Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 1629–1633, March 1984
Biochemistry

Purified reconstituted *lac* carrier protein from *Escherichia coli* is fully functional

(turnover number/biphasic kinetics/symport/proteoliposome/proton electrochemical gradient)

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Communicated by John J. Burns, November 10, 1983

ABSTRACT **Proteoliposomes reconstituted with *lac* carrier protein purified from the plasma membrane of *Escherichia coli* catalyze each of the translocation reactions typical of the β-galactoside transport system (i.e., active transport, counterflow, facilitated influx and efflux) with turnover numbers and apparent K_m values comparable to those observed in right-side-out membrane vesicles. Furthermore, detailed kinetic studies show that the reconstituted system exhibits properties analogous to those observed in membrane vesicles. Imposition of a membrane potential (ΔΨ, interior negative) causes a marked decrease in apparent K_m (by a factor of 7 to 10) with a smaller increase in V_{max} (≈3-fold). At submaximal values of ΔΨ, the reconstituted carrier exhibits biphasic kinetics, with one component manifesting the kinetic parameters of active transport and the other exhibiting the characteristics of facilitated diffusion. Finally, at low lactose concentrations, the initial velocity of influx varies linearly with the square of the proton electrochemical gradient. The results provide quantitative support for the contention that a single polypeptide species, the product of the *lac y* gene, is responsible for each of the transport reactions typical of the β-galactoside transport system.**

β-Galactoside transport across the plasma membrane of *Escherichia coli* is mediated by the *lac* carrier protein or *lac* permease, an intrinsic membrane protein encoded by the *lac y* gene (see refs. 1 and 2 for recent reviews). This protein catalyzes the coupled translocation of substrate with protons in a symport reaction. Therefore, in the presence of a proton electrochemical gradient ($\Delta\bar{\mu}_{H^+}$ interior negative and alkaline), downhill transport of protons in response to $\Delta\bar{\mu}_{H^+}$ drives uphill transport of substrate (i.e., active transport). Downhill transport of substrate drives uphill transport of protons with generation of $\Delta\bar{\mu}_{H^+}$, the polarity of which reflects the direction of the substrate concentration gradient.

The *lac* carrier protein has been purified to homogeneity in a functional state and shown to be the product of the *lac y* gene (3, 4). Proteoliposomes reconstituted with this polypeptide catalyze essentially all of the transport activities observed in intact cells and right-side-out membrane vesicles. Thus, in addition to counterflow, proteoliposomes reconstituted with *lac* carrier protein accumulate lactose against a concentration gradient in the presence of artificially imposed membrane potentials (ΔΨ, interior negative) or pH gradients (ΔpH, interior alkaline). Moreover, it has been shown directly that downhill movements of lactose drive uphill translocation of protons.

Subsequently, other important similarities between "native" and purified *lac* carrier have been documented. For example, studies with right-side-out membrane vesicles indicate that lactose efflux down a concentration gradient occurs by an ordered process in which lactose is released from the carrier prior to the symported proton, and the carrier appears to recycle in the protonated form during exchange and counterflow (5, 6). The observations on which these conclusions are based have been confirmed and extended recently with the reconstituted system (7, 8). Finally, when proteoliposomes are prepared with purified *lac* carrier protein and a terminal *o*-type cytochrome oxidase purified from *E. coli*, lactose is accumulated against a concentration gradient when $\Delta\bar{\mu}_{H^+}$ is generated via turnover of the oxidase (9). Parenthetically, it is also noteworthy that a secondary structure model for the *lac* carrier protein has been proposed (10), monoclonal antibodies against the purified protein have been prepared and characterized (11), and it has been shown directly that the protein spans the bilayer (12).

Taken as a whole, the observations with purified reconstituted *lac* carrier provide a strong qualitative indication that lactose transport in *E. coli* requires a single polypeptide species, the product of the *lac y* gene. In contrast, evidence suggesting that lactose transport may require additional components has been presented (13–16), and most recently, Wright *et al.* (17), using *lac* carrier partially purified and reconstituted by different techniques, were able to elicit counterflow activity but were unable to show ΔΨ- or ΔpH-driven lactose accumulation. The studies described here were undertaken in an effort to resolve this issue on a quantitative basis.

EXPERIMENTAL PROCEDURES

Purification and Reconstitution of *lac* Carrier Protein. The *lac* carrier protein was purified from *E. coli* T206 by the method of Newman *et al.* (3) using the modifications described by Foster *et al.* (4). Column fractions containing *lac* carrier protein were pooled and kept at 4°C until reconstitution into proteoliposomes (generally no more than 1–2 hr after elution from DEAE-Sepharose). Prior to reconstitution, the pooled fractions were diluted with ice-cold column buffer to a concentration of 25 μg per ml of protein.

Reconstitution of the *lac* carrier into proteoliposomes was carried out with *E. coli* phospholipids as described by Garcia *et al.* (7). The final preparation contained 50 mM potassium phosphate, pH 7.5/1 mM dithiothreitol/37.5 mg of phospholipid per ml/56 μg of *lac* carrier protein per ml. Aliquots were frozen and stored in liquid nitrogen.

Proteoliposomes were thawed at room temperature and sonicated in a bath-type sonicator until the preparation was only slightly opaque (8–15 sec) (4). Where indicated, the proteoliposomes were concentrated by centrifugation for 1 hr at 45,000 rpm in a Beckman type 50 Ti rotor (175,000 g_{max}). The supernatant was discarded, and the pellet was resuspended in 50 mM potassium phosphate, pH 7.5/1 mM dithiothreitol to a given protein concentration.

Abbreviations: $\Delta\bar{\mu}_{H^+}$, proton electrochemical gradient; ΔΨ, membrane potential; ΔpH, pH gradient.

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Transport Assays. All assays were conducted at pH 7.5 in a water bath maintained at 25°C. Lactose efflux, counterflow, and $\Delta\Psi$-driven active transport were carried out as described (7, 8).

For measurements of lactose-facilitated diffusion, sonicated proteoliposomes were concentrated 3- to 5-fold, and valinomycin and nigericin were added to final concentrations of 20 μM and 2 μM, respectively. An aliquot (1 μl) was then diluted 1:100 into 50 mM potassium phosphate (pH 7.5) containing given concentrations of [1-^{14}C]lactose (6 to 59 mCi/mmol; 1 Ci = 37 GBq). Reactions were terminated by rapid dilution with 3 ml of ice-cold 50 mM sodium phosphate (pH 7.5), and the samples were filtered immediately through Millipore type GSTF filters (0.2 μm, Millipore) and washed twice with the same volume of cold buffer. Radioactivity retained on the filters was determined by liquid scintillation spectrometry. Experimental values were corrected for zero-time controls obtained by adding an aliquot (1 μl) of proteoliposomes to reaction mixtures that had already been diluted with 3 ml of cold sodium phosphate (pH 7.5), followed by filtration and washing as described (18).

Protein Determinations. Protein was assayed by a modification (3) of the method of Schaffner and Weissmann (19) using bovine serum albumin as standard.

Internal Volume of Proteoliposomes. The internal volume of proteoliposomes reconstituted with purified *lac* carrier protein was determined from the trapped volume of $^{86}Rb^+$ and [1-^{14}C]lactose as described (7).

Calculations. The magnitude of the $\Delta\Psi$ generated theoretically by a given potassium diffusion gradient in the presence of valinomycin was calculated from the following relationship

$$\Delta\Psi\ (\text{mV}) = -59\ \log[K^+]_{in}/[K^+]_{out}, \qquad [1]$$

where $[K^+]_{in}$ and $[K^+]_{out}$ denote internal and external potassium concentrations, respectively, at the time of dilution.

Steady-state levels of lactose accumulation were converted into $\Delta\mu_{lac}$ (in mV) using the following relationship

$$\Delta\mu_{lac}\ (\text{mV}) = -59\ \log[\text{lactose}]_{in}/[\text{lactose}]_{out}, \qquad [2]$$

where $[\text{lactose}]_{in}$ and $[\text{lactose}]_{out}$ denote internal and external lactose concentrations, respectively, at the steady-state level of accumulation.

RESULTS

Facilitated Diffusion of Lactose in Proteoliposomes. When lactose is added to a suspension of proteoliposomes reconstituted with purified *lac* carrier protein in the absence of an imposed $\Delta\bar{\mu}_{H^+}$, uptake proceeds relatively slowly (Fig. 1), and at least 1 hr is required for internal lactose to equilibrate with the external concentration (not shown). In contrast, addition of valinomycin and nigericin increases the initial rate of uptake 2- to 3-fold, and equilibration is complete within 5–7 min. Because lactose uptake under these conditions is essentially completely blocked by *p*-chloromercuribenzenesulfonate, it is apparent that the equilibration process in the proteoliposomes is almost entirely carrier mediated and that passive influx of the disaccharide occurs at a very low rate. Thus, elucidation of the initial velocity of facilitated diffusion is particularly clear-cut in this system.

The observations are qualitatively similar to those reported with intact cells (20, 21) and right-side-out membrane vesicles (22) and are consistent with the finding that addition of lactose to proteoliposomes containing purified *lac* carrier protein under the same conditions leads to alkalinization of the external medium (4). Clearly, therefore, downhill movement of lactose along a concentration gradient drives the uphill translocation of protons, and in the absence of ionophores a $\Delta\bar{\mu}_{H^+}$ (interior positive and/or acid) is established that acts to slow the symport reaction.

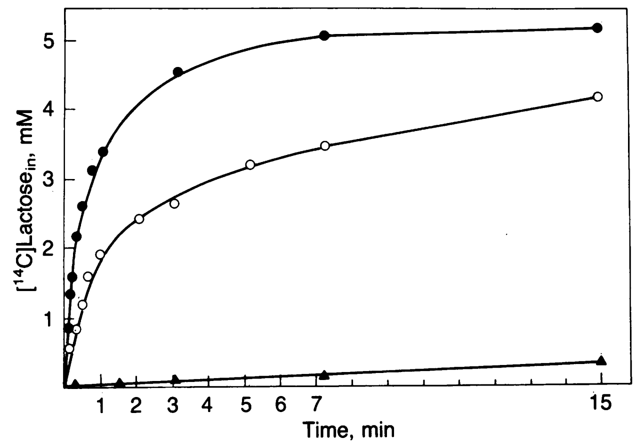

FIG. 1. Facilitated diffusion of lactose in proteoliposomes reconstituted with purified *lac* carrier protein. Proteoliposomes containing purified *lac* carrier were concentrated to 360 μg of protein per ml in 50 mM potassium phosphate (pH 7.5). The following additions were then made to separate suspensions: none (○); valinomycin and nigericin to final concentrations of 20 μM and 2 μM, respectively (●); *p*-chloromercuribenzenesulfonate to a final concentration of 2.5 mM (▲). Aliquots (1 μl) were then diluted 1:100 into potassium phosphate (pH 7.5) containing 5.0 mM [1-^{14}C]lactose (6.6 mCi/mmol) at 25°C. At the times indicated, reactions were terminated and the samples were assayed.

Kinetics of Facilitated Diffusion and $\Delta\Psi$-Driven Active Transport. As shown previously (3, 4, 8), proteoliposomes reconstituted with purified *lac* carrier protein catalyze lactose accumulation against a gradient when a $\Delta\Psi$ (interior negative) is imposed by means of a potassium diffusion gradient ($K^+_{in} \rightarrow K^+_{out}$) in the presence of valinomycin. The data presented in Fig. 2 (*Inset*) contrast the kinetics of facilitated diffusion ($\Delta\Psi$ = 0 mV) with those of $\Delta\Psi$-driven active transport ($\Delta\Psi \simeq -134$ mV). In both cases, initial velocities were measured at pH 7.5, and the data are presented in the form of V vs. V/S plots in which V_{max} is approximated from the y intercept and apparent K_m from the slope of the function. As shown with right-side-out membrane vesicles (5, 6) and intact cells (23), the kinetics of lactose transport in proteoliposomes are dramatically different in the presence and absence of $\Delta\bar{\mu}_{H^+}$ Thus, in comparison to facilitated diffusion, the apparent K_m for $\Delta\Psi$-driven active transport is decreased from $(3.13 \pm 0.66) \times 10^{-3}$ M to $(0.47 \pm 0.13) \times 10^{-3}$ M, while V_{max} is increased from 11.43 ± 0.42 to 35 ± 0.9 μmol of lactose per min per mg of *lac* carrier protein.

In further analogy to the native system (24), when a submaximal $\Delta\Psi$ of about −32 mV is imposed, the system exhibits biphasic kinetics (Fig. 2). One component of the overall process exhibits a low apparent K_m typical of $\Delta\Psi$-driven active transport (*ca.* 0.4×10^{-3} M), while the second exhibits a much higher apparent K_m approximating that observed for facilitated diffusion (*ca.* 1.6×10^{-3} M).

Relationship Between Initial Velocity of Lactose Transport and $\Delta\bar{\mu}_{H^+}$. In addition to causing a decrease in apparent K_m for substrate, $\Delta\bar{\mu}_{H^+}$ (interior negative and/or alkaline) alters the distribution of the *lac* carrier between high and low apparent K_m pathways as a square function (24). That is, over a relatively low range of lactose concentrations, the "apparent" V_{max} varies linearly with the square of $\Delta\Psi$ (interior negative) or ΔpH (interior alkaline). To a reasonable approximation, a similar relationship is observed in proteoliposomes reconstituted with purified *lac* carrier protein (Fig. 3). In the experiments shown, potassium diffusion gradients were imposed in the presence of valinomycin to yield theoretical $\Delta\Psi$

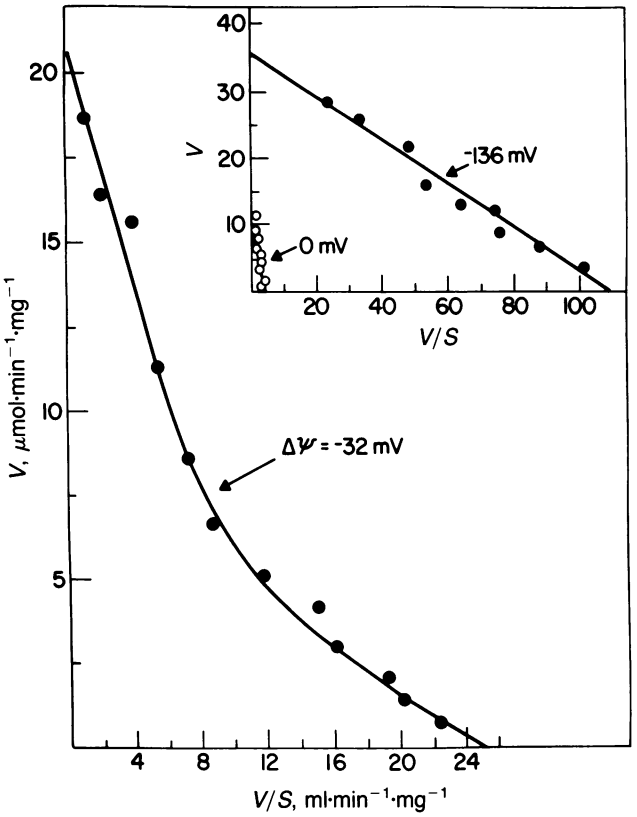

FIG. 2. Kinetics of lactose transport in the presence and absence of $\Delta\Psi$ (interior negative). Proteoliposomes containing purified *lac* carrier protein were concentrated to 167 μg of protein per ml in 50 mM potassium phosphate, pH 7.5/1 mM dithiothreitol. Initial rates of lactose transport were then measured under the conditions given below. Data are presented in the form of V vs. V/S plots, in which the intercept with the y axis represents V_{max} and the slope of the function yields the apparent K_m. Biphasic kinetics of lactose uptake at a submaximal $\Delta\Psi$ are shown. Proteoliposomes were treated with valinomycin at a final concentration of 20 μM, and aliquots (1 μl) were diluted 1:100 into 13.8 mM potassium phosphate (pH 7.5) and 36.2 mM sodium phosphate (pH 7.5) containing [1-^{14}C]lactose (6–59 mCi/mmol) at concentrations ranging from 0.04 to 20 mM. At various times, reactions were terminated and assayed. Data were obtained from linear initial portions of the time courses. (*Inset*) Kinetics of facilitated diffusion (○) and $\Delta\Psi$-driven lactose transport (●). For facilitated diffusion measurements, proteoliposomes were treated with valinomycin and nigericin at final concentrations of 20 μM and 2 μM, respectively, and aliquots (1 μl) were diluted 1:100 into 50 mM potassium phosphate (pH 7.5) containing [1-^{14}C]lactose (6–59 mCi/mmol) at concentrations ranging from 0.27 to 20 mM. At various times, the reactions were terminated and assayed. For $\Delta\Psi$-driven lactose transport, proteoliposomes were treated with valinomycin at a final concentration of 20 μM, and aliquots (1 μl) were diluted 1:200 into 50 mM sodium phosphate (pH 7.5) containing [1-^{14}C]lactose (6–59 mCi/mmol) at concentrations ranging from 0.04 to 1.2 mM. At various times, reactions were terminated and assayed.

values of 0 to −153 mV (interior negative), and initial velocities of lactose transport were measured at a low substrate concentration to minimize the contribution of the high apparent K_m pathway. In addition, steady-state levels of lactose accumulation were measured, and $\Delta\mu_{lac}$ was calculated from Eq. 2. Since the steady-state level of lactose accumulation is in equilibrium with $\Delta\bar{\mu}_{H^+}$ (20, 21, 25), $\Delta\mu_{lac}$ can be used to estimate $\Delta\bar{\mu}_{H^+}$ under the experimental conditions used.

Since imposition of $\Delta\Psi$ (interior negative) causes generation of a ΔpH (interior acid) even in the absence of β-galactosides, the $\Delta\bar{\mu}_{H^+}$ generated under the conditions described is significantly less than $\Delta\Psi$. Generation of a ΔpH (interior acid) at $\Delta\Psi$ values greater than −60 mV has been confirmed in this system by using an entrapped pH-sensitive fluorophore (8-hydroxyl-1,3,6-pyrenetrisulfonate) (26). Although the magnitude of the ΔpH is difficult to quantitate, it is apparent that it increases with increasing values of the imposed $\Delta\Psi$. As a consequence, $\Delta\mu_{lac}$ is in equilibrium with theoretical values of $\Delta\Psi$ generated via potassium diffusion gradients up to −60 mV but deviates from $\Delta\Psi$ in a systematic manner at greater imposed potentials. For these reasons, $\Delta\mu_{lac}$ was used to estimate $\Delta\bar{\mu}_{H^+}$.

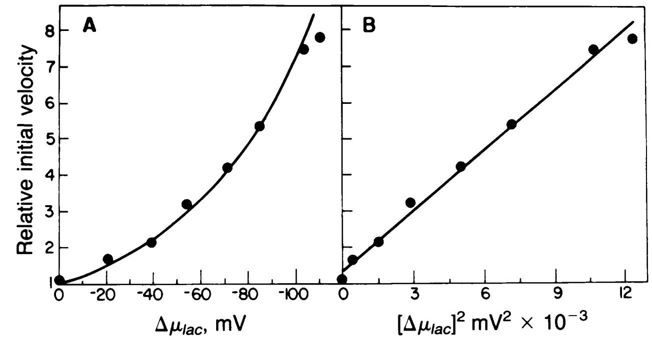

FIG. 3. Relationship between initial velocity of lactose transport and $\Delta\mu_{lac}$. Proteoliposomes containing purified *lac* carrier protein were concentrated to 185 μg of protein per ml in 50 mM potassium phosphate, pH 7.5/1 mM dithiothreitol/20 μM valinomycin. To measure the $\Delta\mu_{lac}$ established by a given $\Delta\Psi$, aliquots (1 μl) were diluted 1:200 to 1:400 into 50 mM phosphate buffer (pH 7.5) that contained various ratios of sodium and potassium as well as [1-^{14}C]lactose (59 mCi/mmol) at a final concentration of 0.075 mM. Reactions were terminated at various times and processed. Regardless of the magnitude of the imposed potassium diffusion potential, maximal levels of [1-^{14}C]lactose accumulation were achieved within 5 min and remained constant between 5 and 15 min. For initial velocity measurements of lactose uptake, aliquots (1 μl) of the proteoliposomes were diluted 1:200 to 1:400 into 50 mM phosphate buffer (pH 7.5) that contained various ratios of sodium and potassium but no radioactive lactose. After a 10-min incubation, [1-^{14}C]lactose (59 mCi/mmol) was added to a final concentration of 0.075 mM to initiate transport. Reactions were terminated at times ranging from 0 to 8 sec and processed. (*A*) Initial velocity vs. $\Delta\mu_{lac}$. Initial velocities of lactose transport and steady-state levels of accumulation were determined at theoretical $\Delta\Psi$ values ranging from 0 to −154 mV. Values of $\Delta\Psi$ and $\Delta\mu_{lac}$ were calculated from Eqs. **1** and **2**. Initial velocities at each value of $\Delta\mu_{lac}$ are expressed relative to that observed in the absence of $\Delta\Psi$ (0.5 μmol of lactose per min per mg of protein). (*B*) Initial velocity vs. $(\Delta\mu_{lac})^2$. Values plotted were derived from the data in *A*.

The data in Fig. 3*A* show that the initial velocity of lactose uptake increases ≈8-fold from 0 to about −120 mV as an upward curvelinear function of $\Delta\mu_{lac}$ (i.e., $\Delta\bar{\mu}_{H^+}$). Furthermore, the relationship is linear when relative initial velocity is plotted as a function of $[\Delta\mu_{lac}]^2$, indicating that the function is exponential to the second power of $\Delta\bar{\mu}_{H^+}$ (Fig. 3*B*).

Turnover Numbers of Purified and Native *lac* Carrier Protein. The proteoliposome values in Table 1 were derived from kinetic experiments (cf. Fig. 2; see refs. 7 and 8) carried out using freeze-thaw/sonicated proteoliposomes reconstituted with *lac* carrier protein purified to a single polypeptide species as described (3, 4). Turnover numbers were calculated for the carrier operating in given modes of translocation and compared to those calculated from published V_{max} values for right-side-out membrane vesicles prepared from *E. coli* ML 308-225. Determination of the amount of *lac* carrier protein in membrane vesicles was based on photolabeling experiments with *p*-nitro[2-^{3}H]phenyl-α-D-galactopyranoside (12, 29), and the value obtained (*ca.* 0.5% of the membrane protein) is in reasonable agreement with that calculated by Overath *et al.* (30). Clearly, both the turnover number of the *lac* carrier and the apparent K_m for lactose are very similar in proteoliposomes and membrane vesicles with respect to $\Delta\Psi$-driven lactose accumulation, counterflow, fa-

Table 1. Comparison of turnover numbers of the *lac* carrier protein: ML 308-225 membrane vesicles vs. proteoliposomes reconstituted with purified carrier

	Turnover numbers, per sec	
Reaction*	Membrane vesicles†	Proteoliposomes‡
$\Delta\Psi$-driven influx ($\Delta\Psi$ = 100 mV)	16 (0.2)	16–21 (0.5 ± 0.1)
Counterflow	16–39 (0.45)	28 (0.6)
Facilitated diffusion	8–16 (20)	8–9 (≈3.1 ± 0.7)
Efflux	8 (2.1)	6–9 (2.5 ± 0.5)

*All reactions were carried out at pH 7.5 and 25°C.

†Determination of the amount of *lac* carrier protein in ML 308-225 membrane vesicles is based on photolabeling experiments with *p*-nitro[2-^{3}H]phenyl-α-D-galactopyranoside, which indicate that the carrier represents about 0.5% of the membrane protein. Kinetic parameters for $\Delta\Psi$-driven influx, counterflow, facilitated diffusion, and efflux, respectively, were taken from Robertson *et al.* (24), Padan *et al.* (27), Garcia *et al.* (28), and Kaczorowski *et al.* (6).

‡Values in parentheses indicate apparent K_m values × 10^{-3} M (±SEM) for determinations conducted on at least two different preparations of purified reconstituted *lac* carrier protein.

cilitated diffusion (i.e., lactose influx under nonenergized conditions), and efflux. [Calculation of turnover numbers in membrane vesicles is highly dependent on the amount of *lac* carrier protein in the membrane, and values in the literature for ML 308-225 vesicles range from 0.5 to 2.0% of the membrane protein (31). Clearly, if the *lac* carrier protein represents a greater proportion of the membrane protein than 0.5% (Table 1), the turnover numbers obtained with proteoliposomes are actually greater than in vesicles.] The only significant discrepancy observed is in the apparent K_m for facilitated diffusion, which is significantly lower in proteoliposomes than in membrane vesicles. It should be emphasized, however, that the *lac* carrier protein was purified from the membrane of *E. coli* T206 (32) and that facilitated diffusion measurements in right-side-out vesicles from this strain yield an apparent K_m that is also lower than that observed in vesicles from ML 308-225 (unpublished observations).

DISCUSSION

Purification of the *lac* carrier protein as described by Newman *et al.* (3) and Foster *et al.* (4) yields a single polypeptide with an amino acid composition closely matching that predicted from the DNA sequence of the *lac y* gene (33). Moreover, the presence of the polypeptide is dependent on expression of the *lac y* gene, and at the phenomenological level, the reconstituted protein catalyzes all translocation activities typical of the β-galactoside transport system (3, 4, 7–9). Nevertheless, rigorous proof that the purified *lac* carrier protein is the only polypeptide responsible for lactose transport is dependent on a demonstration that turnover numbers for the purified *lac* carrier in proteoliposomes are similar to those obtained for the protein in its native environment.

The data in Table 1 testify to the high degree of functionality retained by the purified reconstituted *lac* carrier. Clearly, freeze-thaw/sonicated proteoliposomes prepared by octylglucoside dilution exhibit turnover numbers for $\Delta\Psi$-driven active transport, counterflow, facilitated influx, and efflux that fall within the range of values reported for ML 308-225 membrane vesicles. In addition, with the possible exception of facilitated diffusion, the apparent K_m values obtained with proteoliposomes are similar to those determined with right-side-out membrane vesicles. Taken as a whole, the observations indicate strongly that the *lac* carrier protein retains much of its activity during purification and reconstitution by the methods described.

Additional kinetic similarities between proteoliposomes containing purified *lac* carrier and right-side-out membrane vesicles have also been revealed. (*i*) Monophasic *V* vs. *V/S* plots are observed for lactose transport in the absence or presence of a sufficiently large $\Delta\Psi$, and the kinetic parameters obtained are strikingly different. In the presence of $\Delta\Psi$, the apparent K_m is decreased by a factor of 7 to 10, while V_{max} is increased by a factor of about 3. (*ii*) At a submaximal value of $\Delta\Psi$, biphasic kinetics are observed. One component of the *V* vs. *V/S* plot exhibits the kinetic characteristics of $\Delta\Psi$-driven active transport (i.e., low apparent K_m), while the other exhibits the high apparent K_m typical of facilitated diffusion. The data support the contention (6, 24) that in addition to acting thermodynamically as the driving force for active transport, $\Delta\bar{\mu}_{H^+}$ alters the distribution of the *lac* carrier between two different kinetic pathways. (*iii*) The initial velocity of $\Delta\Psi$-driven lactose transport in proteoliposomes varies linearly with the square of $\Delta\bar{\mu}_{H^+}$ (i.e., $\Delta\mu_{lac}$). As discussed by Robertson *et al.* (24) with respect to similar observations in right-side-out membrane vesicles, a possible explanation that could account for the phenomenon is that the *lac* carrier exists in two forms, monomers and dimers, that the monomer catalyzes facilitated diffusion and the dimer catalyzes active transport, and that $\Delta\bar{\mu}_{H^+}$ promotes aggregation of monomers to dimers. Although this notion is by no means proven, it is interesting that studies using radiation inactivation analysis are consistent with the idea (2, 34, 35).

In any event, regardless of mechanistic interpretations, the activity of purified *lac* carrier protein reconstituted into proteoliposomes is comparable to the activity of the native carrier. Thus, it seems reasonable to conclude that a single polypeptide, the product of the *lac y* gene, is solely responsible for the reactions catalyzed by the β-galactoside transport system. It also follows that purified *lac* carrier reconstitutes with a high degree of fidelity. That is, the orientation of the protein in the reconstituted system must be similar to that in the bacterial cytoplasmic membrane. Importantly, this conclusion is entirely consistent with recent studies using monoclonal antibodies prepared against purified *lac* carrier protein (11). Binding experiments with right-side-out and inside-out membrane vesicles show that the epitope for antibody 4Bl is present virtually exclusively on the periplasmic surface of the membrane. Furthermore, the antibody binds to proteoliposomes reconstituted with purified *lac* carrier with a stoichiometry similar to that observed in right-side-out membrane vesicles (refs. 2 and 36; unpublished observations).

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