Biochimica et Biophysica Acta 859 (1986) 135–142
Elsevier

BBA 73143

Further characterization and chemical purity assessment of the human erythrocyte glucose transporter preparation

Amrit L. Rampal, Edward K.Y. Jung, Jimmy J. Chin, Mark R. Deziel, Harold B. Pinkofsky and Chan Y. Jung §

Biophysics Laboratory, VA Medical Center, Department of Biophysical Sciences, SUNY at Buffalo, Buffalo, NY 14215 and Laboratory of Biochemistry, NHLBI, NIH, Bethesda, MD 20205 (U.S.A.)

(Received January 15th, 1986)

Key words: Erythrocyte; Glucose transporter; Gel filtration; Cytochalasin B binding

Chemical and functional purity of the human erythrocyte glucose transporter preparation obtained by DEAE column chromatography after octyl glucoside solubilization was assessed. The cytochalasin B binding capacity of the preparation indicates that the preparation is 60–85% functional glucose transporter. Gel filtration chromatography on TSK 250 column separates this preparation into at least three major peptide fractions, namely, P0, P1 and P2, with apparent M_r of approx. 80 000, 43 000 and 17 000, respectively. When the preparation is photolabelled with [^{3}H]cytochalasin B prior to the separation only P0 and P1 are labelled. Exposure of the preparation to octyl glucoside or to ultraviolet light irradiation results in an increase in P0 in a time-dependent manner with a concomitant and proportional reduction in P1, without affecting P2 appreciably. For individual preparations, relative abundance of P0 and P1 vary widely in a reciprocal fashion, while that of P2 is practically fixed at approx. 10% of the total protein. The specific activity of cytochalasin B binding of each preparation correlates linearly with the relative abundance of P1 of the preparation, which gives a calculated specific binding activity of 22 nmol/mg protein for this fraction. These results indicate that P1 and P0 are native and denatured transporter, respectively, while P2 is contaminating protein impurities. These results demonstrate that the glucose transporter preparation contains approx. 10% of nontransporter protein impurities, with a varying amount (up to 30%) of denatured transporter, and that the transporter free of the chemical impurities and the denatured transporter can be obtained by a gel filtration chromatography of this preparation.

Introduction

The movement of glucose across human erythrocyte membranes [1] represents a prototype of so-called 'carrier-mediated' transport processes. That this glucose transport is catalysed by a membrane polypeptide known as band 4.5 on NaDodSO_4-polyacrylamide gel electrophoreses [2] is supported by reconstitution of transport activity [3] and glucose-sensitive cytochalasin B binding activity [4] of this polypeptide, and an immunological identification [5]. With the use of nonionic detergents followed by a DEAE-cellulose chromatography, a preparation of this membrane peptide (glucose transporter) has been obtained [6,7] and used widely for the chemical and physicochemical description of the structure and dynamics of the

§ To whom correspondence should be addressed at the Veterans Administration Medical Center, Buffalo, NY 14215, U.S.A.
Abbreviations: DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); EDTA, ethylenediaminetetracetic acid; NaDodSO_4, sodium dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride; Tris, tris(hydroxymethyl)aminomethane.

transporter. This includes a complete amino acid composition [6,7], carbohydrate content [6,8], proteolytic cleavage products [9], and SH reactivities [10,11]. More recent studies also include the proton exchange * and spectral ** properties of this preparation. However, chemical purity of this preparation has not been established [6,7]. Scatchard analysis of the cytochalasin B binding activity of an improved version of this preparation has been reported to give a specific binding of 15.3 nmol/mg protein, indicating that some 30% of the protein in this preparation is nonfunctional as a transporter. As the detergent used for solubilization is known to denature the transporter, an unknown portion of the nonfunctional mass of this preparation is expected to be accounted for by the presence of denatured transporter [7].

The very fact that the chemical purity of the preparation is not known, however, makes results of these chemical and physicochemical studies less than unambiguous. The main objective of the present study is to assess the chemical and functional purities of this preparation. We demonstrate here that the improved preparation of the glucose transporter obtained by the anion exchange column chromatography can be further fractionated into three distinct peptide peaks (P1, P2 and P3) by a gel filtration chromatography. Based on cytochalasin B photolabelling [9] and estimated cytochalasin B binding specific activity [7], we identify these fractions as denatured transporter, native transporter, and nontransporter impurity (respectively). Quantitation of these peaks demonstrates that the chemical impurity of the preparation is consistently approx. 10% by mass, although the denatured transporter may amount to 5 to 30%.

Materials and Methods

Materials. *n*-Octyl-β-D-glucopyranoside (octyl glucoside) was synthesized by modification of the method of Noller and Rockwell [12], but using yellow mercuric oxide (Aldrich Chemical Co.) instead of silver oxide. Cytochalasins B and E, and D-glucose and L-glucose were purchased from Sigma (St. Louis, MO). [^{3}H]Cytochalasin B (spec. act. 13.6 Ci/mmol) was from New England Nuclear. DTNB and PMSF were from Sigma. Ultrapure grades of guanidine-HCl and ammonium sulfate were purchased from Schwarz Mann and Bethesda Research Laboratories, respectively.

Preparation of glucose transporter. Human erythrocyte glucose transporter was prepared as described by Baldwin et al. [7] with a slight modification as follows. White erythrocyte ghosts were prepared from freshly outdated, blood bank whole blood, and treated with 0.2 mM EDTA at pH 12 for 30 min to remove extrinsic proteins. The stripped ghosts were suspended (2 mg protein/ml) in 45 mM of octyl glucoside in 50 mM Tris-HCl (pH 7.4 at 20°C) at 4°C for 30 min, and the detergent extract was separated by centrifugation (80 000 × *g* for 20 min). The extract was applied to a column (25 × 80 mm) of DEAE-cellulose equilibrated with 38 mM octyl glucoside in 50 mM Tris-HCl (pH 7.4) containing 2 mM dithiothreitol. The column was eluted with the same buffer with a flow rate of 1 ml/min. The flow through (100 ml) was diluted with the same buffer minus octyl glucoside but containing 0.1 M NaCl, 1 mM dithiothreitol, 0.1 mM EDTA, and centrifuged (185 000 × *g* for 60 min). Resulting pellets (approx. 0.25 ml) were washed to remove the detergent by suspending in H_2O to a protein concentration of approximately 1 mg/ml and stored in liquid N_2 until use.

Gel filtration chromatography. Bio-Sil TSK 250 columns (Bio-Rad Laboratories) were used with a medium pressure liquid chromatographic system (Pharmacia Fine Chemicals). An aliquot of 50–500 μg protein equivalent of glucose transporter preparation was dissolved in a detergent (either 0.1–0.5% $NaDodSO_4$ or 1% octyl glucoside as specified) solution in a final volume of up to 500 μl and filtered through a membrane filter of 0.20 μm pore size (Gelman Metricel Alpha 200) in a filter holder (GEPT013) or a Centrex centrifugal filter using nylon 66 (S & S). Samples were injected through a 50 to 500 μl loop and U-7 valve (Pharmacia Fine Chemicals). Elution buffer was either 100 mM sodium phosphate or 100 mM Tris-HCl, both pH 7.0, containing 0.1%

* Jung, E.K.Y., Chin, J.J. and Jung, C.Y. (1986) submitted for publication.

** Chin, J.J., Jung, E.K.Y. and Jung, C.Y. (1986) submitted for publication.

$NaDodSO_4$. Absorbances were measured by a Model 441 Detector (Waters Associates) either at 280 or 254 nm, and recorded on a REC-482 two-channel recorder (Pharmacia Fine Chemicals). Flow rates were adjusted between 0.5 to 1.0 ml per min. Fractions (0.2 to 0.5 ml) were collected and absorbance peaks were integrated using Fraction Collector FRAC-100 (Pharmacia Fine Chemicals).

Apparent molecular weight of each separated peak was estimated based on mobilities of seven molecular weight markers on the gel filtration chromatography run in 0.1% $NaDodSO_4$-phosphate buffer in a manner identical to those of Fig. 1. The molecular weight markers used were bovine albumin, egg albumin, glyceraldehyde-3-phosphate dehydrogenase, carbonic anhydrase, trypsinogen, and lactalalbumin with approximate molecular weights of 66 000, 45 000, 36 000, 29 000, 24 000, and 14 200, respectively.

Photolabelling with cytochalasin B. Labelling of band 4.5 preparation with [^{3}H]cytochalasin B was done following Deziel and Rothstein [9] with a slight modification. Typically, 500 μl of aqueous suspension of glucose transporter preparation (100–300 μg protein) was mixed with 5 μl of 10^{-5} M cytochalasin B and 45 μl of [^{3}H]cytochalasin B (1 mCi/ml) at room temperature for 20 min. The mixture in a 2 ml cuvette was positioned in a reflectorized sample chamber of an Aminco-Bowman spectrofluorometer (American Instrument Co.) equipped with a 150 W xenon arc lamp (Hanovia Inc., Neward, NJ) and irradiated at 280 nM using the excitation monochromator for a given time interval, with occasional stirring. The irradiated samples were washed in 2 ml H_2O three times with centrifugation (120 000 $\times$ g for 40 min) in the presence of unlabelled cytochalasin B (10^{-7} M)) to remove unreacted [^{3}H]cytochalasin B. Photolabelling with ghosts was done similarly.

Electrophoretic analysis. $NaDodSO_4$-glycerol polyacrylamide gel electrophoresis was performed as described by Connelly and Kuksis [13]. Typically, 1–2 μg of polypeptide solubilized in 2% $NaDodSO_4$, 10% isopropanol, and 25% glycerol was applied to each sample lane. Gels of 15% acrylamide containing 18% glycerol were run for 16 h at 50 mA per slab. The gels were fixed in 50% methanol, and protein bands were detected by the silver staining technique of Wray et al. [14].

Miscellaneous assay procedures. Cytochalasin B binding activities of the transporter preparations were quantitated as described [15] based on Scatchard analysis of equilibrium binding data obtained at six different ligand concentrations (10^{-8} to 10^{-3} M) using [^{3}H]cytochalasin B as a tracer. Radioactivities of both pellets and supernatants were counted in a liquid scintillation counter (Nuclear Chicago, Mark II). Proteins were assayed by the method of Lowry et al. [16] in the presence of 0.1% $NaDodSO_4$ using γ-globulin as a standard. Phospholipids were measured in terms of lipid phosphorus according to Bartlett [17].

Results

The glucose transporter preparation obtained as described in Materials and Methods, and used in this study, bound cytochalasin B with a dissociation constant (K_d) of $(1.8 \pm 0.6) \cdot 10^{-7}$ M and the total binding capacity (B_T) of 14.3 ± 2.8 nmol/mg protein (average of 14 independent preparations $\pm$ S.D.). B_T of individual preparations varied widely however, with the highest being 18 nmol/mg protein. The overall yield of this preparation, assessed in terms of the glucose-sensitive cytochalasin B binding activity, was 20–50% of that of the EDTA-treated ghosts (550 pmol/mg protein). The preparation contained protein and phospholipid at a mass ratio of 0.31–0.82 to 1. $NaDodSO_4$ polyacrylamide gel electrophoresis of the preparation typically showed a broad Coomassie staining band corresponding to M_r ranging from 45 000 to 70 000 (not illustrated).

The glucose transporter preparation were separated into at least three major discrete protein fractions, P0, P1 and P2 by a gel filtration chromatography in the presence of $NaDodSO_4$ as detailed in the Materials and Methods (Fig. 1). P0, when calibrated against molecular weight markers, showed an apparent molecular weight of approx. 80 000. P1 was the most abundant protein component, and its molecular weight was estimated to be approximately 43 000. P0 and P1 are free of phospholipids (Fig. 2). P2, a second major protein component in most preparations, had an apparent molecular weight of 17 000. P2 contains phospholipids (Fig. 2). P1a, a minor fraction with an estimated molecular weight of 36 000–40 000, often

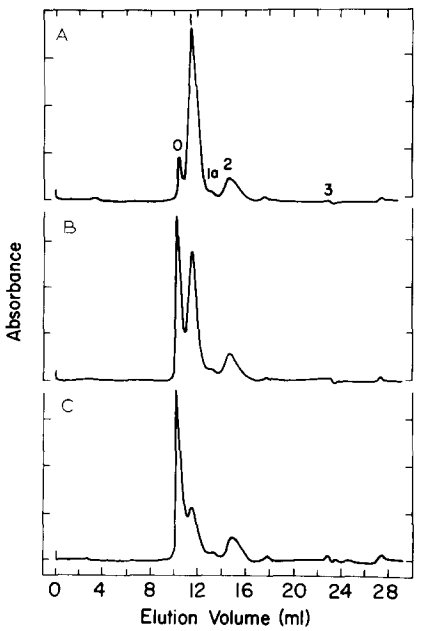

Fig. 1. Gel filtration chromatography of glucose transporter preparation and the effect of octyl glucoside incubations. 56 μg protein equivalent of the glucose transporter preparation (specific cytochalasin B binding capacity of 14.6 nmol/mg protein) was chromatographed in each run. Samples were solubilized in 0.10 ml 0.5% $NaDodSO_4$ prior to injection) and chromatographed at 20°C on TSK SW 3000/600 column. 0.1% $NaDodSO_4$-phosphate buffer was used for elution with a flow rate of 1.0 ml/min. Absorbance at 280 nm with full scale of 0.10. Panel A. Fresh preparation. Numbers indicate peaks 0, 1, 1a, 2 and 3. Panels B and C show the chromatography of the same preparation after incubation in 1% octyl glucoside for 6 and 16 h, respectivelv. at 20°C.

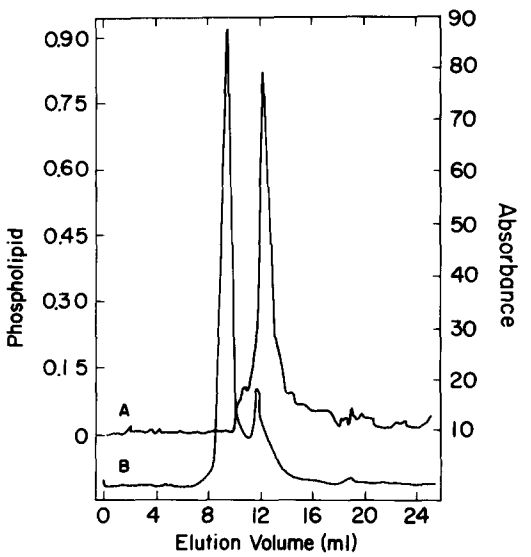

Fig. 2. Phospholipid contents of gel filtration chromatographic peaks of glucose transporter preparation. Fractions of 0.2 ml each were collected and lipid phosphorus were measured in μmol and shown in A as a smooth curve constructed by connecting data points. B shows absorbance at 280 nm of corresponding elution. Bio-Sil TSK 250 (300×7.5 mm) was used. 0.1% $NaDodSO_4$-phosphate buffer was used for elution. The glucose transporter preparation with a cytochalasin B binding capacity of 18.1 nmol/mg protein was used. Note that this preparation showed very little P0. Also shown here is that P0 and P1 are practically free of phospholipids.

appeared as a poorly resolved peak. P3, the smallest constituent of all protein peaks, showed an apparent M_r of less than 10000 when calibrated against molecular markers.

Some of these gel filtration-separated peaks of the transporter preparation were also analyzed by $NaDodSO_4$-glycerol polyacrylamide gel electrophoresis (Fig. 3). Unfractionated glucose transporter showed a broad band of 50000 to 75000 as the major component. P1 also showed a broad band, typical of the major, glycoprotein component of the transporter preparation, but with a slightly fast mobility extending from 45000 to 70000. A minor sharp band with an estimated M_r of 35000 probably corresponds to P1a. P2 appeared as a single species on the gel, with an apparent molecular weight of 16500, agreeing well with a similar value of 17000 derived from its gel filtration chromatographic behavior. The sharp leading edge and diffuse trail of the band suggests that P2 is also a glycoprotein. Interestingly, P2 could not be visualized using the Laemmli gel system (not illustrated), which has been used previously to analyze the transporter preparation [6,7]. Both P1 and P2 were detectable in the $NaDodSO_4$-glycerol electrophoretogram of unfractionated transporter (Fig. 3).

Relative sizes of P0 and P1 varied widely with different preparations. Furthermore, they showed a reciprocal relationship, the sum of the two being practically constant. Relative size of P2 was also practically invariant among different preparations. The fact that P1 is the only single major peptide component of the glucose transporter preparation that can account for the observed high specific

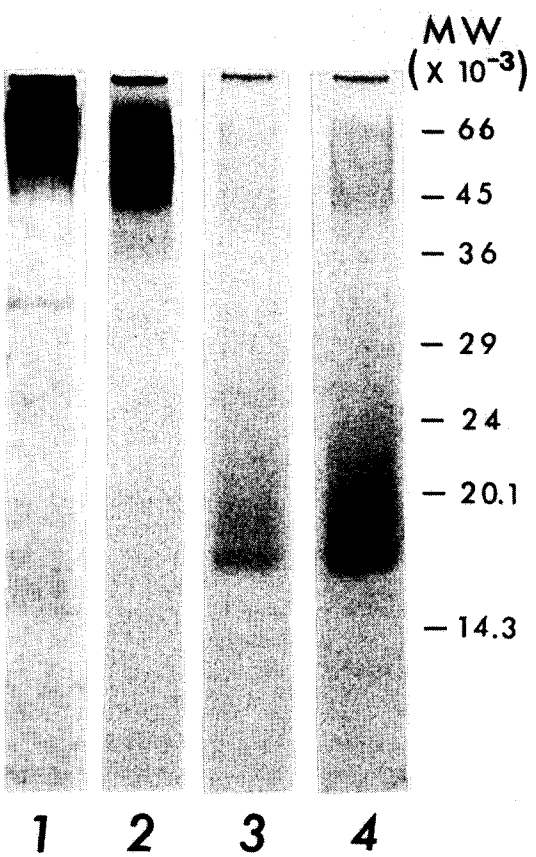

Fig. 3. $NaDodSO_4$-glycerol polyacrylamide gel electrophoresis of unfractionated transporter preparation (Lane 1), and the gel filtration peaks, P1 (Lane 2) and P2 (Lanes 3 and 4, in equivalent and twice equivalent amounts, respectively, based on their relative abundances) of the transporter preparation. Migration of molecular markers is shown on the right.

activity of cytochalasin B binding prompted us to examine a possibility that P1 is the native glucose transporter. The correlation between the relative abundance of P1 mass and the specific cytochalasin B binding activity observed with each of 14 different, freshly-made preparations showed that there is indeed a linear correlation (correlation coefficient of 0.86) between these two parameters (Fig. 4). This linear correlation clearly indicates that P1 largely represents native glucose transporter protein.

When a given transporter preparation was incubated in 1% octyl glucoside solution for a varying time interval prior to the gel filtration chromatography, a progressive reduction in the size of P1, with a concomitant and proportional increase in the size of P0, was observed (Fig. 1). Neither the absorbance of P2 nor the sum of the absorbance of P0 and P1 changed significantly during these incubations (Fig. 1). Analysis of the time-course (Fig. 5A) revealed that the octyl glucoside incubation converts P1 to P0, and that the conversion is a first-order process with a rate constant of approx. 0.087/h at 20°C. Equilibrium binding of cytochalasin B to the preparation measured in parallel experiments (data not illustrated) showed that the detergent incubation reduces the binding capacity with a first order rate constant of approx. 0.088/h. Exposure to ultraviolet light at 280 nm during this incubation caused drastic enhancement of the peak conversion. Photolysis of the preparation in 1% octyl glucoside, the condition used for the cytochalasin B photolabelling discussed below, resulted in an increase of the conversion rate by 6-fold, showing the first order rate constant of approx. 0.35/h at 20°C (Fig. 5B).

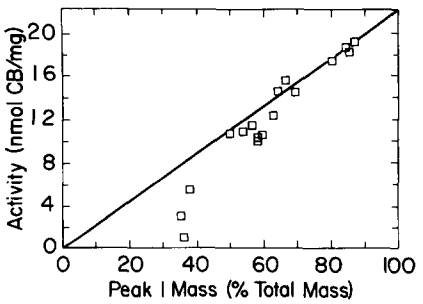

Fig. 4. Correlation of relative mass of P1 against cytochalasin B (CB) binding capacity for 17 different glucose transporter preparations. The relative protein mass was calculated from integration of absorbance scan at 280 nm and observed extinction coefficients 1.40, 1.42, 1.86 and 0.77 per mg protein/ml for P0, P1, P1a and P2, respectively. The three preparations with the binding capacity lower than 8 nmol/mg had been stored at −70°C for more than 8 months prior to the gel chromatography.

The transporter preparation was first photolabelled with [^{3}H]cytochalasin B, then subjected to the gel filtration chromatographic separation, and distribution of the radioactivity among different fractions was examined. Results of such experiments revealed that only P0 and P1 (including P1a, where observed) were photolabelled with cytochalasin B (Fig. 6). The labelling was reduced in

the presence of D-glucose (Fig. 6). Some radioactivity was also seen under P2 region. However, analysis of the radioactivity distribution profile revealed that the radioactivity under P2 is a spill-over from those of P1 (and P1a). This conclusion was confirmed by the experiments where upon rechromatography of the P2, all radioactivity was found to localize at P1 and no radioactivity was associated at P2 (not illustrated).

Time courses of the photolabelling by cytochalasin B at P0 and P1 are shown in Fig. 7. The extent of the labelling at P0 was minimal at the start of the incubation, but increased progressively with time, showing an upwardly concave time-course. The labelling at P1, on the other hand, showed a time-course which is slightly downwardly concave, while total labelling (the sum of P0 and P1 labellings) was virtually linearly proportional to the incubation time. These observations suggest that the cytochalasin B labelling occurs only with P1 peptide, and that the labelling at P0 represents the P1 peptide labelling that was subsequently converted to P0 by the ultraviolet light irradiation.

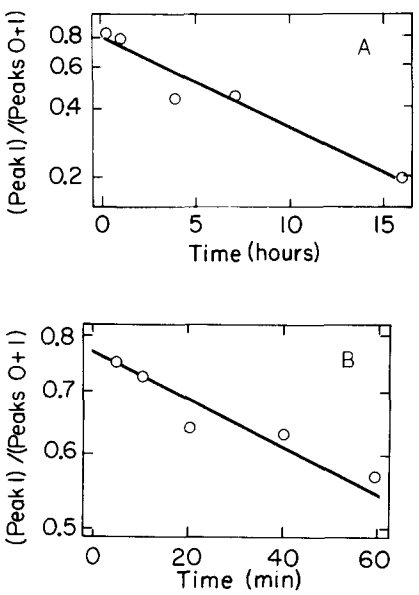

Fig. 5. Time-course of an apparent conversion of P1 to P0 during the incubation of 1% octyl glucoside, without (A) and with (B) ultraviolet light irradiation at 280 nm. 300 μg of a glucose transporter preparation (cytochalasin B binding activity was 16.2 nmol/mg protein) was incubated in 1% octyl glucoside in a 2 ml cuvette at 20°C for an increasing time interval with occasional mixing. Ultraviolet light irradiation was carried out under the condition of cytochalasin B-photolabelling as described in Materials and Methods. At the end of incubation an aliquot of 50 μl reaction mixture (in octyl glucoside) was applied into the column which was pre-equilibrated with 0.1% $NaDodSO_4$-phosphate buffer and eluted with the same buffer. Absorbance (at 280 nm) scan for P0 and P1 wer integrated, from which the ratio of P1/(P0+P1) was calculated. The sum of P0 and P1 was invariant during incubation. Lines drawn are best fits to data points by linear regression, and represent $y = 0.80\ e^{-0.087x}$ (for A) and $y = 0.78\ e^{-0.35x}$ (for B).

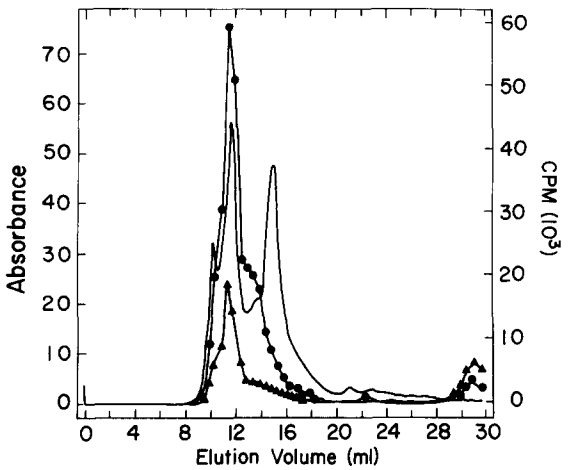

Fig. 6. Gel filtration chromatographic separation of glucose transport preparation after photolabelling with [^{3}H]cytochalasin B. Glucose transporter preparation (cytochalasin B binding activity of 14.2 nmol/mg protein) was photolabelled with [^{3}H]cytochalasin B as described in Materials and Methods, and washed by repeated centrifugation to remove all free ligands. A relatively large amount (200 μg protein) of the labelled preparation was used for each chromatography. Aliquots of 0.5 ml eluents were collected and counted for radioactivity. Photolabelling was in the presence of either 500 mM L-glucose (●) or 500 mM D-glucose (▲). Absorbance scan at 254 nm was superimposed to identify each peak.

Discussion

The human erythrocyte glucose transporter preparation studied here possessed a cytochalasin B binding capacity as high as 18 nmol/mg protein. Assuming that the molecular weight of the transporter is 45 000 and if cytochalasin B binds to the transporter with one-to-one stoichiometry, this would mean that 80% (in mass) of the preparation

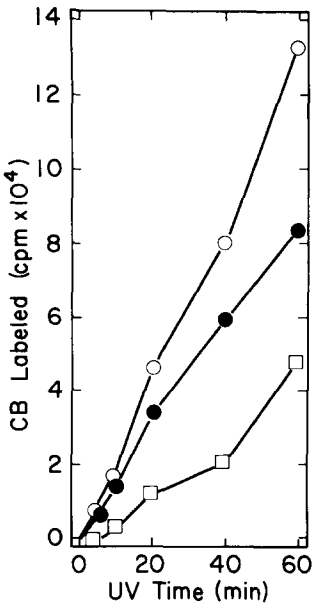

Fig. 7. Time-courses of [^{3}H]cytochalasin B photolabelling associated with peaks 0 and 1. The photolabelling and gel filtration chromatography were carried out as described in legend of Fig. 5. A relatively smaller amount (40 μg protein) of the labelled preparation was used for each chromatography run, in order to give a good separation between peaks 0 and 1. Radioactivities under each peak were integrated and shown in ordinate. Sum of peaks 0+1 (○), peak 0 (□) and peak 1 (■).

is functional transporter. The quality of the preparation, however, was found to be varied unpredictably dependent on ghost preparations, and the temperature and the duration of the incubation with detergent: even slight relaxation in controlling these parameters gave preparations of a specific cytochalasin B binding activity as low as 11 nmol/mg protein. This is reflected in the wide variation in the specific activities observed with individual preparations (Fig. 4). Nevertheless, as a group they gave an average cytochalasin B binding of 14.3 nmol/mg protein, indicating that the preparation studied here is similar to those preparations which were used recently in many different laboratories [6–13] for the molecular characterization of the transporter.

The present study demonstrates that this transporter preparation consists of three distinct protein species, namely, native transporter, denatured transporter, and an impurity (or impurities) of unknown identity, and that each of these is separately obtainable by gel filtration chromatography. A number of observations indicate that P1 is the native transporter. Its apparent molecular weight is approx. 43000. It can be photolabelled by cytochalasin B (Fig. 6). It possesses the diffuse electrophoretic profile typical of a glycoprotein (Fig. 3). The relative size of this peak, which varies widely among different preparations, is directly proportional to the specific activity of cytochalasin B binding of the preparation (Fig. 4). After a prolonged incubation of a particular preparation with octyl glucoside, there is also direct correlation between reduction in the size of this peak (Fig. 5) and reduction in the cytochalasin B binding activity, both showing practically the same first order rate constant (with the $t_{1/2}$ of 8.0 and 7.8 h, respectively, at 20°C).

P0, on the other hand, is most likely a denatured glucose transporter. Its apparent molecular weight of 80000, suggests that it is a dimer of the transporter. The following observations also support this conclusion. The size of this peak is large when the cytochalasin B binding activity of the preparation is low. There is also a reciprocal relationship between the size of P1 and that of P0 for each of different preparations. The size of P0 was also increased when a preparation was incubated in octyl glucoside, and this was accompanied by a concomitant and proportional reduction in the size of P1. The sum of the sizes of these two peaks, on the other hand, remained unchanged, which indicates an apparent conversion of P1 to P0 in the presence of the detergent. The fact that the conversion is greatly enhanced when the preparation was irradiated with ultraviolet light during photolabelling with cytochalasin, suggested that the cytochalasin B labelling associated with this peak is due to a conversion of labelled P1 rather than labelling of P0 itself.

P2, a peptide of an apparent molecular mass of 17000 dalton, represents the major chemical impurity of this transporter preparation. A number of observations support this conclusion. It is not labelled with cytochalasin B (Fig. 6). The size of this peak is fixed to approx. 10% of the total protein of the preparation regardless of its widely varying cytochalasin B binding activity. Spectral characterization of this fraction is quite different

from those of P1 and P0 (data to be published). The diffiuse electrophoretic profile of P2 suggests that this polypeptide is also a glycoprotein. The chemical and functional identity of this impurity is not known. The possibility that P2 is a degradation product of membrane proteins, including the glucose transporter, due to endogenous proteolytic enzymes can not be ruled out. Treatment of the transporter preparation with protease inhibitors such as PMSF, however, did not result in the disappearance of this compound.

The data of Fig. 4 indicate that P1 binds 22 pmol cytochalasin B per mg protein. In the case of the glucose transporter preparation, the assay of Lowry et al. used here for protein determination gives an underestimation by $20 \pm 3.2\%$ ($n = 4$), compared with the value obtained from amino acid analysis (data to be published). After correction for this underestimation, P1 would bind 18.3 nmol cytochalasin B per mg protein. Based on the most recent molecular weight estimate for the transporter of 55 000 [18] one may predict that pure transporter protein would bind 18.2 nmol of cytochalasin B per mg protein. This consideration indicates that P1 is pure functional transporter. However, exact assessment of the purity of P1 as the functional transporter is not possible at present because of the problems in the protein determination and ambiguity in the molecular weight determination. The glucose transporter preparation is known to include the nucleoside transporter, which may account for approx. 3% of its protein mass [19]. Whether P1 includes the nucleoside transporter is yet to be determined.

In conclusion, results of the present study demonstrate that the chemical impurity of the glucose transporter preparation is approx. 10%, although it contains a widely varying amount (up to 30%) of denatured transporters. The presence of the denatured transporter is responsible for the widely varying cytochalasin B binding activity that was observed with each preparation. The study describes for the first time an isolation of chemically almost pure, if not totally pure, glucose transporter of human erythrocytes. Although this chemically pure transporter is yet to be reinserted into lipid bilayer to study its transport function, the preparation will allow unambiguous chemical characterization of this transporter protein.

Acknowledgement

This study was supported in part by a grant from USPHS AM-13376 and the Veterans Administration Medical Center, Buffalo, NY.

References

1 Jung, C.Y. (1975) in The Red Blood Cell (Surgenor, D.M., ed.), Vol. II, pp. 705–751, Academic Press, New York
2 Kasahara, M. and Hinkle, P.C. (1977) J. Biol. Chem. 252, 7384–7390
3 Wheeler, T.J. and Hinkle, P.C. (1981) J. Biol. Chem. 256, 8907–8914
4 Sogin, D.C. and Hinkle, P.C. (1980) Biochemistry 19, 5417–5420
5 Baldwin, S.A. and Lienhard, G.E. (1980) Biochem. Biophys. Res. Commun. 94, 1401–1408
6 Sogin, D.C. and Hinkle, P.C. (1978) J. Supramol. Struct. 8, 447–453
7 Baldwin, S.A., Baldwin, J.M. and Lienhard, G.E. (1982) Biochemistry 21, 3836–3842
8 Lienhard, G.E., Crabb, J.H. and Ransom, K.J. (1984) Biochim. Biophys. Acta 769, 404–410
9 Deziel, M.R. and Rothstein, A. (1984) Biochim. Biophys. Acta 776, 10–20
10 Deziel, M.R., Jung, C.Y. and Rothstein, A. (1985) Biochim. Biophys. Acta 819, 83–92
11 Pinkofsky, H.B. and Jung, C.Y. (1985) Arch. Biochem. Biophys. 240, 94–100
12 Noller, C.R. and Rockwell, W.C. (1938) J. Am. Chem. Soc. 60, 2076
13 Connelly, P.W. and Kuksis, A. (1982) Biochim. Biophys. Acta 711, 245–251
14 Wray, W., Boulikas, T., Wray, V.P. and Hancock, R. (1981) Anal. Biochem. 118, 197–203
15 Pinkofsky, H.B., Rampal, A.L., Cowden, M.A. and Jung, C.Y. (1978) J. Biol. Chem. 253, 4930–4937
16 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
17 Bartlett, G.R. (1959) J. Biol. Chem. 234, 469–471
18 Allard, W.J. and Lienhard, G.E. (1985) J. Biol. Chem. 260, 8668–8675
19 Jarvis, S.M. and Young, J.D. (1981) Biochem. J. 194, 331–339