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Monomeric human red cell glucose transporter (Glut1) in non-ionic detergent solution and a semi-elliptical torus model for detergent binding to membrane proteins

Lars Haneskog [a], Lars Andersson [a,1], Eggert Brekkan [a], Ann-Kristin Englund [a,2], Keiichi Kameyama [b], Lars Liljas [c], Eva Greijer [a], Jorge Fischbarg [d], Per Lundahl [a,*]

[a] *Department of Biochemistry, Biomedical Center, Uppsala University, Box 576, S-751 23 Uppsala, Sweden*
[b] *Institute for Protein Research, Osaka University, Yamadaoka 3-2, Suita, Osaka 565, Japan*
[c] *Department of Molecular Biology, Biomedical Center, Uppsala University, Box 590, S-751 23 Uppsala, Sweden*
[d] *Departments of Physiology and Cellular Biophysics and of Ophthalmology, College of Physicians and Surgeons of Columbia University, 630 West 168th Street, New York, NY 10032, USA*

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Abstract

The self-association state of the human red cell glucose transporter (Glut1) in octaethylene glycol *n*-dodecyl ether ($C_{12}E_8$) and *n*-octyl β-D-glucopyranoside (OG) solution was analyzed in the presence of reductant by gel filtration with light-scattering, refractivity and absorbance detection, and by ultracentrifugation. The $C_{12}E_8$-Glut1 complex was essentially monomeric, whereas OG-Glut1 also formed dimers and larger oligomers. $C_{12}E_8$-Glut1 retained substantial glucose transport activity even after depletion of endogenous lipids by gel filtration, as shown by reconstitution and transport measurements. Removal of endogenous lipids from OG-Glut1 abolished the activity unless phosphatidylcholine was included in the eluent. The binding of $C_{12}E_8$ and OG to Glut1 was determined by gel filtration with refractivity and absorbance detection or with radioactive tracer to be 1.86 ± 0.07 and 1.84 ± 0.09 g/g polypeptide, respectively. A structural model was proposed in which non-ionic detergent forms a semi-elliptical torus (SET) surrounding the transmembrane protein. The torus thickness was assumed to be equal to the radius (short half-axis) of a spherical (oblate ellipsoidal) free detergent micelle and the polar head groups of the detergent molecules were predicted to be situated just outside the hydrophobic surface of the protein. The experimental detergent binding values and those obtained from the SET model together confirmed that Glut1 was monomeric in $C_{12}E_8$ solution and provided constraints on the shape and size of the hydrophobic transmembrane region of Glut1 in α-helical and β-barrel topology models.

Keywords: Detergent binding model; Detergent-protein complex; Glucose transporter; Glucose transport activity; Membrane protein; Phospholipid; (Human red cell)

Abbreviations: BSA, bovine serum albumin; $C_{12}E_8$, octaethylene glycol *n*-dodecyl ether; CMC, critical micelle concentration; DC_8PC, 1,2-dicapryloyl-l-*sn*-glycero-3-phosphocholine; $DC_{10}PC$, 1,2-dicaproyl-l-*sn*-glycero-3-phosphocholine; $DC_{14}PC$, 1,2-dimyristoyl-l-*sn*-glycero-3-phosphocholine; DTE, dithioerythritol; Glut1, human red cell glucose transporter; HTMR, hydrophobic transmembrane region; LALLS, low-angle laser light-scattering; OG, *n*-octyl β-D-glucopyranoside; PC, phosphatidylcholine; PDM, protein-decorated micelle; p*I*, isoelectric point; SET, semi-elliptical torus.

* Corresponding author. Fax: +46 18 552139; e-mail: Per.Lundahl@bmc.uu.se.

[1] Present address: Pharmacia&Upjohn Inc., Biopharmaceuticals, S-112 87 Stockholm, Sweden.

[2] Present address: Pharmacia&Upjohn Inc., Biopharmaceuticals, S-112 87 Stockholm, Sweden.

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1. Introduction

Membrane proteins have been described as associating with non-ionic detergents by insertion into detergent micelles [1]. Recent determinations of the amount of detergent bound to integral membrane proteins indicated that the detergent molecules were instead arranged in a monolayer around the hydrophobic transmembrane region (HTMR) of the protein [2]. Neutron diffraction analyses of

the detergent structure in crystals of bacterial photosynthetic reaction centers showed interconnected detergent rings surrounding the transmembrane protein regions, and the detergent molecules were thought to be arranged in a monolayer with their alkyl chains in contact with the hydrophobic surface of the protein and their polar head groups in contact with the solvent [3,4]. Furthermore, in these crystals the volume ratio between detergent and polypeptide was approximately the same as in the detergent-membrane protein complexes in solution [4] and it seems therefore reasonable to assume that the structure of the detergent toruses of the complexes in solution may be similar to that of the detergent rings in the crystal.

In the present work we propose a refined structural model for the detergent binding to transmembrane proteins. In this model the detergent is organized in a semi-elliptical torus (SET) surrounding the protein transmembrane region, essentially as in the crystals of the photosynthetic reaction center. The hydrophobic moieties of the detergent molecules cover the HTMR and the polar head groups of the detergent molecules along the protein surface are situated in the interfacial region just outside the hydrophobic surface. The torus size is governed by the detergent micellar radius, the length of the detergent polar head and the dimensions of the HTMR. The SET model was applied to the human red cell glucose transporter (Glut1) [5–7] by taking the HTMR dimension from two proposed topology models, one comprising twelve hydrophobic and presumably α-helical polypeptide segments (the 12-helical model) [8–10] and the other a 16-stranded β-barrel (the barrel model) [11–13].

Glut1 is a heterogeneously glycosylated protein with a polypeptide M_r proposed to be 54 117 [8], which value was used for the calculations below, and with an oligosaccharide component of, on the average, $M_r \approx 6800$ (see below). Glut1 is a homotetramer in the cell membrane [14,15] but is solubilized as a homodimer in the presence of cholate and dithiothreitol [16], and mainly as the monomer in the presence of *n*-octyl β-D-glucopyranoside (OG) and dithioerythritol (DTE) [17]. The binding of octaethylene glycol *n*-dodecyl ether ($C_{12}E_8$) and OG to Glut1 was determined by gel filtration with refractivity monitoring or radioactive detergent tracer analysis. Experimental binding data and those obtained from the SET model were compared for certain dimensions of the transmembrane region of the protein. The association state of Glut1 in $C_{12}E_8$ was compared to that in OG solution by gel filtration analysis with detection of low-angle laser light-scattering (LALLS), absorbance and refractivity [18], and by sedimentation velocity analysis. The effect of the presence of phospholipids in detergent-Glut1 solutions on the transport activity observed after reconstitution was studied in extension of earlier work [17,19]. The purpose of the work was dual: to refine recent detergent binding models and to characterize Glut1 in non-ionic detergent solutions for future crystallization attempts.

2. Materials and methods

2.1. Materials

$C_{12}E_8$ was purchased from Nikko Chemicals, Tokyo, and from Fluka. OG was bought from Dojindo Laboratories, Kumamoto, Japan, and from Sigma. *n*-Octyl β-D-[U-^{14}C]glucopyranoside was purchased from American Radiolabeled Chemicals, St. Louis, MO, and was denoted tracer β. *n*-Octyl [1-^{14}C]α-D-glucopyranoside (85%) in mixture with *n*-octyl [1-^{14}C] β-D-glucopyranoside (15%) according to our NMR and thin-layer chromatographic analyses [20] was bought from California BioNuclear, Los Angeles, CA. The mixture was denoted tracer α and was over-represented by 5% in OG micelles, compared to tracer β, according to determinations by equilibrium dialysis at different OG concentrations (data from Ref. [20] with an additional experiment). Nitrophenols [21] were kindly provided by Dr. K. Šlais, Academy of Sciences of the Czech Republic, Brno. Superdex 200 HR columns (1 × 30 cm) were obtained from Pharmacia Biotech. TSK G3000 SW_{XL} columns (0.78 × 30 cm) and TSK SW_{XL} guard columns (0.75 × 7.5 cm) were provided by Tosoh. L-[1-^{3}H(N)]Glucose and D-[^{14}C(U)]glucose were purchased from DuPont NEN. 1,2-Dicapryloyl-, 1,2-dicaproyl-, and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DC_8PC, $DC_{10}PC$, and $DC_{14}PC$, 99%), and phosphatidylcholine (PC) from egg (L-α-lecithin, 95%) were bought from Avanti Polar Lipids, Alabaster, AL. Dialysis membrane (cut-off 3500) was bought from Spectrum, Houston, TX. Microsep 30 K centrifugal ultrafiltration concentrators were purchased from Filtron Technology, Northborough, MA.

2.2. Purification of Glut1

Human red cell membranes were stripped of peripheral proteins [22] and solubilized on ice with 22 mg/ml of $C_{12}E_8$ or OG [23] in 70 mM Tris-HCl (pH 7.0) at 22°C, 1 mM DTE and 3 mM NaN_3 (eluent A). Glut1 was purified by ion-exchange chromatography [17] at 6°C in eluent A supplemented with 1–3.7 mM $C_{12}E_8$ or 38–40 mM OG. The preparations were denoted $C_{12}E_8$-Glut1 and OG-Glut1, respectively. They contained ≈ 0.3 mg polypeptide/ml and ≈ 3 mM endogenous phospholipids and were kept on ice before subsequent experiments.

2.3. Isoelectric focusing of Glut1

$C_{12}E_8$-Glut1 was dialyzed overnight at 6°C against 30 volumes of 3.7 mM $C_{12}E_8$ in water and mixed (7 μl, 1–2 μg) with 14 μl of a solution containing 20 mg/ml (37 mM) $C_{12}E_8$, 6 M urea, 4% Pharmalyte (pH 3–10) and 10 mM DTE that had been adjusted with HCl to pH 4–5. The sample was applied at the anode on an immobilized pH 7–10 gradient gel with 10 mg/ml $C_{12}E_8$, 6 M urea and 1 mM DTE, or similarly in a pH 4–10 gradient gel without

Table 1
Detergent data

	OG	$C_{12}E_8$	SDS
M_D (g/mol)	292.38	538.77	288.38
V_D (nm^3)	0.418 [a]	1.515 [b]	0.651 [c]
b [d] (nm)	1.98 [e]	2.74 [f]	2.4 [g]
c [d] (nm)	0.70 [h]	1.32 [i]	0.51 [k]
$(dn/dc)_D$ (ml/g)	0.138 [l]	0.134 [m]	–
CMC (mM)	36 [n]	0.16 [p]	1.8 [q]

[a] Average calculated by use of the partial specific volumes 0.867 ml/g at 22°C [36], 0.858 ml/g at 20°C [37], and 0.859 ml/g (temperature unknown) [38].
[b] From data for an oblate ellipsoidal micelle (Ref. [33], Table 3, line 4).
[c] Calculated by use of the micellar radius 2.4 nm, obtained by extrapolation to 0.1 M NaCl at 23°C in Fig. 2 in Ref. [39], and by use of $N = 89$ at 0.1 M NaCl (Fig. 7-2 in Ref. [40]).
[d] See Fig. 1 and text.
[e] Linear extrapolation to 6°C of the values determined by light-scattering photometry (instrument LS-601 from Otsuka Electronics with correlator SAI-43A from Kanomax) to be 2.07, 2.11, 2.30 and 2.43 nm at 12, 17, 22 and 36°C, respectively.
[f] For an oblate ellipsoidal micelle (Ref. [33]: Table 3, line 4, 14.2 Å +13.2 Å).
[g] Data from Fig. 2 in Ref. [39] as described in footnote c.
[h] Estimated from molecular model.
[i] Ref. [33]: Table 3, line 4 ($R = 13.2$ Å).
[k] From the volume of the SO_4Na moiety [41], assuming a spherical shape.
[l] According to Ref. [36].
[m] According to Ref. [18].
[n] At 5°C, average of 35 mM (calculated from data in Ref. [36]) and 37 mM (unpublished).
[p] At 10°C [42].
[q] At 25°C in a sodium phosphate buffer of pH 6.8 and ionic strength 0.1 [43].

DTE (since DTE inhibits gel polymerization below pH 6), essentially as described earlier [24]. Isoelectric focusing was done overnight at 20°C at < 3000 V, < 1 mA and < 3 W. The gels were silver-stained [25].

2.4. Glut1 transport activity in detergent solution

Endogenous lipids were separated from $C_{12}E_8$-Glut1 by gel filtration at 5°C on Superdex 200 HR in eluent A supplemented with 3.7 mM $C_{12}E_8$, and similarly from OG-Glut1 in eluent A containing 38 mM OG and 10 mM DTE, with or without PC, as specified below. The sample volume was 1.1 ml and the flow rate 0.25 ml/min. The phospholipid concentration in the eluate fractions was determined by phosphorus analysis [26]. Glut1 was collected, kept at 0–2°C, mixed 1:1 with 260 mM egg phospholipids [23], 260 mM sodium cholate, 200 mM NaCl, 20 mM Na_2EDTA, 2 mM DTE, and 5 mM Tris-HCl (pH 8.4), reconstituted and analyzed for specific D-glucose equilibrium exchange at 23°C during a 2-min incubation period at 50 mM D-glucose concentration, all essentially as described earlier [27].

2.5. Gel filtration analyses of Glut1 association state and detergent binding

$C_{12}E_8$-Glut1 was run in eluent A with 1 mM $C_{12}E_8$ on TSK G3000 SW_{XL} at 0.2 ml/min and 5°C with monitoring of LALLS, absorbance at 280 nm and differential refractivity as described earlier [17,18,28,29] for determi-

Table 2
Detergent binding [a] to Glut1 monomer and dimer according to the SET model (Fig. 1) for Glut1 topology models, and corresponding experimentally determined values

	$C_{12}E_8$ binding	OG binding		SDS binding
	Monomer	Monomer	Dimer	Monomer
α-Helical HTMR [b], d [c] = 0	1.65 ± 0.12	1.66 ± 0.12	1.2 ± 0.1	1.20 ± 0.09
α-Helical HTMR [b], d [c] = 3.5r	1.83 ± 0.14	1.87 ± 0.14	1.1 ± 0.1	1.35 ± 0.10
β-Barrel HTMR [d]	1.76 ± 0.11	1.78 ± 0.11	1.3 ± 0.1	1.27 ± 0.08
Experimental values	1.86 ± 0.07 [e]	1.84 ± 0.09 [f]	1.1 ± 0.1	1.7 ± 0.1 [g]
	(187 ± 7)	(341 ± 17)	(204 ± 19)	(319 ± 19)

The detergent binding values are given in gram detergent per gram polypeptide (polypeptide M_r 54 117 [12]) with standard errors of the mean. Molar ratios are given within parentheses.
[a] The values were calculated by use of Eqs. (1)–(4) with data from Table 1, with the relative error 6%, as derived from estimated error limits of 2% for each of the dimensions h, r, and b (Fig. 1B).
[b] A pore of radius 0.55 ± 0.15 nm was included in the HTMR volume by calculating detergent binding for the pore radii 0.40 nm and 0.70 nm and taking the mean value. The corresponding relative error was added to the relative error of 6% estimated as in footnote a.
[c] Elongation, see Fig. 1.
[d] Dimensions obtained as described in Section 3.2. A relative error of 6% was applied (see footnote a).
[e] Eight determinations. The contribution from the endogenous phospholipids in the Glut1 fractions (0.08 g phospholipid/g polypeptide) has been subtracted.
[f] Average of the photometric value 1.82 ± 0.11 ($n = 6$) g/g polypeptide, with less than 0.003 g phospholipid/g polypeptide, and the tracer value 1.86 ± 0.15 g/g polypeptide (Fig. 6). In the latter experiments the phospholipid concentration in the eluate was less than 2 μM (less than 0.015 g/g polypeptide at the elution volume 26.5 ml, see Fig. 6).
[g] Data from Ref. [52], determined in the presence of 2.0 mM SDS at 23–24°C in a sodium phosphate buffer of pH 6.8 and ionic strength 0.1.

nation of molecular weight and detergent binding. The principle is fully described in Ref. [18]. We assumed that the detergent binding to the protein did not alter the refractivity of either component or the absorptivity of the protein. Bovine serum albumin (BSA) was used as calibrant. OG-Glut1 was similarly run in eluent A containing 38–40 mM OG. The M_r was calculated as described in Ref. [18]. Detergent binding (δ_D) was calculated as outlined in Ref. [28] by use of the absorbance and refractivity signals, whereby the specific refractive index increment of the detergent-protein complex, (dn/dc_p), was expressed as the sum $(dn/dc)_P + \Sigma\delta_i\ (dn/dc)_i$, with $(dn/dc)_P$, the specific refractive index increment of the polypeptide; δ_i, mass ratio of component i and polypeptide (g/g); and $(dn/dc)_i$, the specific refractive index increment of component i; i = S, oligosaccharide; D, detergent, and L, phospholipid. For Glut1, $(dn/dc)_P = 0.187$ ml/g [18], $\delta_S = 0.125$ g/g (the sum of 0.120 g/g for neutral sugars and glucosamine [30], and 0.005 g/g for sialic acid [31]), and $(dn/dc)_S = 0.14$ ml/g [32]. The specific refractive index increments, $(dn/dc)_D$, for $C_{12}E_8$ and OG are given in Table 1. For phospholipids the value $(dn/dc)_L = 0.134$ ml/g was used [18]. The phospholipid concentration was determined by phosphorus analysis [26] and the average phospholipid molecular weight was taken to be 800.

2.6. Sedimentation velocity and dynamic light scattering analyses of Glut1 association state

$C_{12}E_8$-Glut1 was concentrated five-fold by centrifugal ultrafiltration and filtered (0.2 μm). A 450-μl aliquot was applied on a TSK G3000 SW_{XL} at 5°C in eluent A supplemented with 1 mM $C_{12}E_8$. The lipid-depleted monomeric Glut1 was collected and subjected to sedimentation velocity analysis at 40000 rpm (An-60 Ti rotor) with absorbance detection at 5°C in a Beckman Optima XL-A analytical ultracentrifuge. The molecular weight of the complex was calculated according to the Svedberg equation. The estimated density of the solvent, ρ, was 1.009 g/ml. The partial specific volume of the complex, $\bar{v}_c$, was calculated to be 0.886 ml/g according to the protein composition and the detergent binding (Table 2, below). The data used were the partial specific volumes of $C_{12}E_8$, 0.973 ml/g [33]; of the polypeptide, 0.755 ml/g, which was calculated from the polypeptide amino acid composition [8] and the residue partial specific volumes [34]); and of the oligosaccharide, 0.639 ml/g, which was calculated from the partial specific volumes of the sugar residues [34] in the biantennary oligosaccharide structure (Fig. 7 in Ref. [35]) with one sialic acid [31], 18.5 *N*-acetylglucosamines and 0.5 fucose, $M_r \approx 7200$, and in the high-mannose sugar chain (Fig. 7 in Ref. [35]), $M_r = 1900$. We calculated that the average oligosaccharide $M_r \approx 6800$ (0.125×54117, see above) corresponded to ≈ 92% biantennary structure and ≈ 8% high-mannose structure. The diffusion coefficient, D, was obtained by dynamic light scattering analysis of the lipid-depleted Glut1 on the instrument DLS-700, Otsuka Electronics Co., Hirakata, Japan.

2.7. OG binding to Glut1 determined by use of [^{14}C]-tracers

OG-Glut1 was concentrated five-fold by centrifugal ultrafiltration and filtered (0.2 μm). A 0.75-ml sample (with tracer as in the eluent below) was applied at 0.25 ml/min onto two Superdex 200 HR columns in tandem equilibrated at 5°C with eluent A containing 45 mM OG and 100000 cpm/ml of tracer β or tracer α. Two experiments were done with each tracer. The values obtained with tracer α were corrected for the over-representation in OG micelles (see Section 2.1). The first column was bypassed when Glut1 had entered the second column, leaving most of the lipids behind. The polypeptide concentrations (accuracy ±3%) were determined by automated amino acids analysis [27], the phospholipid concentrations by phosphorus analysis [26], and the radioactivity by scintillation counting (standard deviation ±0.04% of the total radioactivity, corresponding to ≈ ±5% of the above-baseline radioactivity in the protein fractions). The detergent binding was calculated.

3. Theory

3.1. Detergent binding model

In the complex between a non-ionic detergent and an integral membrane protein formed at equilibrium above the critical micelle concentration (CMC), the detergent was proposed to form a torus of semi-elliptical cross-section around the protein (Fig. 1). The HTMR height, h, was taken to be equal to the length of the hydrophobic segments traversing the membrane. For a transmembrane protein of given sequence and topology the HTMR volume is

$$V_{HTMR} = \frac{\bar{v}_{HTMR} M_{HTMR}}{N_A} + V_p \quad (1)$$

with $\bar{v}_{HTMR}$, the HTMR partial specific volume calculated from the partial specific volumes of the amino acid residues [34]; M_{HTMR}, the molecular weight of the HTMR according to the amino acid composition; N_A, Avogadro's number; and V_p, the volume of a transport pore. The HTMR radius, r, was calculated from V_{HTMR} and h, assuming a certain elongation, d, of the HTMR cross-section (Fig. 1B). These dimensions can also be taken from crystallographic data, if available, or chosen according to a three-dimensional model. The volume of the semi-elliptical detergent torus, V_{torus}, is

$$V_{torus} = \frac{4\pi}{3} ab^2 + \pi^2 abr + \pi abd \quad (2)$$

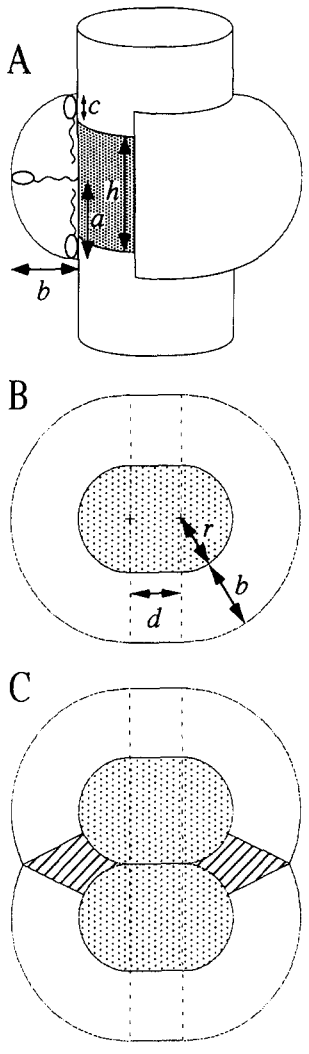

Fig. 1. SET model for detergent binding to a transmembrane protein. (A) Schematic drawing with detergent molecules shown in the torus surrounding the dotted HTMR surface. To cover the central part of the HTMR, detergent molecules were assumed to form a monolayer of a thickness b, equal to the radius of the spherical detergent micelle or the short half-axis of an oblate ellipsoidal detergent micelle (data in Table 1), which defined the length of the horizontal half-axis. The length, a, of the vertical half-axis of the torus was defined by assuming that detergent molecules along the protein surface had their polar head groups (length, c; Table 1) in contact with the polar parts of the protein just outside the HTMR (height, h). (B) Equatorial cross-section of the complex in A. The HTMR cross-section is dotted. While keeping the cross-sectional area constant, the elongation, d, and hence the distance r, were varied in the detergent binding calculations. (C) Cross-section as in B of a detergent-dimer complex (see text).

with the half-axis $a = h/2 + c$, and with b, the micellar radius or short half-axis in the case of an oblate ellipsoidal micelle, and d, the elongation of the cross-section of the HTMR (Fig. 1). For the dimeric model illustrated in Fig. 1C,

$$V_{torus} = 4\left(\frac{\pi - \arccos\left(\frac{r}{r+b}\right)}{2\pi}\right)\left(\frac{4}{3}\pi ab^2 + \pi^2 abr\right) + \pi abd + V_f \quad (3)$$

with V_f, the detergent volume represented by the lined areas, obtained by numerical integration (expression not given).

The detergent binding (δ_D, g/g polypeptide) can be calculated:

$$\delta_D = \frac{V_{torus}\, M_D}{V_D\, M_P} \quad (4)$$

with V_D, the molecular volume of the detergent, M_D, the molecular weight of the detergent, and M_P, that of the polypeptide.

3.2. The Glut1 SET models for $C_{12}E_8$, OG and SDS

In the 12-helical topology model [8] the hydrophobic transmembrane helices were assumed to consist of amino acid residues 12-33, 56-87 (this hydrophobic segment is particularly long and includes a zipper motif), 95-113, 127-145, 154-176, 185-207, 272-291, 307-328, 338-358, 367-392, 402-422 and 433-450 [20] with on the average 22.2 residues per segment. The translation of 0.15 nm/residue gave $h = 3.33$ nm. The weights, volumes and dimensions of the detergent molecules (Table 1) were used in the SET model (Fig. 1) for detergent binding. The long SET half-axis, $a = c + h/2$ (Table 1) was 2.98 nm for $C_{12}E_8$-Glut1, 2.36 nm for OG-Glut1, and 2.17 nm for SDS-Glut1. Furthermore, $M_{HTMR} = 27945$ and $\bar{v}_{HTMR} = 0.782$ ml/g. The pore of volume V_p in Eq. (1) was assumed to be cylindrical with a radius of 0.55 ± 0.15 nm (see Table 2). The corresponding radius, r, for a HTMR of circular cross-section was found to be 1.95 ± 0.04 nm.

A 16-stranded β-barrel model has earlier been proposed [13] in which all transmembrane segments of *Rhodobacter capsulatus* porin ($r = 2.25$ nm, $h = 2.30$ nm, Ref. [44]) were replaced by corresponding putative Glut1 β-strands [11]. For detergent-binding calculations a further adjustment was done by adding amino acid residues to some segments so that their ends corresponded to those derived from a consideration of Chou-Fasman-Prevelige propensity scores [45] and the output of a neural network prediction program [46]. The residues in the putative transmembrane segments were 12-22, 61-72, 77-87, 96-105, 120-132, 137-147, 164-173, 186-195, 271-280, 303-311, 334-343, 348-358, 367-379, 400-408, 413-425 and 433-446. The HTMR height, h, became 3.15 nm and the radius, r, of 2.25 nm was not changed. The pore radius was estimated to be about 0.8 nm.

4. Results and discussion

4.1. Glut1 purity, homogeneity, association state and M_r

Upon SDS-polyacrylamide gel electrophoresis [22] with silver-staining [47] the Glut1 preparations in $C_{12}E_8$ or OG

both gave a broad Glut1 monomer zone, minor zones of self-associated Glut1 (probably dimer and tetramer), and a trace of protein of apparent M_r 18000. The $C_{12}E_8$-Glut1 preparation showed additional minor zones of apparent M_r 28000 and 31000. Isoelectric focusing of $C_{12}E_8$-Glut1 in a pH 4–10 gradient showed a zone of isoelectric point (p*I*) 8.4 (Fig. 2A). In a pH 7–10 gradient the zone was partially resolved into three adjacent zones at p*I* 8.4 ± 0.1 (Fig. 2B), possibly representing Glut1 without or with one or two sialic acid residues (according to the slope of the graph at $q = 0$ in Fig. 8 of Ref. [27]), and showed a streak of self-associated material, in agreement with earlier results [22,24,31,48].

Glut1 in $C_{12}E_8$ solution was eluted in an essentially homogeneous state (Fig. 3) upon TSK G3000 SW_{XL} gel filtration. SDS gel electrophoresis with silver-staining indicated that the first absorbance peak represented Glut1. LALLS, absorbance and refractivity signals indicated a polypeptide M_r of 43000 ± 2000 (S.E., $n = 8$). Glut1 was thus monomeric. Only the material at the very front of the peak was apparently dimeric (Fig. 3). Due to the $C_{12}E_8$ size and hydration [33,49], the $C_{12}E_8$-Glut1 complex was larger than the OG-Glut1 complex, as shown by the elution volumes (not shown). The Glut1 self-association was much slower (not shown) in $C_{12}E_8$ solution than in OG solution. When the entire procedure of stripping and solubilizing human red cell membranes and purifying $C_{12}E_8$-Glut1 or OG-Glut1 was done in the absence of DTE, monomeric detergent-Glut1 complexes were again obtained as shown by the gel filtration elution volumes on TSK G3000 SW_{XL}. This is consistent with the notion that the Glut1 homotetramer [15] is held together by non-covalent interactions [50]. The absence of DTE increased the elution volume slightly (0.2 ml).

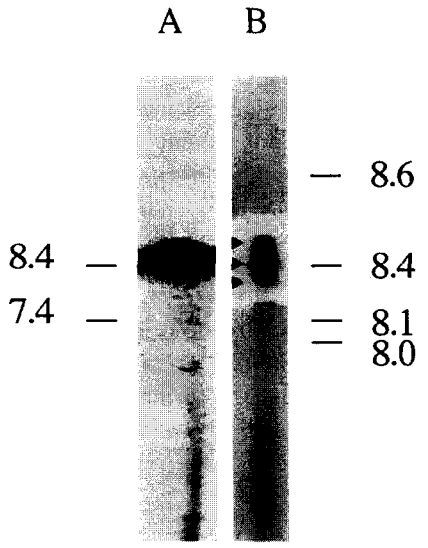

Fig. 2. Isoelectric focusing of Glut1. $C_{12}E_8$-Glut1 was focused in the presence of $C_{12}E_8$ and urea in immobilized pH gradient gels, (A) pH 4–10 (sample pH 4.2) and (B) pH 7–10 (sample pH 4.8). DTE was present in the pH 7–10 gel. The triangles in lane B indicate the protein zones. Only parts of the gels are shown. The markers were horse skeletal muscle myoglobin, p*I* 7.4 [24], sperm whale myoglobin, p*I* 8.4 [31], and nitrophenols, p*I* 8.0, 8.1, and 8.6.

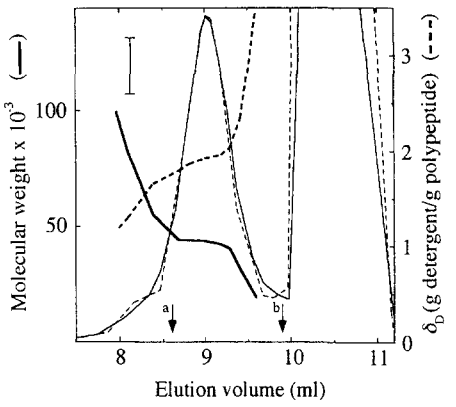

Fig. 3. Gel filtration of Glut1. $C_{12}E_8$-Glut1 was run on TSK G3000 SW_{XL} in the presence of $C_{12}E_8$ and DTE with LALLS (hatched line, arbitrary scale), differential refractivity (not shown) and absorbance (solid line, bar corresponding to $A_{280} = 0.01$) detection. The calculated graphs representing the polypeptide M_r (thick solid line) and the detergent binding, δ_D, (thick hatched line) are included. The second peak represents phospholipid. The arrows a and b mark the elution volume of BSA dimer and monomer, respectively. A typical chromatogram of a gel filtration run started ≈ 4 h after the Glut1 preparation is shown.

The main OG-Glut1 peak (not illustrated, cf. Ref. [17]) represented an apparent polypeptide M_r of 49000 ± 3000 (S.E., $n = 8$). Dimers or larger oligomers were present at the front of the peak. The apparent Glut1 polypeptide M_r values obtained from LALLS, absorbance and refractivity signals in both $C_{12}E_8$ and OG solution were thus somewhat lower than the sequence-deduced M_r 54117 [8].

The sedimentation coefficient $s = 2.07$ S obtained by sedimentation velocity analysis of lipid-depleted $C_{12}E_8$-Glut1 and the diffusion coefficient $D = (2.85 \pm 0.04) \cdot 10^{-7}$ cm^2/s obtained by dynamic light scattering analysis gave the M_r value of 158400 ± 7000 for the complex. The hydrodynamic diameter for $C_{12}E_8$-Glut1 was determined to be 11.0 ± 0.2 nm by dynamic light-scattering analysis at 8 ± 1°C on DLS-700, but increased to 12.4 ± 0.2 nm over one week at 8°C. A hypothetical spherical $C_{12}E_8$-Glut1 complex was estimated to be smaller (diameter 9 nm, with a $C_{12}E_8$ hydration of 0.87 g H_2O/g detergent [33]). By use of the values $\delta_S = 0.125 \pm 0.05$ g oligosaccharide/g polypeptide (above) and $\delta_D = 1.86 \pm 0.07$ g $C_{12}E_8$/g polypeptide (Table 2, below) the M_r of the Glut1 polypeptide was estimated at 53000 ± 4000, which confirms that the Glut1 was monomeric.

4.2. Transport activity of Glut1

The reconstituted specific transport activity was lower, but more stable, for Glut1 in $C_{12}E_8$ solution than in OG solution (Fig. 4). The data obtained do not represent initial rates, which are higher. The freeze-thawed proteoliposomes are heterogeneous in size [51] and low internal

volume of the small proteoliposomes diminishes the specific uptake values. The highest values in the present work were lower than those in Ref. [51], since a longer incubation time was used in the present series of experiments. Gel filtration of $C_{12}E_8$-Glut1 in the absence of eluent lipids lowered the concentration of endogenous phospholipids to less than 10 μM and lowered the stability (Fig. 4).

The effect of lipids on the retention of the OG-Glut1 activity was studied by removal or replacement of the endogenous lipids by gel filtration. With lipid-free eluent less than 10 μM endogenous phospholipids remained in the Glut1 fraction and the activity was abolished (Fig. 5A), in agreement with earlier results [17,19]. However, Glut1 retained its activity in the presence of long-chain egg PC molecules, particularly at 2 mM PC (Fig. 5A). The reason was perhaps partly that endogenous phospholipids remained in the Glut1 fractions in increasing concentration (0.01–0.7 mM) as the egg PC concentration was increased in the eluent. The chromatographic resolution became better with eluent phospholipids of shorter acyl chain length and exchange of essentially all endogenous lipids was achieved with DC_8PC, $DC_{10}PC$ or $DC_{14}PC$. The activity with $DC_{14}PC$ was initially high (108 mmol/g in 2 min) but had decreased to 40% of this value when the reconstitution was done 12 h later (Fig. 5B). The activity became lower the shorter the acyl chains were. Furthermore, the activity was less stable with $DC_{14}PC$ than with egg PC (not shown).

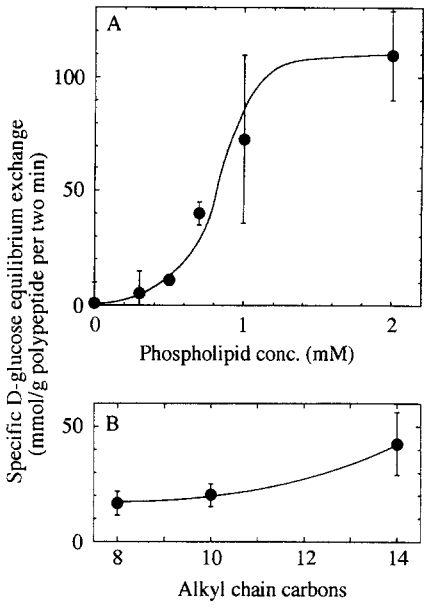

Fig. 5. The stability of OG-Glut1 transport activity after exchange or removal of endogenous lipids. OG-Glut1 was subjected to gel filtration in order to exchange endogenous membrane lipids for various types of PC. Reconstitution was done 12 h after the gel filtration and the specific D-glucose equilibrium exchange was determined. (A) The eluent contained egg PC (average fatty acid chain length $n = 17$) of the given concentration. The Glut1 fraction corresponding to the highest activity contained 0.7 mM endogenous phospholipids due to incomplete separation. (B) The eluent contained DC_nPC ($n = 8$, 10 and 14) in 1.0 mM concentration. Each point corresponds to 2–6 determinations with standard errors for 1–3 reconstitutions.

4.3. Detergent binding to Glut1

The $C_{12}E_8$ binding to monomeric Glut1 was determined by absorbance and refractivity measurements (Fig. 3), and the OG binding both by the photometric procedure (as in Fig. 3 in Ref. [17]) and by use of radioactive tracer (Table 2). The tracer-determined binding to apparently dimeric Glut1 was lower (Fig. 6 and Table 2), consistent with the SET model. The effect of the presence of nucleoside transporter on the observed binding values should be small, since this protein is similar to Glut1 and constitutes only about 5% of the transporter preparations [53].

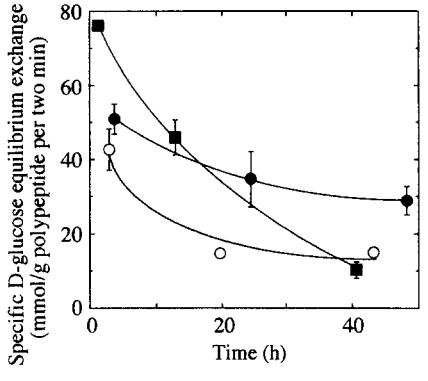

Fig. 4. Stability of Glut1 transport activity. The specific D-glucose equilibrium exchange of $C_{12}E_8$-Glut1 (filled circles) and OG-Glut1 (squares), both with co-purified endogenous lipids, and of $C_{12}E_8$-Glut1 that had been depleted of most of the endogenous phospholipids by gel filtration with lipid-free eluent (open circles) is plotted versus tthe time elapsed before reconstitution. Each point corresponds to 1–6 activity determinations with standard errors for 1–3 proteoliposome samples. The time zero corresponds to the end of the ion-exchange purification or the gel filtration of lipid-depleted material.

The experimental values for binding of $C_{12}E_8$ and OG to the Glut1 monomer both agreed well with the SET model data (Table 2) for an elongated 12-helical topology model ($d \approx 3.5r$) and for the remodelled barrel model. The SET model binding of OG to a barrel dimer was higher than the experimental value (Table 2), but the latter value may be underestimated owing to the presence also of higher oligomers. In the 12-helical model, the cross-sectional area of the Glut1 HTMR including a pore with a radius of 0.55 ± 0.15 nm would amount to (0.99 ± 0.04) nm^2/helix, essentially equal to the value 1.03 nm^2/helix reported for bacteriorhodopsin [54]. The pore would allow passage of the slowly transported glucose analogue 6-deoxy-*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-aminoglucose (NBDG, dimensions $\approx 0.6 \times 0.7 \times 1.4$ nm) [55]. The helices may alternatively form two smaller pores (Fig. 3 in Ref. [6]) of sufficient size.

The monomeric detergent-Glut1 complexes contained 187 $C_{12}E_8$ molecules or 341 OG molecules per Glut1

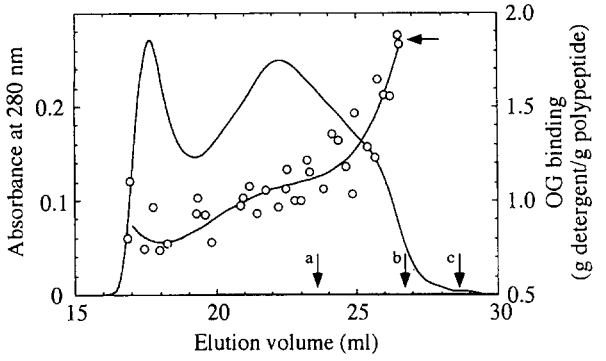

Fig. 6. OG binding to Glut1 determined by gel filtration in the presence of radioactive tracer. A typical elution profile is shown. OG-Glut1 was run on tandem Superdex 200 HR columns equilibrated with eluent supplemented with OG and [^{14}C]tracer. The OG binding data represent four experiments. The vertical arrows mark the elution volume of BSA dimer (a), BSA monomer (b) and hen ovalbumin (c). The OG binding to the Glut1 monomer was taken to be the average for the two highest points (horizontal arrow). The binding graph showed a slope in the monomeric region, probably since the concentration of not yet separated, nascent dimers increased with decreasing elution volume from the very trail of the monomer peak. OG-Glut1 dimer was estimated to be eluted at 23 ml according to a linear calibration diagram with $-\log K_D$ versus M_r for hen ovalbumin, BSA monomer and dimer (elution volumes indicated by vertical arrows), and OG-Glut1 monomer (M_r 158400, see text).

polypeptide (Table 2). These values are significantly higher than the micelle aggregation numbers of 120 [33] and 87 [36] for $C_{12}E_8$ and OG, respectively. This is clearly consistent with the view that the detergent-protein complex cannot be regarded as a detergent micelle that has incorporated the membrane protein. The detergent binding may instead be a measure of the HTMR dimensions, as suggested by Møller and le Maire [2], but also depends on the properties of the detergent. These authors found that the binding of four different detergents, expressed as g detergent/g polypeptide, were similar for a given protein but differed among the four membrane proteins studied, and proposed that the hydrophobic surface of the protein transmembrane region was covered by a detergent monolayer.

Glut1 may become unfolded by SDS to form a protein-decorated micelle (PDM) structure as found by Ibel et al. for a water-soluble protein [43,56]. The PDM structure of water-soluble proteins has been confirmed [57,58] and contains a number of SDS micelles resembling the SDS clusters proposed in the necklace model [59], as discussed by Shinagawa et al. [57]. However, the transmembrane regions of Glut1 and other transmembrane proteins perhaps interact with SDS in a way that has no counterpart among SDS interactions with water-soluble proteins. The observed SDS binding to Glut1 may hypothetically be consistent with the SET model (Table 2), provided that SDS both formed a semi-elliptical SDS torus surrounding the HTMR (1.3 g/g) and also associated with hydrophilic extramembraneous segments of Glut1 to give an additional binding of 0.4 g/g. Such a structure would be compact, which could account for the low apparent molecular weight observed for SDS-Glut1 upon gel filtration [60] and in SDS-polyacrylamide gel electrophoresis [31,61,62].

The SET model was applied to transmembrane regions of circular or elongated cross-sections, but can be modified to represent binding to transmembrane regions of any shape and height. In the absence of a high-resolution structure for Glut1 and other similar membrane proteins, experimental detergent binding data and accompanying SET model calculations may provide useful constraints to which three-dimensional models for these proteins would have to adhere.

5. Conclusions

Glut1 solubilized in $C_{12}E_8$ solution is monomeric and can be reconstituted by the chromatographic detergent-depletion method used for Glut1 solubilized in OG solution. The activity is initially retained even after removal of nearly all endogenous lipids. The transport activity of Glut1 in OG solution disappears upon chromatographic removal of the endogenous lipids, unless suitable lipids are present in the eluent.

The detergent binding to Glut1, expressed as gram detergent per gram polypeptide, was about the same for $C_{12}E_8$, OG and SDS. A semi-elliptical torus model for complexes between the Glut1 and the non-ionic detergents $C_{12}E_8$ and OG accounts for the binding, both for elongated 12-helical and β-barrel topologies. By combining experimental detergent-binding data with binding values according to the model some limitation on the shape of the protein can be set. The higher experimental SDS binding compared to the SET model binding indicates that SDS, does not only bind to the HTMR.

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