J., Lienhard, G.E. and Lodish, H.F. (1985) Science 229, 941–945
23 Allard, W.J. and Lienhard, G.E. (1985) J. Biol. Chem. 260, 8668–8675
24 Simons, K. and Helenius, A. (1970) FEBS Lett. 7, 59–63
25 Robinson, N.L. and Tanford, C. (1975) Biochemistry 14, 369–378
26 Makino, S. and Niki, R. (1977) Biochim. Biophys. Acta 495, 99–109
27 Grefrath, S.P. and Reynolds, J.A. (1974) Proc. Natl. Acad. Sci. USA 71, 3913–3916
28 Kopito, R.R. and Lodish, H.F. (1985) Nature 316, 234–238
29 Kay, M.M.B. (1985) Proc. Natl. Acad. Sci. USA 82, 311–324
30 Young, J.D., Jarvis, S.M., Robins, M.J. and Paterson, A.R.P. (1983) J. Biol. Chem. 258, 2202–2208