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Purification and functional reconstitution of a truncated human Na^+/glucose cotransporter (SGLT1) expressed in *E. coli*

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Abstract A truncated human Na^+/glucose cotransporter (C_5, residues 407–664) was expressed and purified from *Escherichia coli* using a GST fusion vector and glutathione affinity chromatography. The truncated transporter (C_5) was cleaved from GST-C_5 by Factor Xa proteolysis and purified by gel filtration chromatography. Up to 1 mg of purified GST-C_5 was obtained from 1 l bacterial culture. Reconstitution of both GST-C_5 and C_5 proteins into lipid vesicles resulted in 2.5-fold higher initial uptake rates of [^{3}H]D-glucose into C_5-proteoliposomes than into liposomes. Transport was stereospecific, saturable, and inhibited by phloretin. These properties are similar to those obtained for C_5 in *Xenopus laevis* oocytes, and provide additional evidence that the five C-terminal transmembrane helices in SGLT1 form the sugar translocation pathway.

Key words: Human SGLT1; GST fusion protein; Purification; Reconstitution

1. Introduction

SGLT1 is the archetype of a large family of integral membrane proteins with mammalian, bacterial, yeast, insect and nematode origin which transport a wide variety of substrates (sugars, inositol, proline, panthotenate, iodide, urea, etc.). The structural prediction for the primary amino acid sequences of these transporters correlates with the experimentally determined topology of the human Na^+/glucose cotransporter, SGLT1 [1], which includes 14 transmembrane α-helices. The region of highest difference in the amino acid sequences among the cotransporters is located in their carboxy-terminal domain [2]. Replacement of the C-terminus of the low affinity glucose transporter SGLT2 with the corresponding sequence of the high affinity SGLT1 in an SGLT2/SGLT1 chimera increased 10-fold the affinity for αMDG and like SGLT1 the chimera transported D-galactose and 3-*O*-methyl-glucose [3]. In addition, expression of the five C-terminal helices of SGLT1 (C_5, residues 407–664) in *Xenopus laevis* oocytes exhibited a sugar uniporter phenotype [4]. Furthermore, mutation of glutamine 457 residing in C_5 to arginine (Q457R) causes glucose-galactose-malabsorption by blocking sugar translocation [5]. In this study, human C_5 was expressed as a fusion protein in *Escherichia coli* and purified by affinity and gel filtration chromatography. Functional reconstitution of C_5 as well as the fusion protein (GST-C_5) in proteoliposomes revealed similar transport characteristics as described for C_5 when expressed in *Xenopus* oocytes [4].

2. Materials and methods

2.1. Construction of the fusion GST-C_5 plasmid

An 800 bp *Nco*I/*Nco*I fragment, positions 1218-2030 from the human SGLT1 (hSGLT1) clone [6] was isolated, and its 3′-ends 'filled in' with Klenow fragment. This fragment encodes amino acids M407–A664 and includes the last five transmembrane helices (C_5) of hSGLT1. The vector pGEX5X-1 (Pharmacia Biotech) containing the gene for the glutathione S-transferase was linearized with *Eco*RI and also treated with Klenow fragment. After a blunt-end ligation, the recombinant pGEX5X-1/C_5 plasmid encoded the fusion protein GST-C_5. The DNA construct was verified by double stranded DNA sequencing using the chain termination method [7].

One l of LB medium with 100 mg ampicillin was inoculated with 10 ml of an overnight culture of *E. coli* BL21 cells transformed with pGEX5X-1/C_5. Cells were grown at 30°C until reaching an optical density (OD_{600}) of 0.7–0.8 when expression of GST-C_5 was induced by 0.3 mM IPTG. Cells were incubated for an additional 3 h, then harvested by pelleting at 3500×*g* (20 min, 4°C).

2.2. Purification of GST-C_5

Cell pellets (4–5 g) were resuspended in 30 ml Buffer 1 (50 mM Tris-HCl, pH 8; 5 mM EDTA; 50 mM NaCl, supplemented with protease inhibitor cocktail, Sigma) and lysed by sonication with a tip sonicator (Sonic Dismembrator model 550, Fisher Scientific). Large cell debris and unbroken cells (P1, see Fig. 1A) were separated by low speed centrifugation (3500×*g* for 20 min, 4°C). The membrane protein fraction was collected by a subsequent high speed centrifugation step (350 000×*g* for 20 min, 4°C) and membrane proteins (P2) were solubilized in 20 ml Buffer 1 containing 1% Triton X-100 under continuous stirring (4 h, 4°C). The high speed centrifugation was repeated and the pellet re-extracted with 10 ml Buffer 1 containing 1% Triton X-100 (additional 4 h at 4°C). After a high speed centrifugation, both supernatants containing the solubilized GST-C_5 were pooled. GST-C_5 was separated from P2 on 2 ml bed volume glutathione-Sepharose 4B in a batch mode. The beads were pretreated according to the manufacturers protocol (Pharmacia Biotech, see also [8]). To allow complete binding of the fusion protein the suspension was agitated on an orbital shaker (3 h, 22°C). Non-specifically bound material was washed out three times for 30 min with 5 ml PBS/1% Triton X-100 for each wash. GST-C_5 was then eluted with 6 ml glutathione buffer (10 mM glutathione, 50 mM Tris-HCl pH 8, 1% Triton X-100). After addition of 1 mM $CaCl_2$, the fusion protein was digested with 30 units Factor Xa (16 h, 22°C). Subsequent ultrafiltration (Microcon YM-10, Millipore Corp.) reduced the volume to ∼1 ml, removed the glutathione and desalted the sample. 0.75 mg (250 μl) of this sample (containing GST, C_5, undigested traces of GST-C_5, Factor Xa and possible degradation products) was supplemented with 2 mM EDTA, 200 mM NaCl and loaded on a Superose 12HR 10/30 FPLC column (Pharmacia Biotech). 400 μl fractions each were eluted with 10 mM HEPES-Tris pH 8, 2 mM EDTA, 200 mM NaCl, 1% Triton X-100 (or 0.15% decyl β-D-maltopyranoside) at a flow rate of 0.2 ml/min. Fractions containing C_5 (∼200 μg) were pooled and concentrated by ultrafiltration.

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Abbreviations: SGLT1, high affinity Na^+-dependent cotransporter for D-glucose; αMDG, α-methyl-D-glucopyranoside; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis; IPTG, isopropyl 1-thio-β,D-galactopyranoside

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2.3. GST-C_5 detection, protein determination, PAGE and immunoblotting

Extraction of GST-C_5 from the membrane was monitored by measuring GST activity (GST detection module, Pharmacia Biotech). Protein concentration was determined with a BCA protein assay kit (Pierce) using bovine serum albumin as a standard. GST-C_5 protein induction, digestion and purification were followed on 12% SDS-PAGE [9], and identified by silver staining [10] and immunoblotting. The purity of GST-C_5 (and C_5) was accessed by densitometric quantitation of silver-stained SDS-PAGE gels using the software Image-QuaNT (Molecular Dynamics, Inc.). On the Western blots GST-C_5 was identified using anti-GST serum (Pharmacia Biotech) and an anti-peptide antibody, 8821, against residues 602–613 of SGLT1 [11]. Both antibodies were used at a dilution of 1:1000. Secondary antibodies for C_5 (Donkey anti-rabbit IgG peroxidase conjugate, Jackson Immunoresearch Laboratories, Inc.) and GST (alkaline phosphatase conjugate, Calbiochem) were used at a dilution of 1:10 000. Chemiluminescent signals on Hyperfilm ECL (Amersham Pharmacia-Biotech) were visualized using the SuperSignal Kit from Pierce (for horseradish peroxidase substrates) or Western-Light Plus Kit from Trophix (for alkaline phosphatase substrates).

2.4. Vesicle preparation and reconstitution

Twenty mg Cholesterol (Sigma) and 180 mg soy lecithin (Asolectin Purified Soy Phosphatides, Associated Concentrates, Woodside, NY, USA) were dissolved in 2 ml ethyl ether in glass vials. The solvent was evaporated under a stream of N_2 to obtain a thin layer of dry lipid. Ether traces were removed under vacuum in a desiccator overnight. The vials were filled then with N_2 and stored at −20°C for up to 2 weeks. Before each reconstitution, the lipids were first resuspended in 5 ml 10 mM Tris-HEPES pH 8 (final concentration 40 mg/ml) and subsequently sonicated in a N_2-atmosphere in a bath type sonicator (until the suspension became slightly clear and opaque, ∼1 h). Proteoliposomes were formed in a 1 ml reconstitution reaction when 30 μl (40 mg/ml) liposomes were mixed with 970 μl GST-C_5 or C_5 in Buffer 1/1% Triton X-100, containing 1 mM each $CaCl_2$ and $MgSO_4$. The detergent was removed by Bio Beads SM2 (BioRad) pretreated according to [12] in a ratio of 6:1 (wet weight beads:detergent, w/w). The suspension was agitated for 16 h at 4°C. Then the beads were separated by filtration (Evergreen Sci., Los Angeles, CA, USA). Proteoliposomes were collected by centrifugation (350 000 × *g* for 60 min, 4°C), washed twice with KCl buffer (150 mM KCl; 10 mM HEPES-Tris, pH 7.4; 0.1 mM $MgSO_4$; 0.1 mM $CaCl_2$) and finally resuspended in 0.5–1 ml Buffer 2 (2 mM KCl; 1 mM $MgCl_2$; 1 mM $CaCl_2$; 100 mM NaCl; 10 mM HEPES-Tris pH 7.4).

2.5. Functional assays

Downhill glucose transport into proteoliposomes was measured by using [^{3}H]D-glucose (specific activity 33.9 Ci/mmol, NEN) as a substrate. Uptakes of 3 μM [^{3}H]D-glucose into control liposomes or proteoliposomes were started by mixing equal volumes (20 μl) of washed and resuspended control liposomes (or proteoliposomes containing GST-C_5 or C_5) with the radioactive glucose solution. After the indicated time periods, reactions were stopped by addition of 1 ml ice-cold Buffer 2 and passed through nitrocellulose filters (22 μm, Millipore Corp.). The filters were washed with 5 ml Buffer 2, dissolved in 1 ml ethyl acetate and assayed by liquid scintillation counting. Each point in the graphs of Figs. 2–4 represents mean of triplicate measurements during the same experiment. Similar experiments were repeated 2–3 times.

3. Results

C_5 tagged with glutathione S-transferase was expressed in BL21 *E. coli* cells. The purification steps of C_5 are summarized in Fig. 1A and illustrated in a Western blot (left panel) and a silver-stained SDS-PAGE (right panel) in Fig. 1B. The induction of the fusion GST-C_5 protein (∼55 kDa) could only be detected on Western blots (lanes −/+, Fig. 1B) but not on silver-stained gels, indicating its low expression in the bacterial cells. The two minor bands on the Western blot detected with the specific for C_5 antibody (at 45 and 25 kDa, lanes + and P2, Fig. 1B) probably correspond to proteolytic fragments of GST-C_5. These were eliminated after the affinity chromatography purification of GST-C_5 (lane GST-C_5, Western blot, Fig. 1B). As determined by the densitometric quantitation of the silver-stained SDS-PAGE gels (Fig. 1B), GST-C_5 accounted for 90% of the protein. The contaminant bands

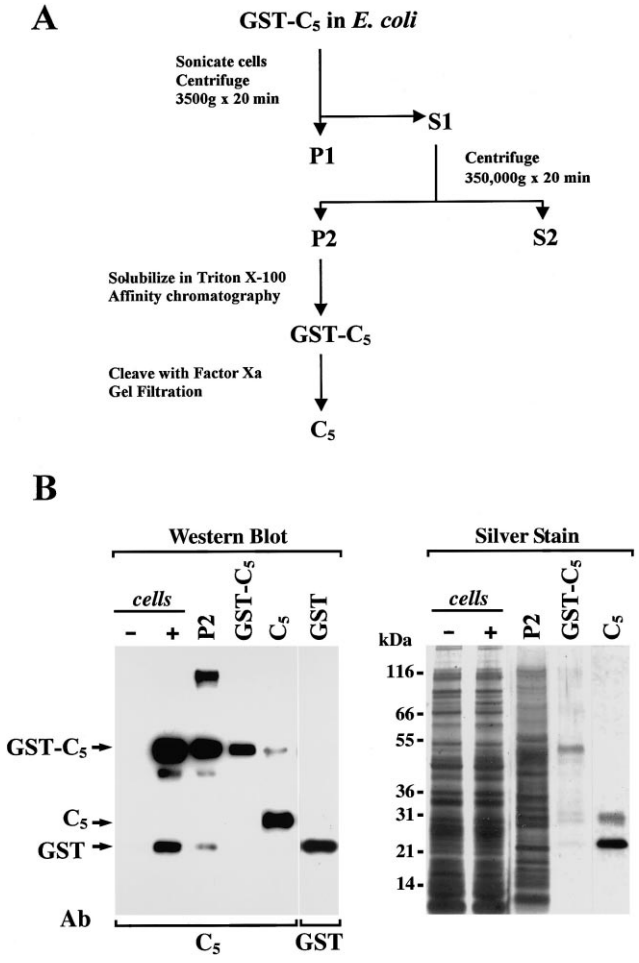

Fig. 1. A: Affinity purification of C_5 tagged with glutathione S-transferase. *E. coli* BL21 cells expressing GST-C_5 were collected and resuspended in Buffer 1. Cells were lysed by sonication and the debris and unlysed cells (P1) were separated by low speed centrifugation. The membrane protein fraction (P2) was obtained by high speed centrifugation of the S1 supernatant. The membrane proteins in the pellet P2 were solubilized with 1% Triton X-100 (80 mg membrane proteins/l culture). The corresponding to P2 supernatant (S2) contained 240 mg soluble protein/l culture. The extracted membrane proteins from P2, containing GST-C_5, were added to glutathione-Sepharose 4B beads. Non-specifically bound proteins were washed out from the matrix and 1.5 mg GST-C_5 was eluted with glutathione buffer (GST-C_5). The GST affinity tag was cleaved from GST-C_5 by digestion with Factor Xa and concentrated by ultrafiltration. The final purification step included gel filtration on a Superose 12HR column (C_5). B: 12% SDS-PAGE with BL21, GST-C_5 and C_5 proteins. Western blot (left panel) illustrating different stages of the purification procedure. GST-C_5, C_5 and GST are depicted by arrows. Lanes −/+, 5 μg of non-induced/IPTG-induced *E. coli* cell proteins; P2, 5 μg solubilized membrane proteins; GST-C_5, 2 μg eluted GST-C_5; C_5, 2 μg purified C_5 after digestion with Factor Xa, probed with antibody 8821; GST, same as C_5 lane, but re-probed with anti-GST. Right panel: corresponding silver-stained gel. Protein amounts loaded were 2 times higher than on the Western blot. Molecular mass markers (Novex, San Diego, CA, USA) are indicated in kDa and correspond to the left and right panels of the figure.

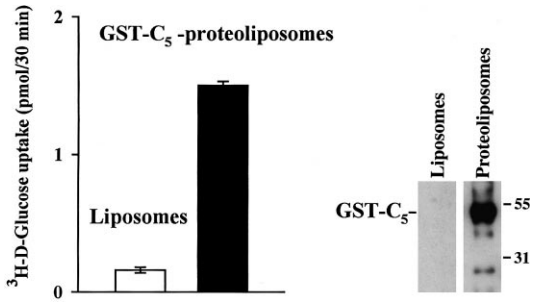

Fig. 2. Reconstitution of GST-C_5 in liposomes. Left panel: GST-C_5 integrated into liposomes transported 1.5 ± 0.03 pmol [^{3}H]D-glucose/30 min of a solution containing 3 μM [^{3}H]D-glucose, whereas the uptake into control liposomes for the same time was 0.16 ± 0.02 pmol. Right panel: Same amounts of liposomes and GST-C_5-proteoliposomes as applied for the uptakes shown in the left panel were separated on a 12% SDS-polyacrylamide gel and electroblotted to nitrocellulose. The Western blot was probed with antibody 8821.

(10% of the total protein) were not immunoreactive with the antibodies.

Of all non-ionic detergents tested: decyl-β-D-maltopyranoside (DEM), octyl-β-D-glucopyranoside (OG), 3-[(-Cholamidopropyl)dimethyl-amino]1-propanesulfonate) (CHAPS) and Triton X-100, GST-C_5 was best solubilized and most active in 1% Triton X-100 (lanes P2, Fig. 1B). After affinity purification of GST-C_5 on glutathione-Sepharose 4B beads (lanes GST-C_5, 1.5 mg/l culture) C_5 was released from GST by digestion with Factor Xa (lanes C_5). Several protocols for the final separation of GST from C_5 were tested. Once the glutathione S-transferase was released from the fusion protein, it associated tightly with C_5. For example, GST revealed similar chromatography characteristics to C_5 on an ion exchange column (Resource Q, Pharmacia Biotech). When we used the Triton X-114 phase separation method [13] GST preferentially partitioned into the detergent-depleted phase as expected, but C_5 also partially distributed in this phase, causing significant loss of C_5.

The final procedure adopted was gel filtration chromatography on a Superose 12HR 10/30 FPLC column, 24 ml bed volume (Pharmacia Biotech). Since the resolution of the Superose 12HR was in the range between 1000 and 3×10^5 Da, a quantitative separation of C_5 (29.2 kDa) from the GST (26 kDa) could not be achieved. To increase the resolution only 0.5–1 mg protein (instead of the 5 mg maximum column capacity) in a minimal volume (250 μl) was loaded per run. The quality of the eluted and pooled fractions is illustrated in Fig. 1B, lane C_5 (containing 5 μg C_5). The intensities of the only two present bands in the lane according to the densitometric scan were 42% for C_5 and 58% for GST. Note that GST runs as an intensively stained band at 26 kDa whereas C_5 is weakly stained and appears more diffuse. The same effect was observed while monitoring the Factor Xa digestion of the fusion protein. Although the molar ratio of GST to C_5 in this case should be 1, GST always stained much more intensely. This causes an overestimate in the GST contamination in the purified C_5 (Fig. 1B). The yield of purified C_5 was ~0.5 mg/l culture.

As shown in Figs. 2, 3 and 4, both proteins (GST-C_5 and C_5) could be reconstituted in proteoliposomes while retaining function. As a part of the fusion protein the GST moiety also remained active with high affinity for its substrates (1-chloro-2,4-dinitrobenzene and glutathione) as determined by the GST detection module (Pharmacia Biotech). The left panel in Fig. 2 shows that the transport of 3 μM [^{3}H]D-glucose into GST-C_5-proteoliposomes was ~10 times higher than in control liposomes (1.5 ± 0.03 versus 0.16 ± 0.02 pmol/30 min). The presence of GST-C_5 in the proteoliposomes is evident from the Western blot in the right panel.

Fig. 3A illustrates the functional reconstitution of C_5. C_5-proteoliposomes transported 3.5 ± 0.2 pmol/15 min, whereas glucose uptake in control liposomes was 1.6 ± 0.1 pmol/15 min. In order to find an optimal C_5-protein-to-lipid ratio (w/w) several C_5 concentrations were tested while the lipid content was held constant (160 μg). Highest uptakes were obtained when 160 μg lipids were mixed with 2 μg protein (protein-to-lipid ratio 1:80). This ratio was maintained in all subsequent reconstitution experiments.

The time course of [^{3}H]D-glucose uptake into liposomes and C_5-proteoliposomes is shown in Fig. 3B. The uptake into liposomes was linear for 210 min. The uptake into proteoliposomes was higher than into liposomes over the course of the

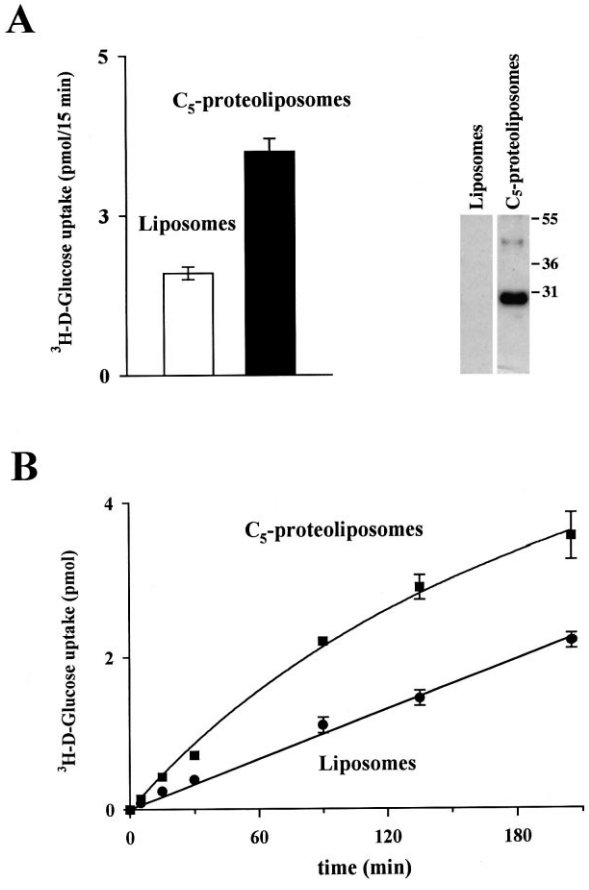

Fig. 3. Reconstitution of C_5 in liposomes. A: D-glucose uptake into liposomes and Western blot. [^{3}H]D-glucose transport (3 μM) into C_5-proteoliposomes (3.5 ± 0.2 pmol) was measured for 15 min and was two times higher than the uptake into control liposomes (1.6 ± 0.1 pmol). The signal at 31 kDa on the photograph in the right panel corresponds to the reconstituted C_5 protein and was obtained after probing the Western blot with antibody 8821. B: Time course of glucose uptake. Uptake of 3 μM [^{3}H]D-glucose into C_5-proteoliposomes was followed for 0, 5, 15, 30, 90, 135 and 210 min, when it reached 3.6 ± 0.3 pmol. In the same time period uptake by control liposomes was linear and reached 2.2 ± 0.1 pmol.

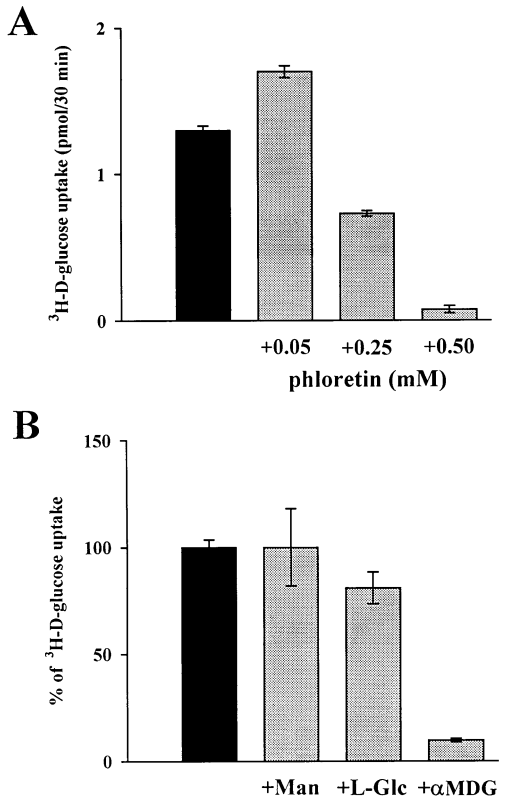

Fig. 4. Characteristics of C_5 mediated D-glucose transport. A: Inhibition. The 30 min uptake of 3 μM [^{3}H]D-glucose by C_5-proteoliposomes (1.3 ± 0.1 pmol, black bar) was not significantly affected by addition of 0.005 (not shown) and 0.05 mM phloretin (1.70 ± 0.15), but inhibited to 0.73 ± 0.05 pmol by 0.25 mM phloretin or to 0.08 ± 0.03 by 0.5 mM phloretin. Control uptake in liposomes was 0.6 ± 0.03 pmol. B: Sugar substrates. The 15 min uptake of 3 μM [^{3}H]D-glucose by C_5-proteoliposomes (black bar) was competed with 100 mM each of mannitol, L-glucose or αMDG. In order to compare measurements from several different experiments, data were normalized to the values obtained for the [^{3}H]D-glucose uptake (3.5 ± 0.2 pmol) over 15 min, taken as 100%. Mannitol had no effect on the uptake (100 ± 18%), whereas L-glucose and αMDG inhibited the uptake by 19 ± 2% and 90 ± 9%, respectively. Shown is the mean of three experiments.

experiment. Uptake was linear over the first 15–30 min and then the rate decreased with time. Regression analysis of the uptakes into proteoliposomes over 210–780 min indicated that the sugar uptake reached a maximum of 8–10 pmol with a half time of approximately 300 min. In five experiments the initial rate of glucose uptake into the proteoliposomes was about 2.5-fold higher than into liposomes, i.e. 0.031 ± 0.003 versus 0.013 ± 0.003 pmol/min. Glucose transport into C_5-proteoliposomes was inhibited by phloretin with an apparent K_i of ~250 μM (Fig. 4A). The 30 min uptake (1.3 ± 0.1 pmol) was reduced ~50% by 0.25 mM phloretin (0.73 ± 0.1 pmol) and was almost completely eliminated by addition of 0.5 mM phloretin (0.08 ± 0.03 pmol). In three experiments with control liposomes, glucose uptake was reduced 10–20% by 0.5 mM phloretin.

[^{3}H]D-glucose transport into C_5-proteoliposomes was blocked by 100 mM α-methyl-D-glucose (αMDG), but not by 100 mM mannitol or 100 mM L-glucose (Fig. 4B). D-Glucose uptake in the presence of mannitol remained unchanged (100 ± 18%; $n = 3$), whereas L-glucose reduced the uptake by 19 ± 2% ($n = 3$) and αMDG reduced it by 90 ± 9%. D-Glucose uptake into control liposomes was only inhibited ~20% by the addition of 100 mM αMDG, L-glucose or mannitol. 100 mM mannitol or L-glucose also did not significantly inhibit D-glucose uptake into GST-C_5-containing proteoliposomes. For example, the 15 min uptake of [^{3}H]D-glucose (1.41 ± 0.1 pmol) remained unchanged in 100 mM mannitol (1.45 ± 0.15 pmol) and was only reduced to 1.11 ± 0.01 pmol in 100 mM L-glucose (not shown).

4. Discussion

In this study, we have purified functional human C_5 after its heterologous expression in *E. coli*. The putative sugar translocation domain (C_5) of SGLT1 was expressed as a fusion protein in BL21 cells, purified by affinity chromatography and gel filtration and reconstituted into liposomes.

Both the GST-C_5 fusion protein and C_5 protein purified from *E. coli* were functional when reconstituted into liposomes. The sugar transport properties of reconstituted C_5 were similar to those for C_5 expressed in *X. laevis* oocytes [4]. The C_5 mediated sugar uniport into oocytes was stereospecific, saturable and sensitive to the inhibitor phloretin (K_i ~0.5 mM). Transport of 50 μM ^{14}C-αMDG was reduced 50% by 50 mM αMDG ([4], Fig. 3C) or 100 mM D-glucose ([4], Fig. 3D), suggesting a $K_{0.5}$ of ~50 or 100 mM, respectively. Incorporation of the GST-C_5 and C_5 proteins into lipid vesicles, as confirmed by Western blotting (Figs. 2 and 3A), resulted in initial rates of D-glucose transport into the vesicles higher than into liposomes (Figs. 2 and 3). The estimated K_i for phloretin inhibition of D-glucose uptake in C_5-proteoliposomes was ~0.25 mM. This K_i is less than the K_i determined for αMDG uptake into oocytes (K_i ~0.5 mM, [4]), which may be due to the different affinity of C_5 for the two sugar substrates (for $K_{0.5}^{\alpha MDG}$ ~50 mM and $K_{0.5}^{D-glc}$ ~100 mM). As judged by the effects of phloretin and the specificity of sugar substrates (αMDG versus L-glucose and mannitol), C_5 mediated a stereospecific and saturable sugar transport across the lipid membrane.

The yield of fusion GST-C_5 (~1 mg/l) was significantly lower than that expected for soluble proteins (~10-fold less than manufacturer's description), and represented ~1.2% of the total membrane protein (P2, 80 mg, see Fig. 1A). The lower expression level is probably due to the fact that cells can not tolerate large amounts of the foreign protein within their membranes and thus may be toxic to the cells. Milligram quantities of membrane proteins have only been obtained by overexpressing the protein in inclusion bodies, IBs [14]. Proteins derived from IBs are denatured and have to be re-folded to the native state to obtain functional protein. The presence of the large polar cytoplasmic loop in the C_5 protein decreases the hydrophobicity of C_5 and could explain the higher amounts of GST-C_5 detected in the soluble fraction S2 (0.9% from the 240 mg total S2 protein, Fig. 1B). The soluble GST-C_5 decreases the apparent yield of final C_5 isolated only from the membrane fraction (P2, Fig. 1B). Under the conditions for growth (30°C) and induction (addition of 0.3 mM IPTG at 0.8 OD_{600}) the amount of soluble GST-C_5 was at its minimum. While contamination of foreign proteins/degradation products during the purification of C_5 was not significant (10% of the total protein, see Fig. 1B), separation of C_5 from its fusion partner became a daunting task. The strong ten-

dency of both proteins to copurify was difficult to be resolved. GST also copurified during the purification procedure of another transmembrane protein – the olfactory receptor – when expressed as a fusion protein [15]. This tight association of GST with C_5 and the noticeable quantity of GST-C_5 in the soluble protein fraction indicate that the pGEX expression system is probably not the expression system of choice for further purification of C_5.

In summary, this study demonstrates that a human truncated transporter (C_5) may be overexpressed in *E. coli*, purified to homogeneity, and reconstituted into proteoliposomes in its functional form. The transport properties of the reconstituted protein resemble those previously reported for C_5 expressed in *X. laevis* oocytes, and this provides additional evidence that the C-terminal five transmembrane helices form the sugar translocation pathway through the Na^+/glucose cotransporter. The GST fusion system provides a fair yield of purified recombinant protein (up to 1 mg per l of culture, 90% purity). However, as reported for another membrane protein, it has proven difficult to separate C_5 from GST, thereby limiting the utility of the system for biochemical and structural studies of the transporter. Our future approach will include overexpression of the transporter in *E. coli* and purification of C_5 (and SGLT1) using Ni^+ affinity chromatography. This has been recently demonstrated for another member of the Na^+/glucose cotransporter family, the Na^+/proline transporter of *E. coli* [16].

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