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HIGH-PERFORMANCE AGAROSE GEL CHROMATOGRAPHY IN OCTYL GLUCOSIDE OF INTEGRAL MEMBRANE PROTEINS FROM HUMAN RED CELLS, WITH SPECIAL REFERENCE TO THE GLUCOSE TRANSPORTER

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SUMMARY

Integral membrane proteins and lipids from human red cells were fractionated in the presence of octyl glucoside by high-performance gel chromatography on a 22-ml column of the small-bead cross-linked agarose gel Superose 6, at 5°C, pH 7.6 and 30–50 m*M* detergent. To avoid aggregation a relatively high flow-rate, 9 ml/h, was chosen. At low ionic strength four main fractions were resolved, which contained anion transporter multimers(I), glycophorin oligomers(II), glucose transporter dimers(III) and phospholipids(IV). In 0.5 *M* sodium chloride the resolution was lower but the yield of the glucose transporter was markedly higher, and chromatography of partially purified glucose transporter gave a protein recovery of about 90%. The apparent M_r values for the octyl glucoside complexes of the main components were: anion transporter, 900000; glycophorin A, 210000–360000, dependent on ionic strength; glucose transporter, 110000–160000; lipids, 70000. Some components aggregated with time: at a flow-rate of 1 ml/h mainly glycophorins and the glucose transporter were eluted, but no anion transporter, and fractionation performed 20 h after solubilization showed extensive aggregation of proteins. Superose-6 chromatography of glucose transporter and membrane lipids that had been isolated on DEAE-cellulose partially resolved the transporter and the phospholipid fractions. In this case, the resolution was better with 50 than with 30 m*M* detergent. The maximum glucose transport activity was approximately one-tenth of that observed before fractionation and appeared in two main fractions, at the main transporter fraction as well as at the overlap between the transporter and the lipids. The activity level was the same in both fractions, although the protein concentration was much lower in the second one. Addition of 2 m*M* egg-yolk phospholipids to the eluent did not increase the activity. This strongly indicates that the glucose transporter needs some specific membrane lipids to retain high transport activity. At the concentration of *ca.* 0.3 mg/ml used, the glucose transporter was probably eluted as a dimer in the absence of phospholipids and as a monomer in their presence.

INTRODUCTION

The solubilization and fractionation of membrane proteins requires the use of

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detergents. For chemical investigations, sodium dodecyl sulphate has been used in the fractionation of membrane proteins[1–5], but this detergent denatures most proteins[6]. To retain biological activity, a milder detergent must often be used, for instance cholate[7], Triton X-100[8–11] or octyl glucoside[12–15].

Previously we reported on the separation of integral membrane proteins from human red cells on Superose 6, in sodium dodecyl sulphate[5]. We now report the use of the Superose 6 gel to separate these proteins in the presence of *n*-octyl-β-D-glucopyranoside (octyl glucoside), a non-ionic detergent of high critical micelle concentration[15]. This detergent was chosen because it can easily be removed in lipid vesicle reconstitution experiments[12,13,16,17], and also since it can be kept bound to membrane proteins during crystallization[18,19]. However, a major obstacle to the use of non-ionic detergents is that the protein–detergent complexes often form aggregates with time. The high flow-rate that can be used on Superose 6 shortens the separation time, which reduces the aggregation effect during fractionation.

The aim of our study was to find suitable conditions for fractionation on Superose 6 of the integral membrane proteins in octyl glucoside. Special emphasis was placed on the purification of the active glucose transporter and on observations of its state of aggregation, which is of interest for crystallization experiments.

Recently Chen *et al.*[20] reported the fractionation of human red cell ghost proteins on another size-exclusion medium, TSK 3000SW, in octyl glucoside.

MATERIALS AND METHODS

Materials

Human red cell concentrate (stored for 4–5 weeks) was supplied by the Blood Bank of the Academic Hospital, Uppsala. Octyl glucoside (No. 8001) and dithioerythritol (No. 8255) were obtained from Sigma (St. Louis, MO, U.S.A.). Sodium dodecyl sulphate for electrophoresis (No. 822050, 90% detergent, 10% inorganic salts) was obtained from Merck-Schuchardt (Darmstadt, F.R.G.). Several calibration proteins (high- and low-molecular-weight markers) for chromatography and electrophoresis were obtained from Pharmacia (Uppsala, Sweden), carbonic anhydrase and β-galactosidase (Grade VI) from Sigma.

All solutions were passed through 0.2-μm filters (Sartorius, SM 11107) and degassed. Unless otherwise stated the chemicals were of analytical grade.

Methods

Preparation of integral membrane proteins. Human red cell membranes were prepared and stripped of peripheral proteins by a tandem-column procedure in combination with centrifugation[13]. The integral membrane proteins and membrane lipids were immediately frozen in liquid nitrogen and stored at −70°C.

Solubilization. These procedures were performed at 2°C. The stripped membranes were stirred for 20 min with 50 m*M* octyl glucoside in 50 m*M* Tris–HCl (pH 7.0, measured at 22°C) and 1 m*M* dithioerythritol (DTE) (*cf.*, ref. 12). The membrane protein concentration was 4 mg/ml. Insoluble material was sedimented during 60 min at 160 000 *g*. The supernatant was collected and fractionated on DEAE-cellulose or Superose 6.

Gel filtration. High-performance gel filtration (molecular-sieve or size-exclu-

sion chromatography) was performed at 5°C on a 28 cm × 1.0 cm Superose 6 column (Pharmacia). Injection valve V-7, pump P-500 and detector UV-1 from Pharmacia were used. Unless otherwise stated, the column was equilibrated with 50 m*M* octyl glucoside in a solution which we denote Tris–DTE, containing 50 m*M* Tris–HCl (pH 7.6, measured at 22°C) and 1 m*M* DTE. The chromatographic experiments were carried out at a flow-rate of 9 ml/h and the sample volumes were 0.5 or 1 ml. These relatively large volumes were chosen with the object of retaining measurable amounts of glucose transport activity after fractionation. The column was calibrated in 50 m*M* Tris–HCl (pH 7.6) containing 0.5 *M* sodium chloride, with tobacco mosaic virus (distribution coefficient, $K_d = 0$), thyroglobulin (molecular weight, M_r 669000 and 335000), β-galactosidase (dimer: M_r 232000 and 116000), bovine serum albumin (dimer: M_r 132000 and 66200), carbonic anhydrase (M_r 29000), RNase (M_r 13700) and potassium dichromate ($K_d = 1$). A plot of $-\log K_d$ *versus* $M_r^{2/3}$ (*cf.*, refs. 21 and 22) was constructed. The non-ionic detergent Triton X-100 does not bind to water-soluble proteins[23]; the same is probably true for octyl glucoside, since calibrations with or without octyl glucoside gave the same elution volumes for RNase, bovine serum albumin and β-galactosidase. The calibration was linear from RNase ($M_r^{2/3} = 572$, $-\log K_d = 0.097$) to thyroglobulin ($M_r^{2/3} = 7650$, $-\log K_d = 0.607$) (not shown). All apparent M_r values in this paper refer to chromatographic estimations, if not otherwise stated.

Preparation of glucose transport protein. A 2.3-ml sample of octyl glucoside/solublized integral membrane proteins and lipids was applied to a 5.7-ml column of DEAE-cellulose (Whatman DE-52), equilibrated with 30 m*M* octyl glucoside at pH 7.0 (measured at 22°C)[12,13]. The material that passed straight through the column contained mainly the glucose transporter and some membrane lipids and was collected. The purification was performed at 5°C.

Electrophoresis. Electrophoretic analyses were carried out as described in refs. 4 and 5, except that DTE was not added to the electrophoretic samples and the proteins were not heated before electrophoresis. Accordingly, the DTE concentration in the samples was 1 m*M* (see *Gel filtration*) before the addition of iodoacetic acid. In this way, the formation of artefactual zones in the region of the glucose transporter was avoided. The electrophoretic migration of the glucose transporter was not visibly affected (*cf.*, ref. 5). The calibration proteins that contain disulphide bonds migrate slower than they do following reduction; the electrophoretic calibrations are therefore approximate. The gel slabs were silver-stained as described[24].

Reconstitution and transport assay. Reconstitution of the glucose transport system in lipid vesicles and measurement of the stereospecific uptake of D-glucose were carried out essentially as described[25]; *i.e.*, glucose transport protein was reconstituted in egg-yolk lipid vesicles. which, after freezing and thawing, were incubated for 2 min with [^{14}C]D-glucose. The protein/lipid vesicles were then separated from free D-glucose and collected for scintillation measurements. These procedures were repeated with [^{14}C]L-glucose using a separate aliquot of the vesicles.

Chemical analyses. Phospholipid concentrations were measured by phosphorus analyses according to the method of Bartlett[26].

RESULTS

Fractionation of integral membrane proteins from human red cells at low ionic strength

Octyl glucoside-solubilized integral membrane proteins were applied to the Superose 6 column, which had been equilibrated with 50 m*M* octyl glucoside in Tris–DTE. The proteins were separated into three main fractions, I–III (Fig. 1A). The broad fraction I consisted mainly of the anion transporter (band 3 in the notation of Steck[27]) in the form of poorly resolved protein multimers of different sizes complexed with the detergent, as judged by electrophoretic analysis (Fig. 1B). These complexes were eluted at an apparent M_r of at least $1.0 \cdot 10^6$ (Fig. 1A and B). Fractions II and III contained detergent complexes of glycophorin A, apparent chro-

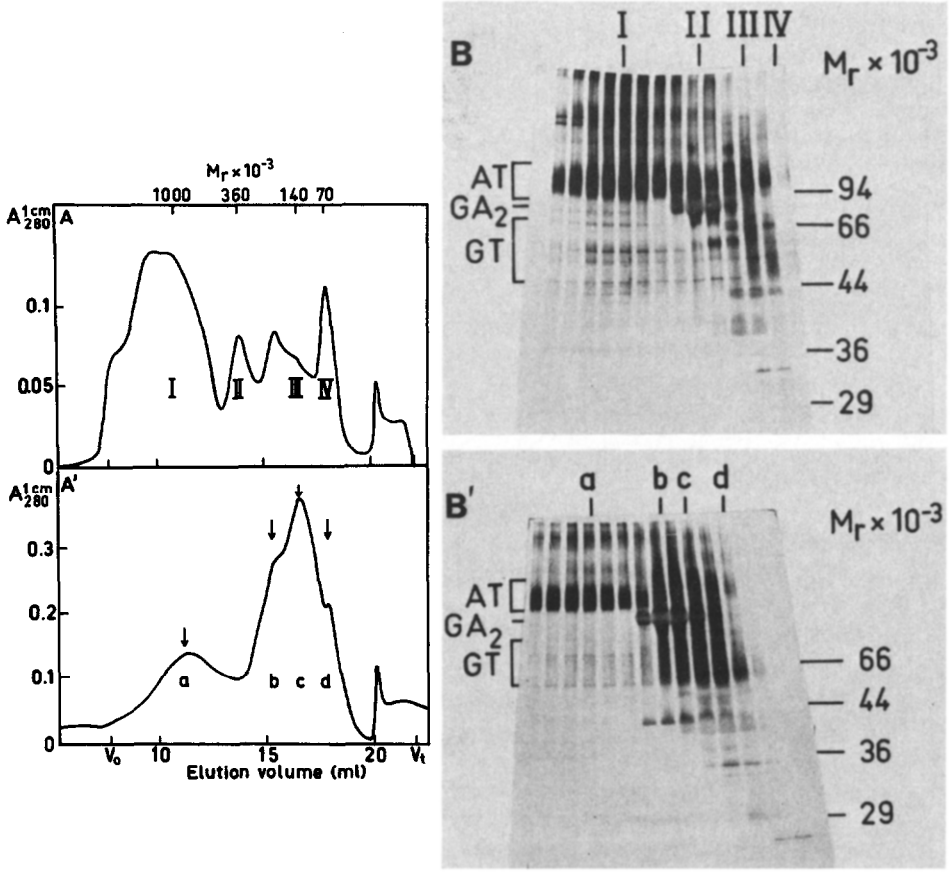


Fig. 1. (A, A') High-performance gel filtration on Superose 6 of octyl glucoside complexes of integral membrane proteins and lipids from human red cells. Eluents: (A) 50 m*M* octyl glucoside in Tris-DTE (see *Methods*), (A') 50 m*M* octyl glucoside and 0.5 *M* sodium chloride in Tris–DTE. Protein concentration: 2 mg/ml. Sample volume: (A) 0.5 ml, (A') 1.0 ml. Flow-rate: 9 ml/h. The apparent M_r values for fractions I–IV are indicated in (A). (B) SDS-PAGE of 0.7-ml fractions between the elution volumes 7.7 and 18.2 ml in (A). (B') SDS-PAGE of 0.7-ml fractions between the elution volumes 9.8 and 20.3 ml in (A') (see arrows). The electrophoretic zones of the anion transporter (AT), the dimer of glycophorin A (GA_2) and the glucose transporter (GT) are indicated in (B) and (B').

matographic M_r 360000, and the glucose transporter (band 4.5), respectively (Fig. 1A, *cf.*, Fig. 1B). The apparent chromatographic M_r for the glucose transporter was 140000. The transporter may appear in the form of a dimer complexed with octyl glucoside (see Discussion) and possibly also with some membrane lipids (IV in Fig. 1A). The above M_r values are collected in Table I.

Fractionation of integral membrane proteins from human red cells at ionic strength 0.5 M

Superose 6 chromatography of the integral membrane proteins in 50 m*M* octyl glucoside in Tris–DTE with the addition of 0.5 *M* sodium chloride resulted in two main fractions (a and b–d in Fig. 1A'). Electrophoretic analysis (Fig. 1B') showed that fraction a contained mainly complexes of the anion transporter and detergent which were eluted at an apparent M_r of 900000 (Fig. 1A'). Fractions b–d were found to contain mainly glycophorin A (b), the glucose transporter (c) and membrane lipids (d), partially separated [Fig. 1A' and B'; phosphorus determination (not shown)]. The apparent chromatographic M_r of the glucose transporter–detergent complex was 140000, exactly the same as that at low ionic strength (*cf.*, above and Table I).

Glycophorin A showed an apparent chromatographic M_r of 360000 in the absence of sodium chloride and 210000 in 0.5 *M* sodium chloride (II in Fig. 1A and b in Fig. 1A', respectively). The increase in the elution volume for glycophorin A that accompanied the addition of 0.5 *M* sodium chloride to the sample and eluent reflects the decrease in size of the negatively charged carbohydrate part of the protein, which would be the result of lowered electrostatic repulsion, and possibly also a decrease in the number of monomers in the protein–detergent complex. The apparent chromatographic M_r values show that the anion transporter (M_r from sequence data 103000[28]) and glycophorin A (131 amino acids, 60% carbohydrate by weight[29], M_r

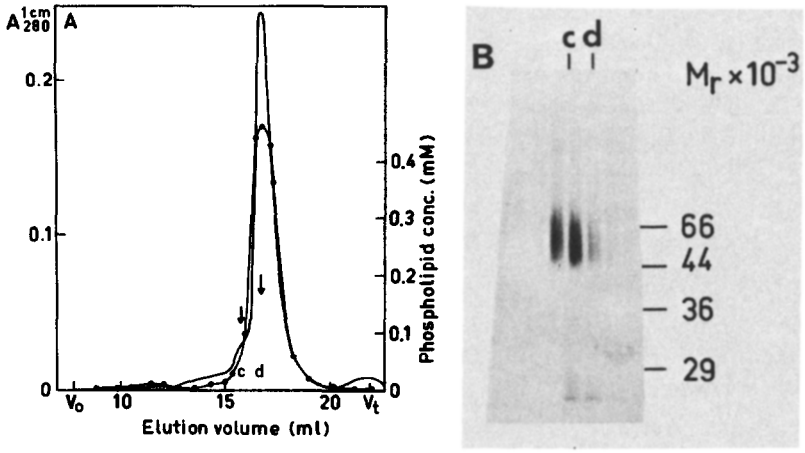


Fig. 2. (A) Chromatography on Superose 6 of octyl glucoside complexes of partially purified glucose transporter and part of the lipids from human red cells (see *Methods*). Eluent: 30 m*M* octyl glucoside in Tris–DTE. Sample volume: 1 ml. Flow-rate: 9 ml/h. —, Absorbance; ●—●, phospholipid concentration. (B) SDS-PAGE of 0.7-ml fractions between the elution volumes 13.3 and 18.2 ml in (A). Positions c and d correspond to the fractions in (A) (see arrows).

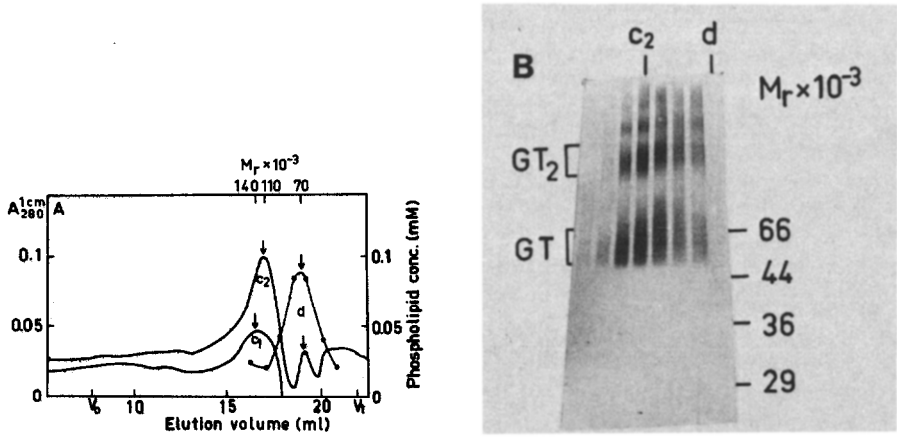


Fig. 3. (A) Chromatography on Superose 6 of octyl glucoside complexes of partially purified glucose transporter and part of the lipids from human red cell membranes. Eluent: 50 m*M* octyl glucoside–0.5 *M* sodium chloride–2 m*M* egg-yolk phospholipids in Tris–DTE (upper line); 50 m*M* octyl glucoside–0.5 *M* sodium chloride in Tris–DTE (lower line). ●—●, Phospholipid concentration in the experiment corresponding to the lower A_{280} curve. (B) SDS-PAGE of 0.7-ml fractions between the elution volumes 14.0 and 19.6 ml in (A). The positions of c_2 and d correspond to the fractions in (A) (upper line, arrows). Electrophoresis of fractions collected from the experiment shown in Fig. 3A, lower line, gave the same pattern (not shown).

ca. 37000) form multimers complexed with octyl glucoside. All the apparent chromatographic M_r values are given in Table I.

Fractionation of partially purified red cell glucose transporter

Glucose transporter was partially purified on DEAE-cellulose and chromatographed on the Superose 6 column in Tris–DTE with 30 m*M* octyl glucoside (Fig. 2A). The transporter preparation also contained membrane lipids. Since the glucose transporter (c in Fig. 2A) overlapped considerably with the membrane lipids (d in Fig. 2A), the elution volume of the transporter was estimated by means of electrophoresis (Fig. 2B). The glucose transporter formed the well known broad 4.5 zone (*cf.*, ref. 27). In this case, the glucose transporter-detergent complex was eluted at an apparent M_r of 160000 (see Table I).

Experiments were also carried out with 50 m*M* octyl glucoside in Tris–DTE (Fig. 3A, lower line). Sodium chloride (0.5 *M*) was also added. This increased the separation of the glucose transporter from the membrane lipids. The transporter–detergent complex was eluted at an apparent M_r of 150000 instead of 160000, a small but significant difference (*cf.*, Table I). Electrophoretic analysis of the fractions that were collected in the experiment illustrated in Fig. 3A, lower line, showed silver-stained zones corresponding not only to monomeric (band 4.5), but also to di-, tri- and tetrameric glucose transporter (not shown). The same pattern was obtained in the electrophoretic analysis of fractions collected from the experiment in Fig. 3A, upper line (Fig. 3B, *cf.*, Fig. 2B). This analysis (Fig. 3B) also indicates that the heterogeneously glycosylated glucose transporter[30] is anomalously eluted; the transporter molecules containing large carbohydrate parts are eluted later than the ones with smaller parts. Glucose transporter containing small carbohydrate parts migrates

faster in sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) than does transporter with large ones[31]. A possible explanation for the chromatographic behaviour is that more dimers may be formed when the carbohydrate part is small, for steric reasons.

Measurements of glucose transport activity

The D-glucose transport activity decreased upon fractionation on the Superose 6 column. The specific uptake of D-glucose was at most 0.25% of the D-glucose with which the protein/lipid vesicles were incubated (Fig. 4), whereas the corresponding value was 2.5 ± 0.2% for the material that was applied to the column. Accordingly, the maximum activity was reduced ten-fold, whereas the dilution factor was *ca.* 3. The activity yield in Fig. 4A was approximately 20%. The activity seemed to be separated into two main and one minor fractions (Fig. 4A and B), one of the main fractions (c_1 and c_2) coinciding with the centre of the glucose transporter fraction and the second one (d) with the zone where the transporter and the membrane lipids overlapped (*cf.*, Fig. 3A). The activity was almost the same in the two main fractions (Fig. 4), despite the lower protein concentration in the second one (Fig. 3B). The glucose transporter needs a milieu of lipids to retain its activity[7,10,32]. In one of the experiments shown in Fig. 3A the Superose 6 column was equilibrated with 50 m*M* octyl glucoside, including 2 m*M* egg-yolk phospholipids and 0.5 *M* sodium chloride, in Tris–DTE (Fig. 3A, upper line). No significant increase in activity (Fig. 4A), compared to the experiment lacking egg-yolk phospholipids (Fig. 3A, lower line and Fig. 4B), was observed. This strongly indicates that the glucose transporter requires an environment of red cell membrane lipids or some specific type or types of these lipids (*cf.*, ref. 33). When fractionated in this egg-yolk phospholipid-containing eluent, the glucose transporter was eluted at an increased volume, corresponding to an apparent M_r of 110000 (Fig. 3A, upper line, Table I), possibly reflecting elution in monomeric form.

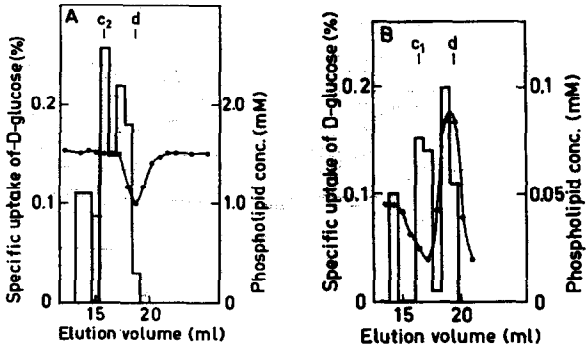


Fig. 4. D-Glucose transport activity after fractionation of DEAE-purified glucose transporter on Superose 6 (A) in the presence of 50 m*M* octyl glucoside and 2 m*M* egg-yolk phospholipids (Fig. 3A, upper line) and (B) in the absence of egg-yolk phospholipids (Fig. 3A, lower line). ●—●, Phospholipid concentration. The concentration of egg-yolk phospholipids in the eluent in the experiment in Fig. 3A, upper line, was higher than the maximum concentration of eluted red cell membrane phospholipids. The latter lipids therefore show a decrease in the concentration of phospholipids.

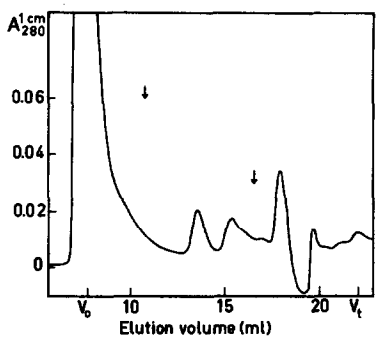
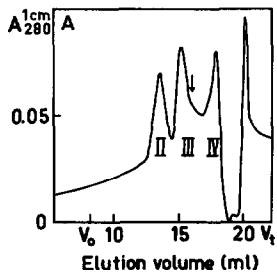


Fig. 5. Chromatography on Superose 6 of octyl glucoside complexes of integral membrane proteins and lipids from human red cells. The sample was incubated for 20 h at 37°C before fractionation. Eluent: 50 m*M* octyl glucoside in Tris–DTE. Sample volume: 0.2 ml. Flow-rate: 9 ml/h. The arrows indicate the elution volumes of fractions I and III in Fig. 1A.

Time-dependent aggregation

When the octyl glucoside-solubilized membrane proteins were incubated for 20 h at 37°C and fractionated on Superose 6 in 50 m*M* octyl glucoside in Tris–DTE, a large portion of the applied material was eluted at or close to the void volume (Fig. 5). When freshly solubilized membrane proteins and lipids were fractionated at a low flow-rate, 1 ml/h, the protein yield decreased considerably (Fig. 6). Hardly any anion transporter was eluted as seen in electrophoretic analysis (Fig. 6B) of fractions collected in this chromatographic experiment (Fig. 6A). The integral membrane proteins and lipids tend to aggregate with time. At low flow-rates this aggregation may proceed during the chromatographic fractionation to the extent that large aggregates formed within the gel beads cannot leave the beads.



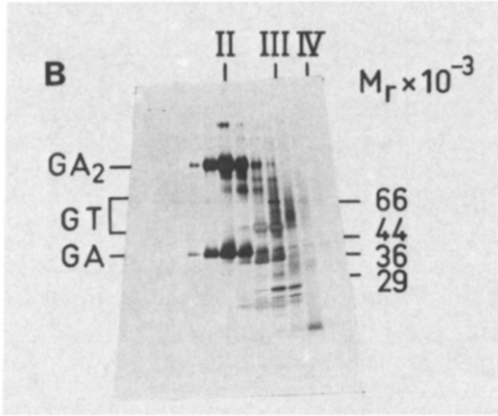


Fig. 6. (A) Chromatography on Superose 6 of octyl glucoside complexes of integral membrane proteins and lipids from human red cells. Chromatographic conditions as in Fig. 1A, except that the flow-rate was 1 ml/h. (B) SDS-PAGE of 0.7-ml fractions between the elution volumes 9.1 and 18.2 ml in (A). The positions of II, III and IV correspond to the fractions in (A). The electrophoretic zones of the dimer (GA_2) and the monomer (GA) of glycophorin A and the glucose transporter (GT) are indicated.

Protein recovery

The areas under the absorbance curves in Fig. 1A, A′ and Fig. 3A were compared with the corresponding area in Fig. 3, thick line, in ref. 5, for which amino acid analysis was carried out. The resulting yield estimations were: Fig. 1A, 80%, possibly overestimated due to light scattering by large complexes of the anion transporter; Fig. 1A′, 40%; Fig. 3A, 90%. The latter value refers to purified glucose transporter. The recovery of the glucose transporter was higher in the presence of 0.5 *M* sodium chloride (Figs. 1A′ and 3A) than in the absence of sodium chloride (Fig. 1A).

DISCUSSION

Size of the glucose transporter–detergent complex

Our results indicate that the glucose transporter is eluted as a monomer or a dimer upon Superose 6 chromatography in the presence of octyl glucoside, as discussed below. We assume that the membrane-spanning part of the D-glucose transporter is a cylinder with a height of 3.15 nm, which binds to the octyl group of the detergent. This cylinder is composed of 12 α-helices each having 21 amino acid residues[34]. The partial specific volume of average proteins, 0.73 cm^3/g, and the molecular mass of the cylinder then give a radius of 1.83 nm. The sizes of the hydrophilic parts of the protein were estimated in a similar way. The length of an octyl glucoside molecule was estimated at 1.89 nm when the octyl chain is extended to 80% of its full length (*cf.*, ref. 35). The radius of a sphere encompassing the protein–detergent complex would be 3.7 nm when the carbohydrate part is disregarded, which corresponds to M_r 80000 according to a plot of $M_r^{1/3}$ *versus* the Stokes' radius for globular proteins. The apparent chromatographic M_r of the complex between the glycosylated glucose transporter (polypeptide monomer M_r according to sequence data 54000[34]) and octyl glucoside was 150000 ± 10000 (Table I). The carbohydrate part (*cf.*, ref. 36) may increase the apparent M_r from 80000 to 150000, or the transporter may appear as a dimer. The latter seems more likely. However, in the presence of 2 m*M* egg-yolk phospholipids, the glucose transporter was eluted at an apparent M_r of 110000 (Fig. 3A, upper line and Table I), which corresponds to a monomer. Accordingly, the transporter is probably solubilized and chromatographed initially as a monomer–detergent complex, apparent M_r 110000, and a dimer–detergent complex may be formed when the lipid concentration in the protein zone decreases below a certain value, which results in an average apparent M_r of 150000.

Glucose transport activity and lipids

The glucose transporter was eluted as a tailing band, which overlapped with the membrane lipid band. The two main fractions which showed transport activity (Fig. 4A and B) probably correspond to the dimer and the monomer of the glucose transporter, where the monomer appears in the tail of the elution zone. Since the activity was equal in both fractions, in spite of the much lower protein concentration in the overlap zone, some specific membrane lipid(s), needed to stabilize the transporter, may be bound to the glucose transporter in this zone. In the other active fraction the transporter is partly inactivated. This inactivation may be caused by a small change (at the active site) and need not necessarily contribute to any drastic

TABLE I

APPARENT M_r VALUES ON SUPEROSE 6 OF OCTYL GLUCOSIDE COMPLEXES OF THE MAJOR INTEGRAL MEMBRANE COMPONENTS FROM HUMAN RED CELLS, ACCORDING TO CALIBRATIONS WITH WATER-SOLUBLE PROTEINS (SEE METHODS)

The M_r values are collected from Figs. 1A, A', 2A and 3A, OG = Octyl glucoside; EYPL = egg-yolk phospholipids.

Eluent	*Apparent M_r value*			
	Anion transporter	*Glucose transporter*	*Glycophorin A*	*Membrane phospholipids*
Tris–DTE with				
30 m*M* OG	–	160 000	–	120 000
50 m*M* OG	1 000 000	140 000	360 000	70 000
50 m*M* OG–0.5 *M* sodium chloride	900 000	140 000*, 150 000**	210 000	70 000*,**
50 m*M* OG–0.5 *M* sodium chloride–2 m*M* EYPL	–	110 000**	–	70 000**

* Value from fractionation of integral membrane proteins and lipids.
** Value from fractionation of purified glucose transporter and lipids.

changes in the overall structure of the glucose transporter or in the apparent chromatographic M_r.

The transport activity was considerably reduced by chromatography in 30 or 50 m*M* octyl glucoside, and the addition of lipids to the eluent in the presence of 50 m*M* detergent did not retain the activity. We therefore presume that the use of a detergent concentration lower than 30 m*M* may be the only way to preserve potential transport activity in octyl glucoside, *i.e.*, a lower detergent concentration than in the solubilization procedure.

Resolution and aggregation

The resolution of the major integral membrane proteins upon chromatography on Superose 6, in octyl glucoside, was relatively high. The anion transporter appeared in large aggregates in a 2-h fractionation (Fig. 1A), whereas in the case of the glucose transporter the amounts of trimers and larger oligomers were negligible (Figs. 2 and 3). The aggregation of the anion transporter increased with time (*cf.*, Fig. 5). Chen *et al.*[20] have recently reported that the anion transporter can be separated from the glucose transporter on a TSK 3000SW column in 51 m*M* octyl glucoside and that binding of cytochalasin B coincides with glucose transport activity. Their resolution between ghost proteins is difficult to estimate, since no electrophoretic analysis is shown.

An earlier report indicates a higher apparent M_r, 400 000, for the active glucose transporter in 12.5 m*M* cholate[7] than we have found in 30–50 m*M* octyl glucoside. This could be attributed to the low detergent concentration, which was chosen to retain transport activity and which caused formation of oligomers.

Shape of protein–detergent complexes

The elution volume of the monomeric glucose transporter in 100 m*M* sodium dodecyl sulphate was 13.85 ml[5] and in 50 m*M* octyl glucoside was 16.65 ml. According to calibrations performed in the absence of detergent, these elution volumes correspond to apparent M_r values of 320 000 and 110 000, respectively. The sodium dodecyl sulphate complex of the transporter is probably very elongated[37] (*cf.*, ref. 38), which explains why its apparent M_r is three times higher than that of the compact octyl glucoside–transporter complex.

CONCLUSIONS

Table I shows that the anion transporter as well as glycophorin A were eluted at high apparent molecular mass in octyl glucoside, which indicates that oligomers were formed, regardless of the ionic strength. The membrane phospholipids were eluted at the same volume with salt or additional lipids as without these components. However, the size of the lipid–detergent complex increased when the octyl glucoside concentration was decreased from 50 to 30 m*M*. The glucose transporter on the other hand was eluted essentially as a dimer in 30 and 50 m*M* detergent. The ionic strength did not appreciably affect the apparent M_r value. Inclusion of egg-yolk phospholipids in the eluent, at high ionic strength, did decrease the apparent size of the transporter–octyl glucoside complex. In this case, the transporter was probably eluted as a monomer. The yield of the glucose transporter increased with increasing ionic strength.

At high flow-rates the Superose 6 column afforded good resolution of the

integral membrane proteins and gave moderate aggregation during the fractionation. The separation between the glucose transporter and the membrane lipids was associated with a decrease in transport activity.

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