*Proc. Natl. Acad. Sci. USA*
Vol. 86, pp. 6087–6091, August 1989
Biochemistry

# Characterization and functional reconstitution of a soluble form of the hydrophobic membrane protein *lac* permease from *Escherichia coli*

**(bioenergetics/symport/overexpression/T7 RNA polymerase/circular dichroism)**

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*Contributed by H. Ronald Kaback, May 15, 1989*

ABSTRACT **Lac permease, a polytopic membrane protein from *Escherichia coli*, has been purified in soluble form by overexpressing the *lacY* gene by means of the T7 RNA polymerase system. Soluble permease is dissociated from membranes with urea or other chaotropes and appears after the membrane is saturated with newly synthesized permease. Remarkably, this form of the permease appears to remain soluble in phosphate buffer at neutral pH after removal of urea, although it aggregates in a time- and concentration-dependent manner. Importantly, soluble permease behaves as a monomer during size-exclusion chromatography with or without urea, contains <3 mol of organic phosphate per mol of protein, and is largely helical. Soluble permease binds *p*-nitrophenyl α-D-galactopyranoside ≈40% as well as permease in the native environment of the membrane and can be reconstituted into phospholipid vesicles that catalyze lactose counterflow or active transport in response to a membrane potential (interior negative). The results suggest that *lac* permease can assume a nondenatured conformation in aqueous solution.**

The *lac* permease of *Escherichia coli* is a polytopic transmembrane protein that catalyzes symport of a single β-galactoside molecule with a single $H^+$ (for reviews, see refs. 1 and 2). Based on CD studies and hydropathy analyses (3), a secondary-structure model has been proposed in which the permease has a hydrophilic amino terminus, 12 transmembrane hydrophobic domains in α-helical conformation connected by more hydrophilic loops, and a hydrophilic carboxyl-terminal tail. Evidence supporting some of the general features of the model has been obtained from laser Raman and Fourier-transform infrared spectroscopy, limited proteolysis, binding studies with monoclonal and site-directed polyclonal antibodies, and chemical labeling. Most recently, J. Calamia and C. Manoil (personal communication) have provided more specific support for the model by characterizing a series of *lacY–phoA* and *lacY–lacZ* fusions.

A typical integral membrane protein, *lac* permease is extremely hydrophobic, requires detergents for solubilization, and aggregates even in the presence of detergent. Despite these problems, the protein has been solubilized and purified to homogeneity in a functional state (4). The procedure involves preextraction of membranes with urea and cholate to remove contaminants, followed by solubilization with octyl β-D-glucopyranoside in the presence of *E. coli* phospholipid and passage through an anion-exchange column. The purified protein can be reconstituted into proteoliposomes and retains full activity as a monomer (4, 5). Another method uses solubilization with dodecylmaltoside and purification by cation-exchange chromatography (6), but this preparation also contains excess phospholipid and tends to aggregate. Although permease can be prepared with minimal phospholipid and the preparations bind substrate, transport activity has not been characterized (7, 8).

In this communication, we characterize an unusual form of *lac* permease, isolated after overproduction, that appears to be soluble in aqueous solution as a monomer. Soluble permease has little associated phospholipid, is largely helical, binds ligand, and can be reconstituted in a functional state. The provocative conclusion is that hydrophobic membrane proteins may be able to adopt a nondenatured conformation in aqueous solution.

## MATERIALS AND METHODS

**Materials.** [1-$^{14}$C]Lactose and *p*-nitro[2-$^3$H]phenyl α-D-galactopyranoside ([$^3$H]NPG) were synthesized by Y.-Y. Liu under the direction of A. A. Liebman (Isotope Synthesis Group, Hoffmann–La Roche). Plasmids pGP1-2 and pT7-6 were provided by S. Tabor and C. C. Richardson (Harvard Medical School). All other materials were reagent grade and purchased from commercial sources.

**Growth of Cells and Overexpression of *lacY*.** Overexpression of *lacY* was accomplished by using the T7 RNA polymerase expression system of Tabor and Richardson (9) as described (10). The *lacY* gene was restricted from plasmid pGM21 [*lac* $\Delta(I)O^+P^+\Delta(Z)Y^+\Delta(A)$, *tet*$^r$] (11) with *Pvu* II and *Eco*RI and ligated into pT7-6 digested with *Eco*RI and *Sma* I. This step results in unidirectional insertion of a 1.6-kilobase-pair fragment containing the entire *lacY* gene and an inoperative *lac* promoter/operator region downstream from the T7 promoter/operator. The resulting construct, pT7-6(*lacY*), was transformed into *E. coli* T184 [$lacI^+O^+Z^-Y^-(A)$, $rpsL,met^-,thr^-,recA,hsdM,hsdR/F',lacI^qO^+Z^{U118}(Y^+A^+)$] (11) harboring plasmid pGP1-2.

*E. coli* T184 harboring pGP1-2 and pT7-6(*lacY*) was grown at 30°C on Luria broth containing 50 μg of both kanamycin and ampicillin per ml to an $OD_{590}$ of 0.5–1.0. The cells were harvested by centrifugation, washed three times in M9 salts (12), and resuspended to an $OD_{590}$ of 1.0 in M9 medium supplemented with 0.01% each of 18 amino acids with the exception of methionine and cysteine. Suspensions were incubated at 30°C for 90 min to starve the cells for methionine and heat-shocked at 42°C for 15 min. Rifampicin was added to 200 μg/ml, and incubation was continued at 42°C for an additional 15 min. Cells were then transferred to 30°C for 20 min, [$^{35}$S]methionine (≈1 Ci/mmol; 1 Ci = 37 GBq) was added to a final concentration of 50 nM, and incubation was continued for 2–3 hr. The cells were harvested by centrifu-

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Abbreviations: NPG, *p*-nitrophenyl α-D-galactopyranoside; [$^3$H]-NPG, *p*-nitro[2-$^3$H]phenylα-D-galactopyranoside; ΔΨ, membrane potential.

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gation, washed in M9 salts, and either frozen in liquid $N_2$ and stored at −20°C or kept at 4°C as a pellet for up to 12 hr.

**Preparation of Soluble *lac* Permease.** Typically, cells from 1.0-liter cultures, treated as described, were resuspended to 40 ml in 50 mM potassium phosphate (pH 7.3) containing 200 μM phenylmethylsulfonyl fluoride, 10 mM lactose, and 1 mM dithiothreitol and lysed by passage through a French pressure cell at 16–20,000 psi (1 psi = 6.9 kPa). Unlysed cells were removed by centrifugation at 2100 rpm for 10 min in a GS-3 rotor (Sorvall). Membranes (≈40 mg of total membrane protein) were harvested by centrifugation at 150,000 × $g_{max}$ for 45 min in a 45 Ti rotor (Beckman). Crude soluble permease was prepared by resuspending the membrane pellet in 2.0 ml of 100 mM potassium phosphate (pH 7.3)/5.0 M urea/1.0 mM dithiothreitol/10 mM lactose, incubating at room temperature for 10 min, and centrifuging at 150,000 × $g_{max}$ for 45 min in a 70.1 Ti rotor (Beckman). Where indicated, the urea extract was dialyzed overnight at 4°C against 200–250 vol of 100 mM potassium phosphate (pH 7.3)/1.0 mM dithiothreitol/10 mM lactose.

Soluble permease was purified further from the urea extract by size-exclusion chromatography on Sephacryl SF-200 (Pharmacia). Where indicated, the column buffer contained either 100 mM potassium phosphate (pH 7.3)/1.0 mM dithiothreitol/10 mM lactose or 100 mM potassium phosphate (pH 7.3)/5.0 M urea/1.0 mM dithiothreitol/10 mM lactose. A 35 × 0.5 cm column ($V_0$ = 27.5 ml) was used to purify permease from a 1.0-liter culture of cells; the column was run at a flow rate of 0.3 ml/min. Calibration was achieved with lysozyme, ovalbumin, and creatine phosphokinase, each at 100 μg/ml.

**Reconstitution of Soluble Permease.** After dialysis, purified soluble permease (75–100 μg of protein per ml, final concentration) was added to washed *E. coli* phospholipids (4) suspended in 50 mM potassium phosphate (pH 7.3)/1.0 mM dithiothreitol/10 mM lactose at a concentration of 50 mg/ml. The suspension was stirred at 37°C for 45 min, cooled slowly to room temperature, and diluted rapidly into a 50-fold excess of 50 mM potassium phosphate (pH 7.3) at room temperature and centrifuged at 175,000 × $g_{max}$ for 90 min in a 70.1 Ti rotor (Beckman). The supernatant was aspirated and discarded, and the proteoliposomes were resuspended in 50 mM potassium phosphate (pH 7.3) to a final concentration of phospholipid at 50 mg/ml and of protein at 75–100 μg/ml. Aliquots (50 μl) were sonified for 10 s in a bath sonicator (4) before assay.

**NPG Binding.** Binding of [$^3$H]NPG was assayed by flow dialysis at 4°C (13).

**Lactose Transport.** Active transport and counterflow in proteoliposomes were assayed as described (4).

**NaDodSO$_4$/PAGE.** NaDodSO$_4$/PAGE, silver staining, and autoradiography were done as described (4, 10).

**CD.** CD spectra were obtained at 4°C in a Cary 61 spectrometer. An aliquot (500 μl) of purified soluble permease in 100 mM potassium phosphate (pH 7.3) at 40 μg/ml was placed in a quartz cuvette that was water-cooled by a circulator (Brinkman Instruments); the scan rate was 10 nm/min.

**Phosphate Analysis.** An aliquot (1.0 ml) of purified soluble *lac* permease (150 μg of total protein) was precipitated with 10% (vol/vol) trichloroacetic acid. The protein was collected by centrifugation for 15 min in a Beckman Microfuge and washed twice in distilled water. Alternatively, an identical aliquot was dialyzed extensively against HPLC-grade water, the sample was extracted with chloroform/methanol (3:1, vol/vol), and phosphate analyses were performed on the dried extract. Phosphate was determined by the method of Chen *et al.* (14) by using potassium phosphate as standard; both samples yielded similar values.

**Protein Determinations.** Protein was determined with amido black (4).

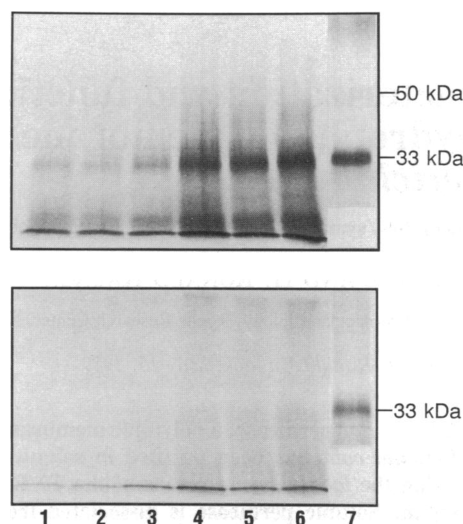


FIG. 1. Time course of appearance of membrane-inserted (*Top*) and urea-soluble (*Bottom*) forms of *lac* permease (33 kDa). *E. coli* T184/pGP1-2/pT7-(*lacY*) overexpressing the *lacY* gene were grown as described, and [$^{35}$S]methionine (500 Ci/mmol) was added to initiate synthesis of the permease. At given times, the suspensions were frozen in liquid nitrogen and subsequently thawed during sonication in a bath-type sonifer (4). Membranes were harvested by centrifugation (≈100 μg of total protein), resuspended in 100 mM potassium phosphate (pH 7.3)/5.0 M urea, and centrifuged for 45 min in a Beckman Airfuge at 100,000 × $g_{max}$. (*Bottom*) Supernatants were aspirated, precipitated with 10% trichloroacetic acid, and the precipitates were solubilized in 35 μl of 1% NaDodSO$_4$/10% (vol/vol) glycerol/1% 2-mercaptoethanol. (*Top*) Membrane pellets were solubilized in 35 μl of the same solution, and both sets of samples were incubated at 37°C for 30 min. Aliquots (25 μl) were then subjected to NaDodSO$_4$/PAGE (4, 10). The gels were dried, and autoradiography was done at −70°C for 10 hr. Cells frozen 2 s (lane 1), 5 s (lane 2), 15 s (lane 3), 30 s (lane 4), 45 s (lane 5), 60 s (lane 6), and 2 hr (lane 7) after addition of [$^{35}$S]methionine.

## RESULTS

By using the T7 RNA polymerase expression system of Tabor and Richardson (9), *lac* permease was labeled specifically with [$^{35}$S]methionine, and the stability of permease mutants truncated at specified positions in the carboxyl terminus was studied (10). During the experiments, overproduction of *lac* permease was seen to result in two distinct forms of permease, one that fractionates with the membrane on extraction with 5.0 M urea and a second that is solubilized by the chaotrope. Because the latter form of the permease is not present under the conditions described previously (4), this material was characterized more completely.

**Kinetics of Formation of Membrane-Inserted and Urea-Soluble Permease.** The autoradiograms in Fig. 1 illustrate the time course of [$^{35}$S]methionine incorporation into membrane-inserted (*Top*) and soluble (*Bottom*) forms of *lac* permease. As shown previously (10), incorporation into the membrane-inserted form occurs rapidly, and within 30 s (*Top*, lanes 1–4) the membrane exhibits a full complement of permease. In contrast, urea-soluble permease is not visible until 1–2 min after initiation of *lacY* transcription, and the intensity of the 33-kDa band continues to increase throughout the time course of the experiment;‡ by 2 hr, ≈40% of the permease synthesized is in this form (*Bottom*, lanes 1–7). Thus, urea-soluble permease probably is not a precursor of the membrane-inserted form. Rather, the urea-soluble form appears

‡Although the molecular mass of *lac* permease is ≈46.5 kDa, as determined from the sequence of *lacY*, the protein electrophoreses with a molecular mass of ≈33 kDa under the conditions described (4).

to result from "spill-over" after the membrane is saturated with permease. Although not shown, similar results were obtained with 4.0 M KBr.

Remarkably, when the urea extract is dialyzed against 100 mM potassium phosphate (pH 7.3), no precipitation is seen. Furthermore, the urea extract and the dialyzed preparation are stable for at least a week at 4°C without visible aggregation, and functional proteoliposomes can be prepared even after a week of storage. However, when the protein is concentrated to ≈1 mg/ml in potassium phosphate (pH 7.3), precipitation occurs within 48 hr. Moreover, at 100–150 μg/ml, aggregation occurs slowly, as evidenced by centrifugation at 175,000 × $g_{max}$ for 60 min. One day after isolation, only ≈15% is sedimented; after 1 week, ≈75% is sedimented. In contrast, no material is sedimented from 100 mM potassium phosphate (pH 7.3)/5.0 M urea after 5 days at 4°C.

**Soluble Permease Is Monomeric.** Size-exclusion chromatography of urea-soluble permease on Sephacryl SF-200 before or after dialysis reveals that the protein migrates as a monomer with a molecular mass of 48 kDa (Fig. 2), a value close to that predicted from the sequence of *lacY* (15). Similar results are obtained with chromatography on Bio-Gel P-150 (Bio-Rad), suggesting the results are not due to interaction with the gel matrix (data not shown). Furthermore, chromatography on Sephacryl SF-200 results in purification of the permease (Fig. 3). The [35S]methionine-labeled fractions from the Sephacryl SF-200 column are essentially pure radiochemically, except for a small amount of β-lactamase precursor (Fig. 3 *Bottom*, lanes 4–6), although other minor contaminants are revealed by silver staining (Fig. 3 *Top*, lanes 4–6). It is also important that organic phosphate determinations on purified soluble permease reveal <3 mol of phosphate per mol of permease. Therefore, it is unlikely that soluble permease contains significant amounts of bound phospholipids or lysophosphatides.

**CD Spectroscopy.** Purified, soluble permease is largely helical, as evidenced by the strong negative peaks seen at 208 and 223 nm in the CD spectrum (Fig. 4, solid line). The CD spectrum from the water-soluble preparation is even more characteristic of helical structure than that from permease solubilized from the membrane (Fig. 4, broken line). However, light scattering secondary to the phospholipid in the detergent-solubilized material is likely to obscure the minimum at 208 nm (3).

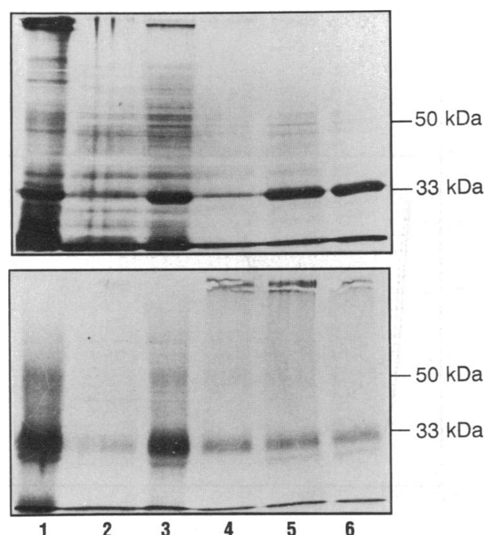


FIG. 3. NaDodSO4/PAGE of selected fractions during isolation and purification of soluble permease. (*Top*) Photograph of the silver-stained gel. (*Bottom*) The same gel after drying and autoradiography at −70°C for 10 hr. Lanes: 1, membrane fraction from *E. coli* T184/pGP1-2/pT7-6(*lacY*) grown on [35S]methionine as described; 2, cytoplasmic fraction after lysis by passage through a French pressure cell, centrifugation, and precipitation with 10% trichloroacetic acid; 3, supernatant after washing membranes with 100 mM potassium phosphate (pH 7.3)/5.0 M urea; 4–6, fractions collected from the Sephacryl SF-200 column at 10, 11, and 12 ml, respectively (compare with Fig. 2). Each lane contains 1/300th of the total material in each fraction. The heavily stained bands at 33 kDa corresponding to *lac* permease (4) at *Top* are the same bands that are heavily labeled with [35S]methionine at *Bottom*. The diffuse band seen at ≈50 kDa at *Bottom* is an aggregate of *lac* permease; the sharp band immediately below the 33-kDa permease in lanes 5 and 6 (*Bottom*) corresponds to unprocessed β-lactamase.

**NPG Binding.** Purified, dialyzed water-soluble permease retains significant ability to bind the high-affinity ligand NPG (Fig. 5). After equilibration with 100 μM [3H]NPG, ≈40% as

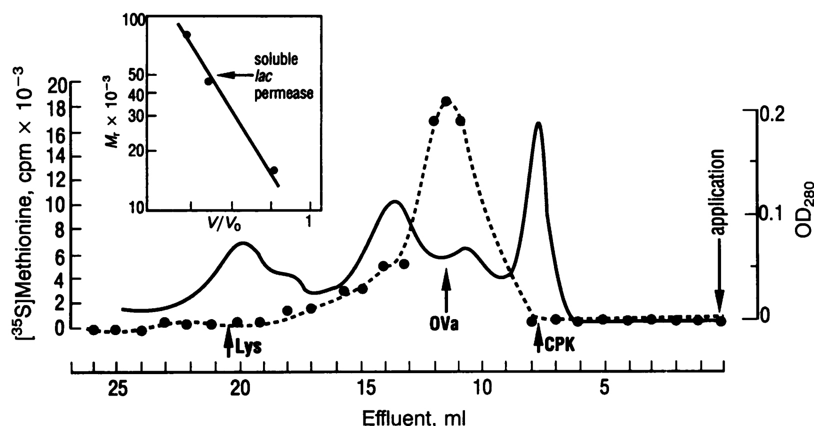


FIG. 2. Size-exclusion chromatography of urea-soluble permease before or after dialysis. Membranes isolated from a 1.0-liter culture of *E. coli* T184/pGP1-2/pT7-6(*lacY*) grown in the presence of [35S]methionine as described were resuspended in 100 mM potassium phosphate (pH 7.3)/5.0 M urea/1.0 mM dithiothreitol/10 mM lactose and centrifuged for 45 min at 150,000 × $g_{max}$ in a 45 Ti rotor (Beckman). One milliliter of the supernatant (≈750 μg of protein) was placed on a Sephacryl SF-200 column, which was then developed with 100 mM potassium phosphate (pH 7.3)/5.0 M urea/1.0 mM dithiothreitol/10 mM lactose as described. Alternatively, the urea-soluble fraction was dialyzed overnight against 100 mM potassium phosphate (pH 7.3)/1.0 mM dithiothreitol/10 mM lactose and chromatographed on a similar column in the same buffer with virtually identical results, except that the yield of permease was ≈0.5. —, Elution profile monitored at $OD_{280}$; ––, radioactivity monitored by liquid scintillation spectrometry. Most radioactivity was seen in the fraction collected at $V/V_0 = 0.4$, which corresponds to the volume at which a globular protein with a molecular mass of 48 kDa should elute (*Inset*, Calibration curve for the column: $V$, volume at which peak elution is seen; $V_0$, exclusion volume of the column). Measurement of the $OD_{280}$ of pooled fractions from a column developed with urea indicates that the yield of purified soluble permease is ≈250 μg from a 1.0-liter culture. Lys, lysozyme (14 kDa); OVa, ovalbumin (44 kDa); and CPK, creatine phosphokinase (81 kDa).

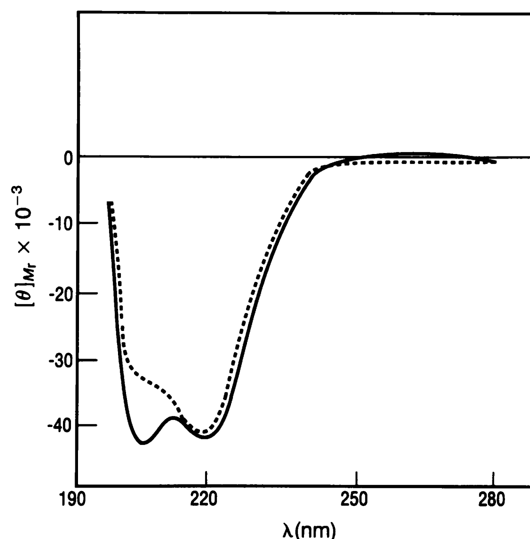


FIG. 4. CD spectrum of purified water-soluble permease (—) compared with that of permease solubilized from the membrane and purified in the presence of octyl β-D-glucopyranoside (OG) and phospholipid (---). The spectrum for permease solubilized and purified in the presence of OG and phospholipid is taken from Foster et al. (3). Purified water-soluble permease was obtained by extracting membranes from *E. coli* T184/pGP1-2/pT7-6(*lacY*) with 100 mM potassium phosphate (pH 7.3)/5.0 M urea as described. The urea extract was then dialyzed and chromatographed on Sephacryl SF-200 with 100 mM potassium phosphate (pH 7.3) as described for Fig. 2, and fractions containing ≥90% pure permease (Fig. 3, lane 6) were pooled. The protein concentration was ≈40 μg/ml. The solid line is scaled relative to the broken line for comparison.

much ligand is competitively dissociated by β-D-galactopyranosyl 1-thio-β-D-galactopyranoside as seen with T206 membrane vesicles containing an approximately equal amount of permease.

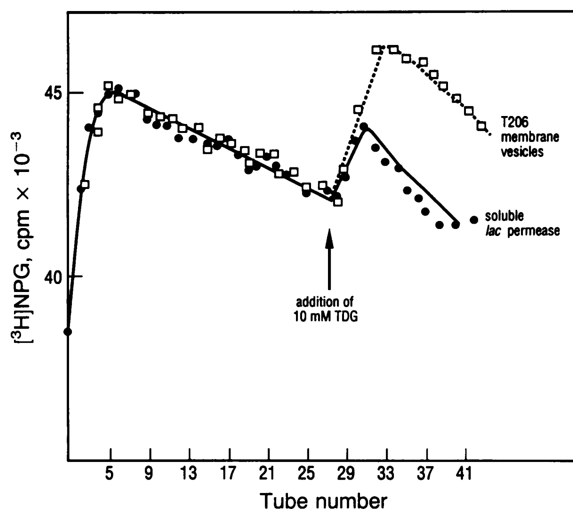


FIG. 5. NPG binding to soluble (●) and membrane-inserted (□) forms of *lac* permease by flow dialysis. For the membrane-inserted permease, right-side-out membrane vesicles from *E. coli* T184/pGM21 were added to the upper chamber of the flow dialysis apparatus, such that the total amount of permease was ≈500 μg; for soluble permease, 500 μg of purified protein dialyzed against 100 mM potassium phosphate (pH 7.3) was added. In each case, [$^3$H]NPG (1 Ci/mmol) was then added to the upper chamber (100 μM, final concentration), and after equilibration was achieved, bound ligand was dissociated by addition of β-D-galactopyranosyl 1-thio-β-D-galactopyranoside (TDG) to 10 mM. Similar results were obtained by addition of 10 mM unlabeled NPG (data not shown).

**Reconstitution of Water-Soluble Permease.** When water-soluble, [$^{35}$S]methionine-labeled permease is mixed with *E. coli* phospholipids and proteoliposomes are prepared, 60–70% of the permease cosediments with the proteoliposomes and is no longer extracted in 5.0 M urea (data not shown). Furthermore, when a membrane potential (ΔΨ, interior negative) is generated, lactose accumulates at an initial rate and to a steady-state level comparable to that of proteoliposomes reconstituted with an equivalent amount of permease purified from the membrane (Fig. 6*A*). When no ΔΨ is generated, lactose accumulation is negligible, and the internal concentration of the disaccharide approximates that of the medium. Similarly, proteoliposomes reconstituted with water-soluble permease catalyze entrance counterflow about as well as proteoliposomes reconstituted with permease purified from the membrane (Fig. 6*B*).

## DISCUSSION

The results presented here demonstrate that *lac* permease, a hydrophobic transmembrane protein that normally requires detergent for solubilization, can be prepared in soluble form when the protein is overproduced at a high rate via the T7 RNA polymerase system of Tabor and Richardson (9). This unexpected form of permease is dissociated from the membrane with 5.0 M urea or other chaotropes and appears after a full complement of permease is inserted into the membrane. EM does not reveal inclusion bodies (data not shown), which are commonly seen after overexpression of soluble or peripheral membrane proteins. Furthermore, urea-soluble permease is associated with right-side-out membrane vesicles

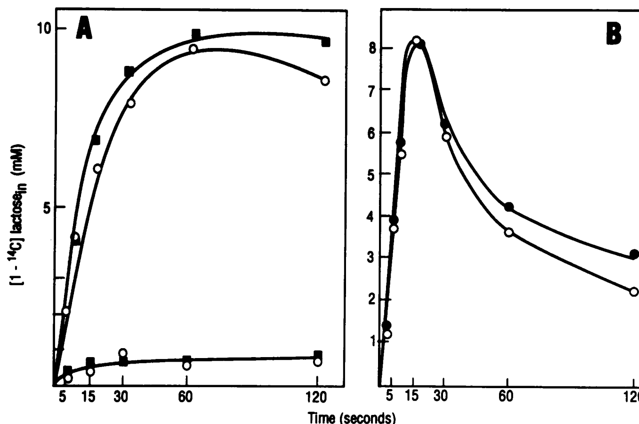


FIG. 6. ΔΨ-driven lactose accumulation (*A*) and entrance counterflow (*B*) in proteoliposomes reconstituted with water-soluble *lac* permease (○) or permease extracted from the membrane with octyl-β-D-glucopyranoside in the presence of *E. coli* phospholipid (■, ●). Permease was extracted from membranes of *E. coli* T184/pGM21 and reconstituted as described (4). Urea-soluble permease was prepared, dialyzed, and reconstituted as described. (*A*) Proteoliposomes containing approximately equal amounts of permease (100 μg/ml protein and 50 mg/ml phospholipid) were equilibrated with 50 mM potassium phosphate (pH 7.3), and valinomycin was added to 10 μM. Aliquots (3 μl) were then rapidly diluted 100-fold into equimolar $Na_3PO_4$ (pH 7.3) (ΔΨ, interior negative) or potassium phosphate (pH 7.3) (no ΔΨ) containing 1.0 mM [1-$^{14}$C]lactose (10 mCi/mmol). Bottom set of points (○, ■) in *A* represents dilution into potassium phosphate (negative control). (*B*) Proteoliposomes containing approximately equal amounts of permease (protein at 100 μg/ml and phospholipid at 50 mg/ml) in 50 mM potassium phosphate (pH 7.3) were equilibrated with 10 mM lactose in the presence of valinomycin and nigericin at final concentrations of 10 μM and 0.2 μM, respectively, and then rapidly diluted 100-fold into 50 mM potassium phosphate (pH 7.3) containing 1.0 mM [1-$^{14}$C]lactose (10 mCi/mmol). At the times indicated, the reactions were quenched, and the samples were assayed as described (4).

(16) and comigrates with the membranes on sucrose density-gradient centrifugation. It seems likely, therefore, that this form of permease has not been inserted into the membrane, but is peripherally associated like D-lactate dehydrogenase, which is also extracted by chaotropes and contains no bound phospholipid (17).

Strikingly, urea-soluble permease remains in solution after urea is removed, and the solution remains clear for a week at 4°C in potassium phosphate, although the material aggregates in a time- and concentration-dependent manner. Moreover, the protein behaves as a monomer during size-exclusion chromatography with or without urea, and its CD spectrum is typical of a largely helical protein. Water-soluble permease binds ligand in solution. Moreover, it associates with *E. coli* phospholipids in a form no longer extractable in urea, and when proteoliposomes are prepared, they catalyze lactose accumulation in the presence of $\Delta\Psi$ (interior negative) and entrance counterflow. Taken together, the results provide support for the heretical notion that this very hydrophobic membrane protein retains much of its native conformation in aqueous solution.

Polytopic transmembrane proteins are generally extremely hydrophobic (50–80% hydrophobic residues; see ref. 18) and aggregate in the absence of detergent. Although we have no precise explanation for the properties of the permease described here, at least two possibilities should be considered. (*i*) Soluble permease has significant amounts of bound fatty acid, which may act as detergent. (*ii*) The hydrophilic loops and the amino and carboxyl termini may fold around the surface of the permease to electrostatically shield the hydrophobic domains from water. In any case, permease solubilized from the membrane in detergent and phospholipid is unable to adopt a water-soluble conformation. Perhaps once the protein is associated with detergent and/or phospholipid, it cannot interact with water without undergoing irreversible and deleterious conformational alterations. The observation that soluble permease is no longer extracted into urea after reconstitution is consistent with this idea.

It is noteworthy that the polytopic ADP/ATP carrier is synthesized as a cytosolic protein before uptake and insertion into the inner mitochondrial membrane (19). Furthermore, the colicins and melittin, as well as other toxins, exist in both water-soluble and integral membrane forms. The recent crystallization of a water-soluble form of the thermolytic fragment of colicin A (20) has led to the proposal that helices in the soluble conformation are oriented perpendicular to the plane of the membrane by electrostatic fields before insertion and subsequently insert by means of a "helical hairpin" (21). An important postulate from this study and prior CD measurements on the two forms of the colicin A thermolytic fragment (22) is that the soluble fragment may have a secondary structure similar to that of the membrane-inserted form. Furthermore, the membrane-interacting hydrophobic regions of the soluble fragment are buried within a cluster of more hydrophilic residues. In any event, whatever the explanation for the properties of this unusual form of *lac* permease, utilization of the procedures may lead to solubilization and purification of other transmembrane proteins and, ultimately, to their crystallization.

**Note Added in Proof.** While this manuscript was in press, we made similar observations with the melibiose permease and the tetracycline-resistance protein from *E. coli.*

We thank S. Tabor and C. C. Richardson for generously providing plasmids pGP1-2 and pT7-6, J. Durkin for help in performing phosphate analyses, V. Mandiyan and M. Boublik for help with the CD measurements, W. Hellman and M. Boublik for EM, and H. K. Sarkar for helpful discussions. We also thank C. Manoil for allowing us access to unpublished data. P.D.R. is a Fellow of the Jane Coffin Childs Memorial Fund for Medical Research (New Haven, CT).

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