The lactose carrier protein of *Escherichia coli* was purified by a simple procedure employing differential solubilization and ion-exchange chromatography and reconstituted into liposomes by octylglucoside dilution. The proteoliposomes exhibited both membrane potential-driven lactose transport and lactose counterflow. Furthermore, the purified protein was identified as the product of the *lac* y gene. These and other results demonstrate that the lactose carrier is the only polypeptide species essential for energy-coupled lactose transport and counterflow.

The transport of β-galactosides across the cytoplasmic membrane of *Escherichia coli* is mediated by the lactose transport system (1). During the last decade, a large body of evidence has accumulated to support Mitchell's hypothesis (2) that sugar-proton symport by this system is driven by a transmembrane electrochemical gradient of protons (see Ref. 3 for review). The kinetics, substrate specificity, and genetics of this transport system have been extensively studied, and in addition, Kennedy and his collaborators (4, 5) have identified a membrane-bound protein as the product of the *lac* y gene. Most recently, the *lac* y gene has been cloned in a bacterial plasmid and its product amplified (6). The sequence of the carrier protein was deduced from the DNA sequence, and the purification of the carrier in an inactive form was reported (7). Although it has been demonstrated that the lactose carrier plays an essential role in lactose transport, evidence has been presented suggesting that an additional component(s) may be required for energy-coupled transport (8).

The elucidation of the subunit composition and molecular mechanism of the lactose transport system will require the purification and reconstitution of the protein(s) responsible for lactose transport. A step in this direction was the recent solubilization and reconstitution of the lactose transport system by Newman and Wilson (9). Furthermore, Kaczorowski *et al.* (10) were able to label the lactose carrier specifically with the photoaffinity reagent 4-nitro[2-3H]phenyl-a-n-galactopyranoside. Using these techniques in concert, we have now purified a single protein that was identified as the product of the *lac* y gene on the basis of its activity, inducibility, electrophoretic mobility, and amino acid composition.

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EXPERIMENTAL PROCEDURES

**Bacteria—** *E. coli* T206 (12), which carries the *lac* y gene in a recombinant DNA plasmid, was provided by Dr. Peter Overath, Max-Planck-Institut für Biologie, Tübingen, West Germany.

**Materials—** Sodium cholate and octylglucoside were obtained from Calbiochem. DEAE-Sephadex CL-6B was from Pharmacia. Urea (ultra-pure) was from Bethesda Research Laboratories. Crude chloroform/methanol-extracted *E. coli* lipids were either purchased from Avanti Biochemicals or prepared from *E. coli* phage. L-[3H]NPG was synthesized by Yu-Ying Lui (Isotope Synthesis Group, Hoffmann-La Roche under the direction of Arnold Liebman and had a specific activity of 30 Ci/mmol (1 Ci = 3.7 x 10^10 becquerels). All other materials were obtained as described (9).

**Preparation of Membrane Vesicles—** Cells were grown and induced for the lactose transport system as described by Teather *et al.* (12). Vesicles were prepared either by osmotic lysis (rightside-out; Refs. 13 and 14) or by passage through a French pressure cell (9).

**Preparation of Acetone/Ether-Washed E. coli Lipid—** Crude chloroform/methanol-extracted *E. coli* lipid was acetone/ether-washed as described (9).

**Photoaffinity Labeling of the Lactose Carrier—** *E. coli* membranes were mixed in a 98:2 (protein/protein) ratio with [3H]NPG-labeled membranes. The specific activity of the mixture was 50-100 nCi/mg of membrane protein. In a typical experiment, 12.5 mg of protein of the above mixture was added to 10 mg of lactose/ml in 50 mM potassium phosphate, pH 7.5, 0.5 mM dithiothreitol, 10 mM lactose. An equal volume of 10 mM urea (room temperature) was added dropwise while blending on a vortex mixer. This suspension was incubated on ice for 10 min on ice and centrifuged at 175,000 x g for 1 h. The pellet was resuspended (using a glass rod and a 1 ml syringe) in a final volume of 1.75 ml of 50 mM potassium phosphate, pH 7.5. Sodium cholate (30% w/v, pH 7.8) was added to a final concentration of 6% (w/v) while blending on a vortex mixer. This suspension was incubated on ice for 20 min and centrifuged at 25,000 x g for 15 min. The pellet was resuspended in 5 ml of 10 mM potassium phosphate, pH 5.8, and centrifuged again at 26,000 x g for 15 min.

The pellet (occasionally stored under 1 ml of the above buffer overnight on ice) was resuspended in 1.45 ml of 10 mM potassium phosphate, pH 5.8, and this suspension was mixed with 17.5 μl of 100 mM dithiothreitol, 13 mg of lactose, 131 μl of washed *E. coli* phospholipid (50 mg/ml) and blended on a vortex mixer. Octylglucoside (146 μl of a 15% w/v solution in 10 mM potassium phosphate, pH 5.8) was added to a final concentration of 2.5%. The protein/lipid/detergent ratio was approximately 1:3:10. The suspension was blended on a
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Vortex mixer, incubated for 10 min on ice, blended again, and then centrifuged at 175,000 × g for 1 h. The supernatant solution was removed and adjusted to pH 5.8 with 10 mm HClO4 containing 1.25% octylglucoside.

DEAE-Sepharose was prewashed once with 10 volumes of 1 M potassium phosphate, pH 5.8, and 8 times with 10 volumes of 0.1 M potassium phosphate, pH 5.8. A 6-ml column (0.9 × 9 cm) was prepared and equilibrated with 10 M potassium phosphate, pH 5.8. Equilibration was ensured by measuring the pH and conductivity of the effluent. The column was then washed with 18 ml of column buffer: 10 M potassium phosphate, pH 5.8, 1 mM dithiothreitol, 20 mM lactose, 0.25 mg of washed E. coli lipid/mL, 1.25% octylglucoside. One ml of the octylglucoside extract (approximately 300 μg of protein) was loaded on the column, and the lactose carrier was eluted with column buffer at a flow rate of 15 ml/h. Fifteen 1-ml fractions were collected. Fractions were stored at 0°C and were active for reconstitution for at least 1 week.

Reconstitution of Lactose Transport—A rapid filter assay was developed for the determination of carrier activity in column fractions. The procedure utilizes 1/6 the amount of lipid as the previous method (9) and eliminates the proteoliposome centrifugation step. All steps were carried out at room temperature unless otherwise noted. Bathsonicated liposomes (16.5 μl), prepared as described (9), were mixed with 1.4 μl of 15% octylglucoside and 55 μl of an octylglucoside membrane extract or column fraction, blended on a Vortex mixer, and then loaded onto ice for 10 min. The suspension was drawn into an automatic pipette and squirted (diluted) into 2.5 ml of 50 mM potassium phosphate, pH 7.5, 1 mM dithiothreitol, 20 mM lactose which was at room temperature and blended gently on a Vortex mixer. Lactose counterflow in the reconstituted proteoliposomes was measured by filtering 0.5 μl of proteoliposomes on a 25 mm (0.22 μm; Millipore) GSTF filter at 25 inches of mercury vacuum suction, using a 9-mm internal diameter chimney. The use of a small diameter chimney to direct the proteoliposomes and [14C]lactose under the larger diameter chimney during this procedure, resulted in the determination of the carrier activity following solubilization and reconstitution. As soon as the proteoliposomes were filtered, the vacuum pump was turned off and the vacuum was allowed to dissipate. The filter valve was closed and 200 μl of 50 mM potassium phosphate, pH 7.5, containing 0.2 μCi of [14C]lactose (the final lactose concentration was 17 μM) was placed on top of the filter (in the 9-mm chimney). It is important to use a filter device which does not draw the lactose solution through the filter due to residual vacuum. After a 2-min incubation, the pump was turned on and the lactose solution was filtered. The chimney was immediately removed, replaced by a standard (16-mm internal diameter) chimney, and the filter was washed with 5 ml of ice-cold 50 mM potassium phosphate, pH 7.5, and counted as described (9). A background value, determined by assaying proteoliposomes in the presence of 10 μM thiodigalactoside, was routinely subtracted. The rapid filter technique allows the entire transport assay to be carried out on the filter.

The octylglucoside extract was loaded onto a DEAE-Sepharose column which was then developed with column buffer as described under “Experimental Procedures.” The effluent was assayed for protein, [14C]NPG, and counterflow activity (Fig. 1). Although a significant amount of counterflow activity and 60% of the [14C]NPG-labeled material was eluted, most of the protein (74%) was adsorbed by the column. As shown, the elution profile contained two protein peaks. The first, which coincides with the void volume of the column, contained approximately 20% of the protein and 20% of the [14C]NPG

### Table 1

| Fraction                  | Protein [14C]NPG | Purification factor |
|--------------------------|-----------------|--------------------|
| Membrane                 | 12.5 mg (100%)  | 425 (100%)         |
| Urea-extracted membrane  | 5.6 mg (45%)    | 322 (76%)          |
| Urea/cholate-extracted membrane | 2.6 mg (21%) | 259 (61%)          |
| Octylglucoside extract   | 0.4 mg (3.2%)   | 159 (38%)          |
| DEAE column peak         | 56 μg (0.4%)    | 59 (14%)           |

*Fractions 6, 7, and 8 from DEAE column profile shown in Fig. 1.

Determination of protein in E. coli membrane preparations by the Lowry procedure (18) routinely yielded values 1.5-fold higher than those obtained by the procedure of Schaffner and Weissmann. A similar overestimation has been observed in another system (19). The accuracy of the Schaffner and Weissmann procedure was confirmed by the amino acid analysis of the purified lactose carrier (data not shown).

RESULTS

### Purification of Functional Lactose Carrier Protein—Patel et al. (20) demonstrated that E. coli membrane vesicles extracted with 5 M urea retain the capacity to transport lactose. Extraction of E. coli T206 French press vesicles with 5 M urea resulted in the solubilization of 55% of the membrane protein, while approximately 80% of the [14C]NPG label remained in the particulate material after centrifugation (Table I).

It was also shown by Padan et al. (21) that although extraction of membrane vesicles with 6% cholate removes 30% of the membrane protein, substantial lactose transport activity can be recovered in the particulate fraction after reconstitution with soybean phospholipids. Extraction of urea-treated T206 membranes with 6% cholate removed an additional 21% of the original membrane protein, while only 8% of the [14C]NPG label was solubilized. Sequential treatment of the membrane with urea and cholate resulted in a 4-fold enrichment of the [14C]NPG label in the membrane (Table I).

Recent work has demonstrated that the lactose carrier can be solubilized in a functional form with octylglucoside and reconstituted into liposomes (9). When the urea/cholate-treated membrane was extracted with octylglucoside in the presence of washed E. coli lipid according to this procedure, 60–80% of the [14C]NPG label was solubilized. Since this procedure resulted in the solubilization of only 15% of the protein, this step yielded a 4-fold enrichment of the [14C]NPG label, resulting in a 12-fold purification relative to the original membrane (Table I).

The octylglucoside extract was loaded onto a DEAE-Sepharose column which was then developed with column buffer as described under “Experimental Procedures.” The effluent was assayed for protein, [14C]NPG, and counterflow activity (Fig. 1). Although a significant amount of counterflow activity and 60% of the [14C]NPG-labeled material was eluted, most of the protein (74%) was adsorbed by the column. As shown, the elution profile contained two protein peaks. The first, which coincides with the void volume of the column, contained approximately 20% of the protein and 20% of the [14C]NPG

Octylglucoside extraction of right-side-out or French press vesicles which had not been treated with urea or cholate typically resulted in the solubilization of 95% of the [14C]NPG-labeled protein. We do not know why the urea/cholate-treatment leads to a slightly lower efficiency of octylglucoside solubilization. There is no indication that treatment with urea and cholate results in substantial loss of lactose transport activity following solubilization and reconstitution.

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1 Larger amounts of octylglucoside-treated protein were occasionally placed on the column after concentrating the octylglucoside extract to 4–6 M, with a minicon B-15 (Amicon) concentrator. Concentration of the extract did not result in significant loss of [14C]NPG label or counterflow activity.
The determination of a single time point by the rapid filter assay can only yield a semiquantitative estimation of the total counterflow activity, as the time course and maximum accumulation for counterflow is affected by the concentration of carrier used in the reconstitution (22).

### Table II

| Amino acid | Determined | DNA sequence |
|------------|------------|--------------|
| Asx        | 23         | 22           |
| Thr        | 18         | 19           |
| Ser        | 24         | 29           |
| Glx        | 22         | 22           |
| Pro        | 12         | 12           |
| Gly        | 38         | 36           |
| Ala        | 36         | 35           |
| Cys        | 8          | 8            |
| Val        | 32         | 29           |
| Met        | 5.6        | 14           |
| Ile        | 24         | 33           |
| Leu        | 52         | 54           |
| Tyr        | 11         | 14           |
| Phe        | 51         | 56           |
| His        | 4          | 4            |
| Lys        | 12         | 12           |
| Arg        | 11         | 12           |
| Trp        | ND         | 6            |

* Amino acid composition predicted by DNA sequence (7).

* A 2-fold increase in methionine was observed from 24 to 72 h of hydrolysis; value given is from analysis of 72-h hydrolysate.

pooled material from the second peak (fractions 6, 7, and 8) was 405 nmol of lactose/mg of protein. This value was 2.8 times higher than the specific activity of proteoliposomes reconstituted with the octylglucose extract (144 nmol of lactose/mg of protein). Although this is only a rough estimation of specific activity, the difference corresponds well with the purification factor reported for the DEAE column step (Table I) and indicates that the carrier did not lose activity during DEAE-Sepharose chromatography. There was excellent correspondence between protein concentration, [\( ^{3}H \)] NPG concentration, and counterflow activity in the second peak.

As described in Table I, the fractions of the second peak containing the highest counterflow activity (fractions 6, 7, and 8 in Fig. 1) contained 14% of the [\( ^{3}H \)] NPG label and 0.4% of the protein originally present in the membranes. This represents a 35-fold purification of the [\( ^{3}H \)] NPG label relative to the starting material. Photolabeling studies with [\( ^{3}H \)] NPG have indicated that 3% of the protein in *E. coli* T206 membranes is lactose carrier (data not shown). A 35-fold enrichment of the [\( ^{3}H \)] NPG label suggests that a high degree of purification was achieved. This was confirmed by SDS-polyacrylamide gel electrophoresis of the purified material. Fig. 2 shows the results from SDS-polyacrylamide gel electrophoresis of urea/cholate-extracted membranes, the octylglucose extract, and pooled DEAE fractions. The pooled DEAE fractions yielded a single broad band when stained with a highly sensitive silver procedure. The purified protein had an apparent molecular weight of M, = 33,000, which is in close agreement with published values for the molecular weight of the carrier as determined by SDS-polyacrylamide gel electrophoresis (5, 6). When membranes were prepared from cells which had not been induced with isopropyl-thiogalactoside, the band corresponding to the purified carrier was only a minor constituent of the octylglucose extract of urea/cholate-treated membranes (data not shown). This indicates that the purified protein is induced by isopropyl-thiogalactoside, a property expected of the product of the lac y gene in the recombinant plasmid (12). Finally, the

![Image of SDS-polyacrylamide gel electrophoresis of various fractions obtained during the purification of the lactose carrier.](image-url)

![Image of purification profile of the DEAE-Sepharose column.](image-url)
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A Single Polypeptide Is Required for Lactose Counterflow and Energy-coupled Transport—When the purified carrier protein was reconstituted into liposomes as previously described (9), the resultant proteoliposomes exhibited counterflow activity, and the activity was completely inhibited by thiodigalactoside (Fig. 3 A). Quantitation of the protein and [\(^{14}\)C]lactose suspension without proteoliposomes, a second blank (to correct for the presence of [\(^{3}\)H]lactose in the proteoliposomes), was subtracted from all points. 

DISCUSSION

These experiments describe a relatively simple procedure for the purification of the lactose carrier protein in functional form. Moreover, the results demonstrate that only one polypeptide species, the product of the lac y gene, is necessary for lactose counterflow and energy-coupled lactose transport. In light of this finding, it is difficult to explain how certain mutations mapping outside the lactose operon (8) result in the pleiotropic loss of energy-coupled accumulation of solutes by several transport systems, including the lactose carrier, despite the ability of these cells to maintain a transmembrane electrochemical proton gradient. One possible explanation is that the transport systems in these mutants have been altered by a mutation in some component of a common regulatory system.

This and previous work (20, 21, 25) have demonstrated that several E. coli membrane proteins are not irreversibly denatured by treatment of the membrane with urea or cholate, although significant amounts of protein are solubilized. Furthermore, the lactose and melibiose (26) transport systems have been solubilized with octylglucoside and reconstituted. The results suggest that these procedures may have general applicability to other bacterial transport systems.

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