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Isolation and Functional Reconstitution of Soluble Melibiose Permease from *Escherichia coli*

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ABSTRACT: By use of techniques described recently for *lac* permease [Roepe, P. D., & Kaback, H. R. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 6087], the melibiose permease from *Escherichia coli*, another polytopic integral plasma membrane protein, has been purified in a metastable soluble form after overexpression of the *melB* gene via the T7 RNA polymerase system. As demonstrated with *lac* permease, soluble melibiose permease is dissociated from the membrane with 5.0 M urea and appears to remain soluble in phosphate buffer at neutral pH after removal of urea by dialysis, although the protein aggregates in a time- and concentration-dependent fashion. Moreover, soluble melibiose permease behaves as a monomer during purification by size exclusion chromatography in the presence of urea. Circular dichroism of purified soluble melibiose permease reveals that the protein is highly helical in potassium phosphate buffer and that secondary structure is disrupted in 5.0 M urea. Finally, purified melibiose permease can be reconstituted into proteoliposomes, and the preparations catalyze membrane potential driven H^+/melibiose or Na^+/methyl 1-thio-β,D-galactopyranoside symport. The results provide further support for the notion that hydrophobic transmembrane proteins may be able to assume a nondenatured conformation in aqueous solution and extend the implication that the approach described may represent a general method for rapid isolation and reconstitution of this class of membrane proteins.

The facultative anaerobe *Escherichia coli* utilizes four types of membrane transport mechanisms [cf. Neidhart et al. (1987)]: (i) vectorial phosphorylation via the phosphoenolpyruvate–phosphotransferase system which catalyzes covalent modification of certain sugars during translocation; (ii) periplasmic binding protein mediated transport in which high-energy phosphate is used directly as a source of energy; (iii) primary active transport in which H^+ is extruded by the respiratory chain or the H^+-ATPase leading to the generation of an H^+ electrochemical gradient ($\Delta\bar{\mu}_{H^+}$[1]; interior negative and/or alkaline); and (iv) secondary active transport in which solute is accumulated against a concentration gradient in response to $\Delta\bar{\mu}_{H^+}$.

Accumulation of sugars such as lactose or melibiose by secondary active transport involves cotransport with H^+ or Na^+ in response to $\Delta\bar{\mu}_{H^+}$ (i.e., symport). The process is mediated by substrate-specific polytopic inner membrane proteins called porters, carriers, or permeases which utilize the free energy released from the downhill flow of H^+ or Na^+ to drive uphill accumulation of solute. One well-studied example of a secondary active transport system is the *lac* permease, which has been solubilized from the cytoplasmic membrane, purified to homogeneity, and shown to catalyze the coupled translocation of a single H^+ with a single β-galactoside molecule in monomeric form [cf. Kaback (1988, 1989) for reviews]. The *lacY* gene, which encodes the permease, has been sequenced (Büchel et al., 1980), and on the basis of circular dichroic studies with purified *lac* permease and the hydrophathy profile of the deduced amino acid sequence, it was suggested that the protein is composed of 12 hydrophobic transmembrane α-helices connected by loops containing most of the charged residues (Foster et al., 1983). Evidence supporting some of the general features of the model has been obtained [cf. Kaback (1988, 1989)], and more recently, a series of *lacY–phoA* fusions [cf. Manoil and Beckwith (1986)] has provided strong support for

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[1] Abbreviations: $\Delta\bar{\mu}_{H^+}$, the H^+ electrochemical gradient; CD, circular dichroism; TMG, methyl 1-thio-β,D-galactopyranoside; KP_i, potassium phosphate; DTT, dithiothreitol; NaP_i, sodium phosphate; $NaDodSO_4$, sodium dodecyl sulfate; Ch, choline.

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more specific topological predictions of the model (J. Calamia and C. Manoil, unpublished results). Similar structures have been proposed for the melibiose permease (Botfield, 1989) and a number of other membrane transport proteins all of which appear to contain a 12-transmembrane helical motif (Baldwin & Henderson, 1989).

The melibiose permease is a particularly interesting model system for secondary active transport, as it catalyzes symport with H^+, Na^+, or Li^+ depending upon the substrate. Cotransport of the α-galactoside melibiose occurs with H^+ or Na^+, while cotransport of methyl 1-thio-β,D-galactopyranoside occurs with Li^+ or Na^+ (Tsuchiya & Wilson, 1978). Site-directed mutagenesis studies have begun to identify amino acid residues in melibiose permease that are important for activity, and mutants have been described that exhibit altered cation specificity (Kawakami et al., 1988; Botfield & Wilson, 1988). For continued progress with the system, however, purified protein is needed. Toward this end, Tsuchiya et al. (1982) reported a partial purification of melibiose permease using procedures similar to those used for *lac* permease which entail preextraction of *E. coli* membranes with high concentrations of urea and cholate, followed by solubilization of melibiose permease with octyl β,D-glucopyranoside in the presence of exogenous phospholipids. Although functional reconstitution was achieved, the procedure resulted in only a 4–5-fold purification from the membrane.

Recently, Roepe and Kaback (1989) described a simple method for purifying *lac* permease based on overexpression of the *lacY* gene via the T7 RNA polymerase system (Tabor & Richardson, 1985). In brief, overproduction of *lac* permease in this manner leads to the appearance of a unique form of the protein which appears to be membrane-associated but not inserted into the bilayer. Thus, it is dissociated from the membrane with urea or other chaotropes and can be purified with little or no bound phospholipid. Although *lac* permease does not precipitate after removal of the chaotrope and behaves as a monomer during size exclusion chromatography, aggregation occurs in a time- and concentration-dependent fashion, suggesting that the soluble form of the protein is metastable. Remarkably, it was also found that purified soluble *lac* permease binds ligand in phosphate buffer and that it can be reconstituted into proteoliposomes in a functional state.

We now report that the melibiose permease from *E. coli* can also be obtained in a functional state by using similar techniques. In addition to demonstrating that the approach may be of general use, the results provide further support for the heretical contention that integral polytopic membrane proteins may be able to adopt a nondenatured conformation in aqueous solution.

Materials and Methods

Materials

[^{3}H]Melibiose was a generous gift from G. Leblanc (Laboratoire J. Maetz, Villefranche sur mer, France). Antiserum directed against the melibiose permease carboxyl terminus was a generous gift from T. H. Wilson (Harvard University). [^{3}H]Methyl 1-thio-β,D-galactopyranoside (TMG) was purchased from Amersham/Searle and used without further purification. All other materials were of reagent grade and were obtained from commercial sources.

Methods

Growth of Cells and Overexpression of melB. Overexpression of *melB*, the gene encoding melibiose permease, was accomplished via the T7 RNA polymerase expression system of Tabor and Richardson (1985). Briefly, the T7 polymerase system involves heat-induced derepression of the gene encoding T7 RNA polymerase, which is under control of the λP_L promoter (plasmid pGP1-2), followed by T7 polymerase catalyzed transcription of a given gene, in this case *melB*, which is under the control of the T7 promoter (plasmid pT7-6). The strategy allows controlled high expression of *melB*, as synthesis of T7 polymerase is dependent on derepression of P_L. Furthermore, since *E. coli* RNA polymerase is inhibited by rifampicin and T7 polymerase is resistent, the system allows specific expression of the gene under control of the T7 promoter.

melB was cloned into plasmid pT7-6 by a procedure described by Pourcher et al. (1989). A 3-kbp fragment demonstrated by complementation tests to contain the *melA* and *melB* genes (Hanatani et al., 1984) which encodes α-galactosidase and melibiose permease, respectively, was restricted from a recombinant plasmid in the Clark and Carbon colony bank with *Pst*I and *Eco*RI and ligated into M13mp18 DNA. After mutation by oligonucleotide-directed, site-specific mutagenesis of an upstream region to create a novel *Eco*RI site, the replicative form of mutant M13mp18 DNA was restricted from *Eco*RI, and the 1.5-kbp fragment containing *melB* was ligated into plasmid pT7-6 digested with *Eco*RI. Restriction analysis with *Eco*RV was used to determine the orientation of *melB* in the plasmid, and pT7-6(*melB*) was then transformed into *E. coli* T184 [*lacI*$^+$*O*$^+$*Z*$^-$Y$^-$(*A*),*rpsL*,*met*$^-$,*thr*$^-$,*recA*,*hsdM*,*hsdR*/*F*',*lacI*^{q}*O*$^+$*Z*^{U118}(*Y*$^+$*A*$^+$)] (Teather et al., 1980) harboring plasmid pGP1-2 (Tabor & Richardson, 1985). As shown by Pourcher et al. (1989), pT7-6(*melB*) confers melibiose transport activity to the *melB*$^-$ strain *E. coli* DW2 [Δ*lacZY*,*melA*$^+\Delta$*B*] (Botfield & Wilson, 1988).

E. coli T184 harboring pGP1-2 and pT7-6(*melB*) were grown at 30 °C on Luria broth containing 50 μg/mL each of kanamycin and ampicillin to an OD_{590} of 0.5–1.0. The cells were harvested by centrifugation, washed three times in M9 salts (Miller, 1972), and resuspended to an OD_{590} of 1.0 in M9 medium supplemented with 0.01% each of 18 amino acids except for methionine and cysteine. Suspensions were incubated at 30 °C for 90 min to starve the cells for methionine and then heat-shocked at 42 °C for 15 min. Rifampicin was added to 200 μg/mL, and incubation at 42 °C was continued for 2–3 h. The cells were harvested by centrifugation, washed in M9 salts, and either frozen in liquid nitrogen and stored at −20 °C or kept at 4 °C as a pellet for up to 12 h.

Preparation of Soluble Melibiose Permease. Typically, cells from 1.0-L cultures treated as described above were resuspended to a volume of 40 mL in 100 mM potassium phosphate (KP_i; pH 7.3)/200 μM phenylmethanesulfonyl fluoride/10 mM melibiose/1.0 mM dithiothreitol (DTT) and lysed by passage through a French pressure cell at 16 000–20 000 psi. Unlysed cells were removed by centrifugation at 2100 rpm for 10 min in a GS-3 rotor (Sorvall). Membranes (ca. 50 mg total membrane protein) were harvested by centrifugation at 150000g_{max} for 45 min in a 45Ti rotor (Beckman). Crude soluble melibiose permease was then prepared by resuspending membranes in 5.0 M urea/100 mM KP_i (pH 7.3)/10 mM melibiose/1.0 mM DTT, incubating at room temperature for 10 min, and centrifuging at 150000g_{max} as described above.

Soluble melibiose permease was purified further from the urea extract by size exclusion chromatography on Sephacryl 200SF (Pharmacia). The column buffer contained 5.0 M urea/100 mM KP_i (pH 7.3)/10 mM melibiose/1.0 mM DTT. A 45 × 0.6 cm column was used and run at a flow rate of 0.3 mL/min. The column was standardized by using 100 μg/mL samples of lysozyme (14 kDa), ovalbumin (44 kDa) and creatine phosphokinase (80 kDa, dimeric).

Reconstitution into Proteoliposomes. After dialysis against a 200–300-fold excess of 50 mM KP_i (pH 7.3)/10 mM melibiose/1.0 mM DTT, purified melibiose permease (75–100 μg/mL protein, final concentration) was added to washed *E. coli* phospholipids (Newman et al., 1981; Viitanen et al., 1986) suspended in 50 mM KP_i (pH 7.3)/10 mM melibiose/1.0 mM DTT to a concentration of 50 mg/mL. The suspension was shaken vigorously at 37 °C for 45 min, cooled slowly to room temperature, diluted rapidly into a 50-fold excess of 50 mM KP_i (pH 7.3)/1.0 mM DTT at room temperature, and centrifuged at 175000g_{max} for 90 min in a 70.1Ti rotor (Beckman). The supernatant was aspirated, and the proteoliposomes were resuspended to a final concentration of 50 mg/mL phospholipid and 75–100 μg/mL protein in 50 mM KP_i (pH 7.3)/1.0 mM DTT. Aliquots (50 μL) were sonicated for 10 s in a bath sonifier (Newman et al., 1981; Viitanen et al., 1986) prior to assay.

Transport Assays. Active transport of [^{3}H]melibiose or [^{14}C]TMG in reconstituted proteoliposomes was assayed as described for *lac* permease (Newman et al., 1981; Viitanen et al., 1986). Aliquots (3 μL) of proteoliposomes equilibrated with 50 mM KP_i (pH 7.3) and containing 10 mM valinomycin were rapidly diluted 100-fold into 50 mM KP_i, sodium phosphate (NaP_i), or ChP_i (pH 7.3), as indicated, containing 1.0 mM [^{3}H]melibiose (10 mCi/mmol) or 0.5 mM [^{14}C]TMG (10 mCi/mmol). At given times, transport was quenched by addition of 3.0 mL of ice-cold NaP_i or ChP_i (pH 5.5)/10 mM $HgCl_2$, as indicated, and the samples were immediately filtered through Whatman filters (type WCN 02500, 0.45-μm pore size), followed by one wash with the same buffer. Trapped radioactivity was quantitated by liquid scintillation spectrometry.

Sodium Dodecyl Sulfate (NaDodSO$_4$)–Polyacrylamide Gel Electrophoresis. $NaDodSO_4$–polyacrylamide gel electrophoresis was performed in 12% polyacrylamide gels (10 × 6.5 × 0.15 cm) containing 0.1% $NaDodSO_4$ and 250 mM Tris-HCl (pH 8.0) with a 2.5-cm 5% polyacrylamide stacking gel. Samples were incubated in 1.0% $NaDodSO_4$/10% glycerol/1.0% β-mercaptoethanol/0.02% bromophenol blue for 30 min at 37 °C before application, and the gels were run at 14-mA constant current. Silver staining was carried out as described [cf. Viitanen et al. (1986)].

Immunoblot Analysis. Immunoblot analysis was performed as described (Roepe et al., 1989) by using antiserum directed against the carboxyl terminus of melibiose permease (Botfield & Wilson, 1989) and ^{125}I protein A.

Circular Dichroism (CD). CD spectra were obtained at 4 °C in a Cary 61 spectrometer. An aliquot (500 μL) of purified soluble permease in either 100 mM KP_i (pH 7.3) or 5.0 M urea/100 mM KP_i (pH 7.3) at a concentration of 50 μg/mL protein was placed in a quartz cuvette that was water-cooled by a circulator (Brinkman). The scan rate was 10 nm/min.

Phosphate Analysis. Purified melibiose permease was precipitated with 10% trichloroacetic acid, and the protein was collected by centrifugation for 15 min in a Beckman microfuge and washed three times with high-performance liquid chromatography grade water. Alternatively, the purified protein was extracted with chloroform/methanol (3:1 v/v), and phosphate analyses were performed on the dried extract. Phosphate was determined by the method of Chen et al. (1956) using KP_i as a standard. Both samples yielded similar results.

Protein. Protein was determined with amido black as described [cf. Viitanen et al. (1986)].

Results

Overexpression of melibiose permease at a high rate via the T7 RNA polymerase system yields results similar to those reported previously for *lac* permease (Roepe & Kaback, 1989). Thus, when membrane fractions are isolated from cell cultures harvested at short time intervals after initiation of transcription of *melB*, a full complement of melibiose permease is found in the membrane within 30–60 s [data not shown; cf. Roepe and Kaback (1989)]. Upon continued incubation of the cells with excess methionine, a form of melibiose permease that is dissociated from the membrane with 5.0 M urea begins to appear after about 2 min, and by 2–3 h, 40–50% of the melibiose permease in the cell is dissociated from the membrane with 5.0 M urea.

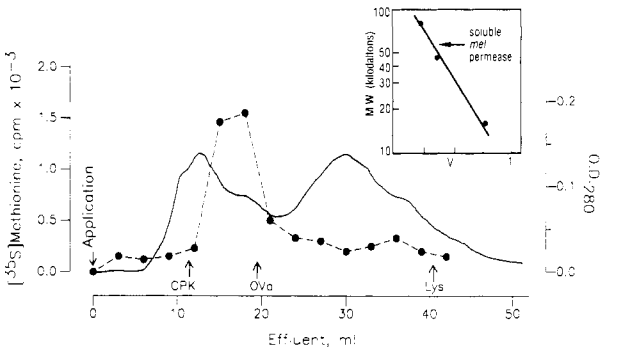

FIGURE 1: Size exclusion chromatography of urea-soluble melibiose permease. Membranes isolated from *E. coli* T184/pGP1-2/pT7-6-(*melB*) grown in the presence of [^{35}S]methionine as described under Methods are resuspended in 5.0 M urea/100 mM KP_i (pH 7.3)/10 mM melibiose/1.0 mM DTT and centrifuged for 45 min at 150000g_{max} in a 45Ti rotor (Beckman). One milliliter of the supernatant (ca. 850 μg of protein) was placed on a Sephacryl 200SF column that was then developed with 5.0 M urea/100 mM KP_i (pH 7.3)/10 mM melibiose/1.0 mM DTT. The solid line represents the elution profile monitored at OD_{280}, and the broken line represents radioactivity monitored by liquid scintillation spectrometry. Most of the radioactivity eluted in the fraction collected at $V = 0.36$, which corresponds to the volume at which a globular protein with a molecular weight of approximately 55 000 should elute (cf. insert showing the calibration curve for the column $V = V_i/V_T$, where V_i is the volume at which a peak is eluted and V_T is the total volume of the column). Although gel filtration in the presence of urea does not necessarily yield a linear relationship between elution volume and log molecular weight, we observe such an empirical correlation with the standards chosen [cf. Creighton (1979)]. Protein assays by amido black [cf. Viitanen et al. (1986)] indicate that the yield of purified urea-soluble melibiose permease is approximately 300 μg from a 1.0-L culture.

Soluble Melibiose Permease Is Monomeric. When the urea extract containing [^{35}S]methionine-labeled melibiose permease is chromatographed on Sephacryl 200SF in the presence of urea, 90% of the radioactivity elutes as a symetrical peak centered at 55 kDa (Figure 1), a value near that predicted from the sequence of *melB* (i.e., 52 kDa; Yazyu et al., 1984). Furthermore, chromatography on Sephacryl 200SF results in substantial purification of the protein, similar to results obtained previously with *lac* permease (data not shown, cf. Roepe and Kaback (1989)]. As shown in Figure 2, the bands heavily labeled with [^{35}S]methionine that migrate at 39 kDa, the M_r reported by Pourcher et al. (1989) for melibiose permease electrophoresed under the same conditions, also react intensely with antiserum directed against the carboxyl terminus of melibiose permease (Botfield & Wilson, 1989). The higher molecular weight band at 88 000 which also reacts with antibody probably represents a dimeric form of melibiose permease, as suggested by Pourcher et al. (1989).

Remarkably, when purified urea-soluble melibiose permease is dialyzed overnight against 100 mM KP_i (pH 7.3), no visible precipitation is observed, a phenomenon reported previously for similar preparations of *lac* permease (Roepe & Kaback, 1989). Furthermore, the urea extract and the dialyzed

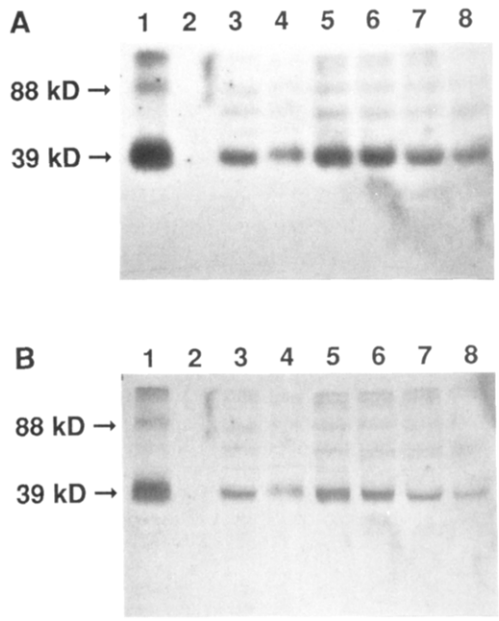

FIGURE 2: Specific [^{35}S]methionine labeling and immunoblot analysis of crude and purified soluble melibiose permease. $NaDodSO_4$–polyacrylamide gel electrophoresis was carried out as described under Methods, and the gel was blotted to nitrocellulose overnight at room temperature. After blocking with 5% bovine serum albumin/100 mM Tris (pH 7.4)/9% NaCl/2% Triton X-100, the nitrocellulose was incubated with 40 μL of anti-C-terminal antiserum, followed by incubation with 6 μL of ^{125}I protein A (30 μCi/μg), both in 10 mL of the blocking solution. After drying, two pieces of Kodak X-AR2 film were placed on the nitrocellulose with a piece of filter paper between them, and autoradiography was carried out for 48 h at −70 °C. The film closest to the nitrocellulose is exposed by both ^{35}S and ^{125}I, while the second film is exposed by ^{125}I only. (A) is a photograph of the first film, (B) of the second. (Lane 1) Membrane fraction from *E. coli* T184/pGP1-2/pT7-6(*melB*) grown in the presence of [^{35}S]methionine as described under Methods; (lane 2) cytoplasmic fraction collected after passage of the cells through a French pressure cell; (lane 3) supernatant obtained after membranes were washed with 5.0 M urea/100 mM KP_i (pH 7.3)/10 mM melibiose/1.0 mM DTT; (lanes 4–8) fractions collected from the Sephacryl 200SF column at 14, 15, 16, 17, and 18 mL, respectively (cf. Figure 1). The bands heavily labeled with ^{35}S at 39 kDa in (A) also react strongly with anti-C-terminal antiserum and correspond to melibiose permease (Pourcher et al., 1989).

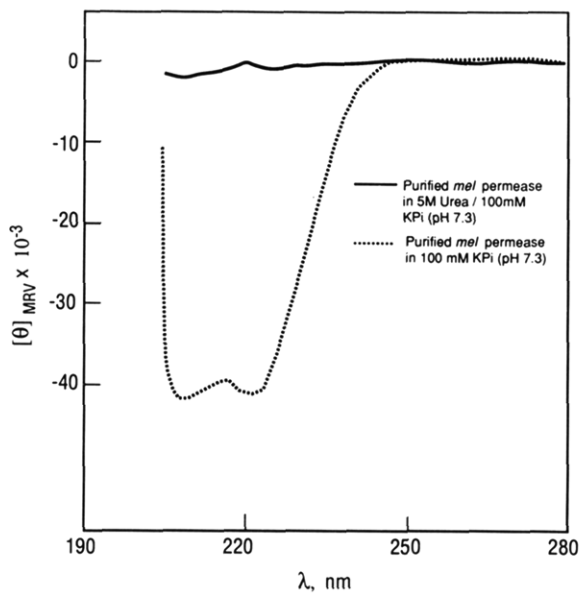

FIGURE 3: CD spectra of purified soluble melibiose permease in the absence (broken line) and presence of 5.0 M urea (solid line). Purified soluble melibiose permease was obtained by extracting membranes from *E. coli* T184/pGP1-2/pT7-6(*melB*) with 5.0 M urea/100 mM KP_i (pH 7.3) as described under Methods. The urea extract was then purified as described in Figure 1, and fractions 16–18 containing purified melibiose permease were pooled. An aliquot was then dialyzed against 100 mM KP_i (pH 7.3) overnight as described under Methods. The protein concentration was approximately 50 μg/mL for both samples. See Methods for further details.

preparation are stable for at least a week at 4 °C without visible aggregation, and functional proteoliposomes can be prepared (see below) even after a week of storage. However, the protein aggregates in a time- and concentration-dependent manner, as evidenced by centrifugation at $175000g_{max}$ for 60 min. One day after storage at 4 °C, about 10% of the protein is sedimented, and after 1 week, approximately 70% of the material is sedimented. Conversely, little of the material is sedimented from 5.0 M urea/100 mM KP_i (pH 7.3)/10 mM melibiose/1 mM DTT after 5 days at 4 °C.

The results are similar qualitatively to those reported previously for *lac* permease (Roepe & Kaback, 1989). It is also noteworthy that organic phosphate determinations carried out on purified soluble melibiose permease after either precipitation with trichloroacetic acid or extraction with chloroform/methanol demonstrate that less than 3 mol of phosphate is present per mole of protein. Therefore, it is unlikely that the protein contains significant amounts of bound phospholipids or lysophosphatides.

CD Spectroscopy. The results presented in Figure 3 represent CD spectra obtained from purified melibiose permease dissolved in 100 mM KP_i (pH 7.3) (broken line) or 100 mM KP_i (pH 7.3)/5.0 M urea (solid line). In the absence of urea, melibiose permease exhibits spectral characteristics typical of a protein that is largely helical in conformation (estimated at >90%). Strikingly, however, in the presence of 5.0 M urea, secondary structure is substantially disrupted. The spectrum obtained for soluble melibiose permease in the absence of urea is very similar to that obtained for *lac* permease under the same conditions (Roepe & Kaback, 1989) and suggests that the two proteins adopt similar conformations in aqueous solution. Furthermore, flow dialysis measurements indicate that soluble melibiose permease is able to bind *p*-nitrophenyl α,D-galactopyranoside significantly, although we have not yet been able to demonstrate that binding is sodium dependent [cf. Tokuda and Kaback (1978) and Damiano-Forano et al. (1986)]. In any event, the data taken as a whole suggest that melibiose permease, like *lac* permease, can adopt a conformation in aqueous solution that is not radically different from its conformation in the membrane.

Reconstitution of Soluble Melibiose Permease into Proteoliposomes. When purified [^{35}S]methionine-labeled melibiose permease in 100 mM KP_i (pH 7.3)/5.0 M urea is mixed with *E. coli* phospholipids and proteoliposomes are prepared, very little radioactivity sediments with the proteoliposomes, and no transport activity is observed when the proteoliposomes are diluted into equimolar NaP_i (pH 7.3). In contrast, when urea is removed by dialysis against 50 mM KP_i (pH 7.3) prior to the mixing with phospholipids, 60–70% of the radioactivity co-sediments with the proteoliposomes in a form that is no longer extracted with 5.0 M urea. Moreover, when the proteoliposomes, which are prepared in KP_i (pH 7.3), are diluted into equimolar NaP_i (pH 7.3) in the presence of valinomycin so that a membrane potential ($\Delta\Psi$, interior negative) is generated, [^{3}H]melibiose accumulation is observed (Figure 4A, solid symbols). On the other hand, when the proteoliposomes are diluted into equimolar KP_i (pH 7.3) so that no $\Delta\Psi$ is generated, melibiose accumulation is negligible (Figure 4A, open symbols), and after several minutes, the internal concentration of sugar approximates that of the external medium.

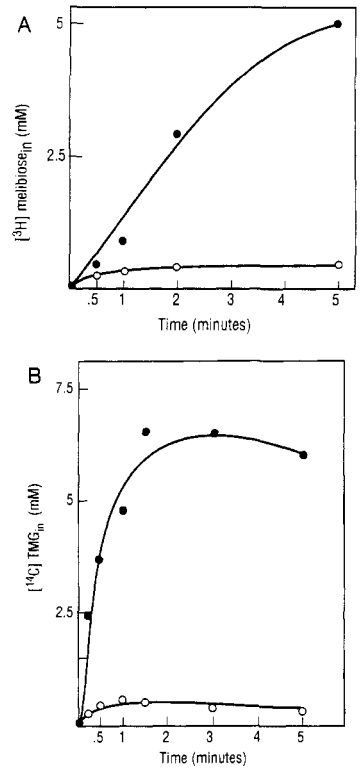

FIGURE 4: $\Delta\Psi$-driven melibiose (A) or TMG (B) accumulation in proteoliposomes reconstituted with purified melibiose permease. Urea-soluble melibiose permease was purified, dialyzed, and reconstituted into proteoliposomes as described under Methods. Proteoliposomes containing approximately 100 μg/mL protein and 50 mg/mL phospholipid were equilibrated with 50 mM KP_i (pH 7.3), and valinomycin was added to a final concentration of 10 μM. In (A), aliquots (3 μL) were rapidly diluted 100-fold into equimolar NaP_i (pH 7.3) (solid circles) or KP_i (pH 7.3) (open circles) containing 1.0 mM [^{3}H]melibiose (10 mCi/mmol); in (B), aliquots (3 μL) were diluted into equimolar NaP_i (pH 7.3) (solid circles) or ChP_i (pH 7.3) (open circles) containing 0.5 mM [^{14}C]TMG (10 mCi/mmol). At times indicated, the reactions were quenched with ice-cold 50 mM NaP_i (pH 5.5) (A) or ChP_i (pH 5.5) (B), and the samples were assayed as described under Methods.

Although data are not shown, the preparations also catalyze entrance counterflow when the proteoliposomes are equilibrated with 10 mM melibiose and then diluted into 1.0 mM [^{3}H]melibiose.

Since melibiose permease catalyzes cotransport of TMG with Na^+ or Li^+ but not with H^+, $\Delta\Psi$-driven TMG accumulation was studied in the presence or absence of Na^+ by diluting KP_i-loaded proteoliposomes containing valinomycin into equimolar NaP_i (Figure 4B, solid symbols) or ChP_i (Figure 4B, open symbols). As shown, when the diluant contains Na^+, rapid uptake of TMG is observed, resulting in 12–15-fold accumulation over the external concentration at the steady state. In contrast, when a comparable $\Delta\Psi$ is generated in the absence of Na^+ by dilution into ChP_i, TMG accumulation is negligible.

Discussion

The results presented in this paper demonstrate that melibiose permease can be isolated in soluble form by using procedures described for the preparation of soluble *lac* permease (Roepe & Kaback, 1989). In analogy with *lac* permease, under the growth conditions used soluble melibiose permease appears after a full complement of molecules is inserted into the membrane, electron microscopy (not shown) does not reveal the presence of inclusion bodies which are commonly observed after overexpression of soluble or peripheral membrane proteins, and protein is clearly associated with the membrane fraction of the cell. Since the protein is readily dissociated from the membrane in 5.0 M urea, an operation that does not solubilize the membrane-inserted form of *lac* permease to any extent whatsoever (Newman et al., 1981; Viitanen et al., 1986), it seems likely that this new form of melibiose permease is associated with the membrane but not inserted into the bilayer.

As reported for *lac* permease (Roepe & Kaback, 1989), melibiose permease is partially purified in monomeric form by size exclusion chromatography in urea, and the protein remains in solution after urea is removed by dialysis. The purified material also remains optically clear for a week in KP_i (pH 7.3) at 4 °C, although it aggregates in a time- and concentration-dependent manner, as judged by centrifugation studies. Purified melibiose permease is largely helical in conformation when dissolved in 100 mM KP_i, as evidenced by circular dichroism, but exhibits little helical structure in 5.0 M urea. Interestingly, the purified protein does not reconstitute into proteoliposomes from 5.0 M urea, thereby suggesting that helical structure may be important for association with phospholipids.

Although the approach described has been applied to only two membrane proteins thus far, the *lac* and melibiose permeases, it is apparent that the techniques may be useful for other polytopic membrane proteins, particularly those that fall into the superfamily containing a 12-transmembrane helical motif (Baldwin & Henderson, 1989). Furthermore, the observations that melibiose permease remains soluble in phosphate buffer in a largely helical conformation and that it appears to be able to bind ligand to a significant extent in aqueous solution provide further support for the heretical notion that polytopic transmembrane proteins may be able to adopt a near-native conformation in aqueous solution, a point raised in the earlier study with *lac* permease (Roepe & Kaback, 1989). To explain similar metastable properties of *lac* permease, Roepe and Kaback (1989) suggested that the hydrophilic amino and carboxy termini, as well as the hydrophilic loops connecting the transmembrane domains, electrostatically "screen" the exposed hydrophobic surfaces of the protein, thus slowing aggregation. Alternatively, the possibility has not been excluded that significant amounts of noncovalently bound fatty acid may play a role in the phenomenon. In any case, it is clear that similar explanations apply to soluble melibiose permease.

Regardless of the precise reason for the metastable water-soluble behavior of the *lac* and melibiose permeases after overexpression via the T7 RNA polymerase system, it is noteworthy that the integral membrane forms of both proteins are apparently unable to adopt water-soluble conformations after extraction from the membrane with detergent. It is proposed that once these proteins interact with detergent and/or phospholipid, they assume a stable conformation and cannot be converted into soluble forms without irreversible and deleterious conformational alterations.

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Registry No. H^+, 12408-02-5; Na, 7440-23-5; melibiose permease, 9055-24-7; melibiose, 585-99-9; methyl 1-thio-β,D-galactopyranoside, 155-30-6.

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