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Direct Measurement of Lactose/Proton Symport in *Escherichia coli* Membrane Vesicles: Further Evidence for the Involvement of Histidine Residue(s)†

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ABSTRACT: Addition of lactose to *Escherichia coli* ML 308-225 membrane vesicles under nonenergized conditions induces transient alkalinization of the medium, and the initial rate of proton influx is stimulated by valinomycin and abolished by nigericin or carbonyl cyanide *m*-chlorophenylhydrazone. A functional *lac y* gene product is absolutely required as the effect is not observed in ML 308-225 vesicles treated with *N*-ethylmaleimide nor with vesicles from uninduced *Escherichia coli* ML 30. Furthermore, the magnitude of the phenomenon is enhanced about 3-fold in vesicles from *Escherichia coli* T206, which contain amplified levels of the *lac* carrier protein. Kinetic parameters for lactose-induced proton influx are the same as those determined for lactose-facilitated diffusion, and quantitative comparison of the initial rates of the two fluxes indicates that the stoichiometry between protons and lactose is 1:1. Treatment of ML 308-225 vesicles with diethyl pyrocarbonate causes inactivation of lactose-induced proton influx. Remarkably, however, treatment with the histidine reagent enhances the rate of lactose-facilitated diffusion in a manner suggesting that the altered *lac* carrier catalyzes lactose influx without the symport of protons. The results are consistent with the hypothesis that acylation of a histidyl residue(s) in the *lac* carrier protein dissociates lactose influx from proton influx and indicate that this residue(s) play(s) an important role in the pathway of proton translocation.

According to the chemiosmotic hypothesis (Mitchell, 1961, 1966, 1968, 1973), a proton electrochemical gradient ($\Delta\bar{\mu}_{H^+}$,[1] interior negative and alkaline) is the immediate driving force for accumulation of β-galactosides in *Escherichia coli* (Mitchell, 1963). Thus, transport is postulated to be catalyzed by a β-galactoside-specific membrane protein (the product of the *lac y* gene) that translocates β-galactosides with protons in a symport reaction, the protons moving down their electrochemical gradient and driving the uphill translocation of sugar. Over the past decade, virtually unequivocal experimental support for this concept has been presented.

Several laboratories have investigated proton/β-galactoside symport in intact *E. coli* by studying substrate-induced proton fluxes. West (1970) and West & Mitchell (1972, 1973) demonstrated that addition of lactose to cells under energy-limited conditions causes alkalinization of the medium. Other workers have demonstrated accumulation of the lactose analogue methyl 1-thio-β-D-galactopyranoside (TMG) in response to an artificially imposed membrane potential ($\Delta\psi$) or pH gradient (ΔpH) (Flagg & Wilson, 1977). Similarly, efflux of TMG from preloaded cells drives accumulation of other solutes because coupled proton movements lead to the generation of $\Delta\bar{\mu}_{H^+}$ (Flagg & Wilson, 1978).

Proton/β-galactoside symport has also been studied intensively in cytoplasmic membrane vesicles from *E. coli*. Right-side-out (Kaback, 1971, 1974a; Short et al., 1975; Owen & Kaback, 1978, 1979a,b) and inverted (Hertzberg & Hinkle, 1974; Rosen & McClees, 1974) vesicles generate a $\Delta\bar{\mu}_{H^+}$ of similar magnitude but opposite polarity (Ramos et al., 1976; Ramos & Kaback 1977a; Reenstra et al., 1980), and in both instances, β-galactoside transport is coupled to $\Delta\bar{\mu}_{H^+}$ (Ramos & Kaback, 1977b,c; Lancaster & Hinkle, 1977a,b). Lactose accumulation in right-side-out vesicles leads to partial collapse of $\Delta\psi$ (Schuldiner & Kaback, 1975) and ΔpH (Ramos &

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[1] Abbreviations: $\Delta\bar{\mu}_{H^+}$, transmembrane proton electrochemical gradient; $\Delta\psi$, membrane potential; ΔpH, pH gradient; TMG, methyl 1-thio-β-D-galactopyranoside; NNM, *N*-(2-naphthyl)maleimide; DEPC, diethyl pyrocarbonate; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

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Kaback, 1977a), and artificial imposition of $\Delta\psi$ and/or ΔpH drives β-galactoside accumulation (Hirata et al., 1974; Schuldiner & Kaback, 1975; Schuldiner et al., 1975; Kaczorowski et al., 1979; Robertson et al., 1980) and alters the rate of lactose efflux (Kaczorowski et al., 1979). Furthermore, carrier-mediated lactose efflux down a concentration gradient results in the formation of a $\Delta\psi$ (interior negative) in a manner that is consistent with an ordered proton symport mechanism in which lactose dissociates first and protons dissociate second (Kaczorowski & Kaback, 1979). Finally, proteoliposomes reconstituted with purified *lac* carrier protein catalyze counterflow, $\Delta\psi$- and/or ΔpH-driven lactose accumulation, and lactose-induced proton influx (Newman et al., 1981; Foster et al., 1982), thus providing a strong indication that the *lac y* gene product is the only protein in the cytoplasmic membrane of *E. coli* required for lactose/proton symport.

Despite the abundance of evidence favoring proton/β-galactoside symport, extensive efforts to measure lactose-induced pH changes in isolated membrane vesicles have been uniformly negative [cf. Kaback (1972)]. Recently, however, Horne (1980) and Daruwalla et al. (1981) reported that addition of galactose, arabinose, or fucose to anaerobic suspensions of the appropriate membrane vesicles causes an alkaline pH change, thus demonstrating for the first time that it is possible to observe proton/substrate symport directly in a vesicle system. These findings and the need to further characterize the effects of histidine reagents on lactose transport (Padan et al., 1979; Garcia et al., 1982) stimulated us to reinvestigate lactose-induced proton movements in membrane vesicles.

In this study, lactose-induced proton influx is demonstrated in right-side-out membrane vesicles from *E. coli*, and evidence is presented supporting the suggestion that a histidyl residue(s) in the *lac* carrier play(s) an important role in the phenomenon.

Experimental Procedures

Materials

DEPC and lactose were obtained from Sigma Chemical Co., and [1-^{14}C]lactose (57.7 mCi/mmol) was purchased from Amersham/Searle. Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and valinomycin were obtained from Calbiochem. Nigericin was generously provided by Dr. John Wesley, Hoffmann-La Roche, Inc. All other materials were reagent grade obtained from commercial sources.

Methods

Growth of Cells and Preparation of Membrane Vesicles. *E. coli* ML 308-225 ($i^-z^-y^+a^+$) and ML 30 ($i^+z^+y^+a^+$) were grown on minimal medium A (Davis & Mingioli, 1959) containing 1.0% disodium succinate (hexahydrate). *E. coli* T206, which carries the *lac y* gene in a recombinant plasmid, was grown and induced as described by Teather et al. (1980). Membrane vesicles were prepared as described (Kaback, 1971; Short et al., 1975). Where indicated, the vesicles, which were prepared and stored in 0.1 M potassium phosphate (pH 6.6), were washed and resuspended in 150 mM KCl containing 10 mM $MgSO_4$ as rapidly as possible (i.e., the vesicles were exposed to unbuffered salts for approximately 45 min before the inception of the experiments, unless otherwise noted). The vesicles were thawed at 40–45 °C for a few minutes, diluted 5-fold with 150 mM KCl containing 10 mM $MgSO_4$, and collected by centrifugation (48000*g* for 30 min). After the supernatant was discarded, the pellet was resuspended in 50 mL of the same salt solution, centrifuged, and resuspended to a given protein concentration by means of a hypodermic syringe fitted with a 15-gauge needle.

Lactose-Induced Proton Influx. pH determinations were performed in a closed, temperature-controlled (25 °C) electrode vessel that was continuously flushed by a stream of water-saturated nitrogen. A suspension of membrane vesicles (2.0 mL final volume) containing 10–12 mg of protein in 150 mM KCl and 10 mM $MgSO_4$ was placed in the electrode vessel. An aliquot of a stock lactose solution (0.5 M, freshly prepared) was added to the membrane suspension through a lateral inlet by means of a Hamilton syringe. Mixing was achieved with a magnetic stirrer. A Radiometer pH meter (pHm 84) connected to a Radiometer pH electrode (GK 2401 B) and a Radiometer recorder (REC 61 Servograph) was used to monitor pH continuously. All solutions were prepared in 150 mM KCl containing 10 mM $MgSO_4$, degassed, and kept under a stream of water-saturated nitrogen throughout the experiment. The pH of the 0.5 M stock lactose solution was adjusted to the pH of the membrane suspension (between pH 6.6 and 6.8) with a KOH solution. Solutions of inophores were prepared in absolute ethanol, and the final ethanol concentration in the reaction mixture after addition of the ionophores was less than 0.5%. Calibraton of the pH changes observed was performed at the end of each experiment by adding 10 μL of a 1.0 mM HCl solution.

Lactose-Facilitated Diffusion. Facilitated diffusion measurements were performed under the same conditions as those described for lactose-induced proton influx except that the reactions were carried out in air. Thus, membrane vesicles were concentrated to 15 mg of protein/mL in 150 mM KCl containing 10 mM $MgSO_4$, and valinomycin was added to a final concentration of 1 μM. Aliquots (100 μL) of the membrane suspension were incubated at 25 °C, and [1-^{14}C]lactose was added at concentratons ranging from 0.5 to 12.5 mM. Initial rates of transport were determined from time points taken during the first 15 s of the reaction. Because of the high protein concentration, 47-mm diameter filters were used for the assay (Kaczorowski et al., 1979). Where indicated, the data were corrected for passive permeability by performing identical experiments with vesicles in which the *lac* carrier had been inactivated by treatment with 0.1 mM *N*-(2-naphthyl)-maleimide (NNM) for 30 min at 25 °C (Cohn et al., 1981). Otherwise, the data were corrected for background radioactivity as described (Kaback, 1971, 1974b).

Treatment with Diethyl Pyrocarbonate (DEPC). Vesicles were resuspended in 100 mM potassium phosphate at the desired pH and adjusted to a final membrane concentration of about 2.0 mg of protein/mL. Small aliquots of 2.0 M DEPC (freshly prepared in absolute ethanol) were added to the membrane suspensions to given final concentrations. The suspensions were immediately vortexed and incubated at room temperature for appropriate periods of time. Reactions were terminated by 5-fold dilution with ice-cold 100 mM potassium phosphate (pH 7.0) and immediate centrifugation at 48000*g* for 30 min. The supernatants were discarded and the pellets washed twice with at least a 250-fold excess (v/v) of 150 mM KCl containing 10 mM $MgSO_4$ that was previously degassed by continuously flushing with water-saturated nitrogen. The final pellets were resuspended in the same medium to an appropriate protein concentration just before the experiment.

Protein Determinations. Protein was measured as described by Lowry et al. (1951) with crystalline bovine serum albumin as standard.

Results

Lactose-Induced Proton Influx in E. coli Membrane Vesicles. In order to elicit a direct demonstration of lactose/proton symport in *E. coli* membrane vesicles, an approach similar to

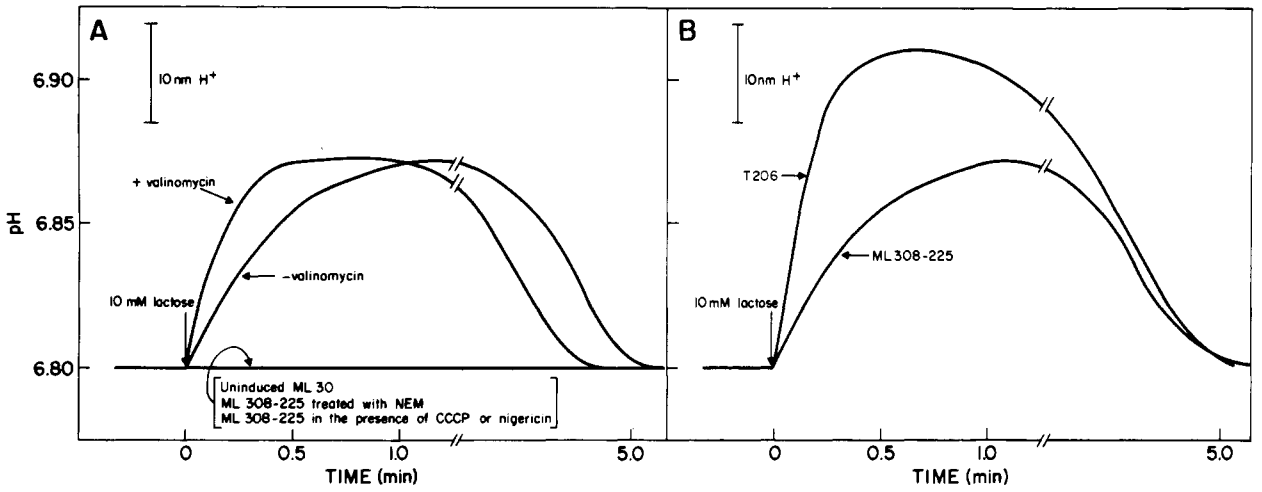

FIGURE 1: Lactose-induced proton influx in *E. coli* membrane vesicles. (A) A 2.0-mL suspension of *E. coli* ML 308-225 membrane vesicles containing 10–12 mg of protein in 150 mM KCl and 10 mM $MgSO_4$ was placed in a closed electrode vessel that was continuously flushed with a stream of water-saturated nitrogen and maintained at 25 °C. Where indicated, valinomycin was added to a final concentration of 1 μM. The reaction was started by addition of lactose to a final concentration of 10 mM, and the pH of the solution was registered continuously as described under Experimental Procedures. In parallel experiments, the same measurements were made with vesicles from uninduced *E. coli* ML 30 and with ML 308-225 vesicles in the presence of nigericin, CCCP, or *N*-ethylmaleimide (NEM) at final concentrations of 0.25 μM, 5.0 μM, or 1.0 mM, respectively. The pH change was calibrated at the end of each experiment by addition of 10 μL of 1.0 mM HCl. (B) A 2.0-mL suspension of *E. coli* T206 membrane vesicles containing 10–12 mg of protein in 150 mM KCl and 10 mM $MgSO_4$ was placed in the electrode vessel, and lactose was added to a final concentration of 10 mM. For comparison, the identical experiment was carried out with ML 308-225 vesicles in the absence of valinomycin. Other experimental conditions were as described above and under Experimental Procedures.

that described originally for intact cells was used (West, 1970; West & Mitchell, 1972, 1973), except that the vesicles were exposed to unbuffered medium for as short a period of time as possible (cf. below). When lactose is added to ML 308-225 vesicles, transient alkalinization of the medium is observed, and the pH tracing reaches maximum displacement in 0.5–1.0 min and returns to the base line after approximately 5 min (Figure 1A). Importantly, the rate of the phenomenon is enhanced when valinomycin is added to the reaction mixture, suggesting that a $\Delta\psi$ (interior positive) is generated during lactose/proton symport in the absence of the ionophore and that the $\Delta\psi$ acts to limit the process. Similar stimulation is observed in the presence of 10 mM thiocyanate (data not shown). In contrast, nigericin or CCCP abolishes lactose-induced alkalinization when added prior to the disaccharide (Figure 1A).

Lactose-induced transient alkalinization is not observed when the same experiment is carried out with membrane vesicles prepared from uninduced *E. coli* ML 30 vesicles which do not transport lactose or with ML 308-225 vesicles treated with *N*-ethylmaleimide under conditions that completely abolish *lac* carrier function (Kaback & Barnes, 1971; Cohn et al., 1981) (Figure 1A). On the other hand, with vesicles prepared from *E. coli* T206, a strain harboring a hybrid plasmid encoding the *lac y* gene that overproduces the *lac* carrier (Teather et al., 1980), lactose-induced alkalinization is considerably enhanced relative to ML 308-225 vesicles (Figure 1B). Thus, it is apparent that the phenomenon is absolutely dependent upon the presence of a functional *lac y* gene product and that its magnitude is related to the amount of *lac* carrier present in the membrane.

As implied above, demonstration of lactose-induced alkalinization with isolated membrane vesicles is not straightforward, and the phenomenon was missed for many years [cf. Barnes & Kaback (1971) and Kaback (1972)]. At least one reason for the problem is that the vesicles lose considerable activity with time when suspended in unbuffered media (Figure 2). In the experiment shown, vesicles were treated as described in Figure 1 and either assayed immediately or incubated in unbuffered salts for given periods of time prior to assay. Clearly, the vesicles lose about 50% of the control activity in 1 h, and only about 40% of the initial activity remains after 100 min. It should be emphasized that the loss of activity observed is probably underestimated, as the control sample was also exposed to unbuffered medium for a minimum of about 45 min before the experiment was begun. Since earlier attempts to demonstrate lactose-induced alkalinization involved extensive washing of the vesicles to remove buffer, it is not surprising that the previous experiments were unsuccessful.

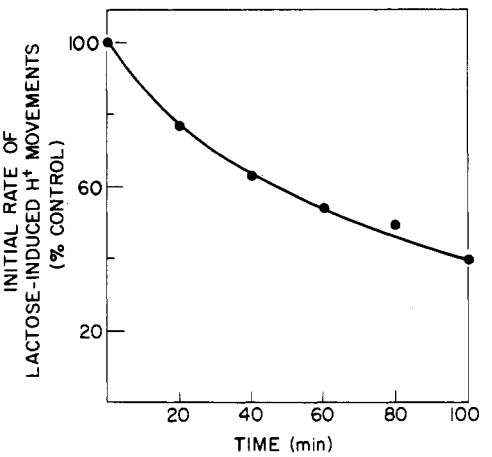

FIGURE 2: Loss of lactose-induced proton influx with time. *E. coli* ML 308-225 membrane vesicles were washed and resuspended in 150 mM KCl and 10 mM $MgSO_4$ to a final concentration of 5–6 mg of protein/mL as described under Experimental Procedures and kept on ice under a stream of water-saturated nitrogen. At the times indicated, 2.0 mL of the membrane suspension was placed in the electrode vessel and assayed for proton influx after addition of lactose to a final concentration of 10 mM as described under Experimental Procedures and in Figure 1. Results are plotted as a percentage of the initial rate of lactose-induced proton influx observed in samples that were assayed at "zero time".

Lactose-Facilitated Diffusion. The experiments presented in Figure 3 represent time courses of lactose influx in the

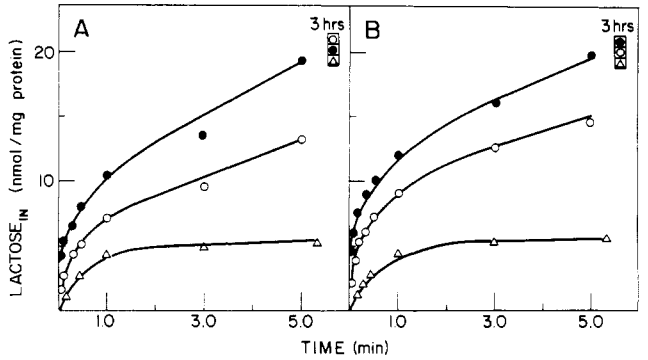

FIGURE 3: Lactose-facilitated diffusion in *E. coli* ML 308-225 membrane vesicles (A) and *E. coli* T206 membrane vesicles (B). Vesicles were washed and resuspended in 150 mM KCl containing 10 mM $MgSO_4$ to a final concentration of 15 mg of protein/mL. Aliquots (100 μL) of the suspensions were incubated at 25 °C, and at zero time, [1-^{14}C]lactose (1.7 mCi/mmol) was added to a final concentration of 10 mM. At the indicated times, samples were assayed as described and corrected for background radioactivity (Kaback, 1971, 1974b). (○) Control; (●) experiment carried out in the presence of CCCP at a final concentration of 10 μM; (△) experiment performed with vesicles in which the *lac* carrier was inactivated by treatment with NNM (0.1 mM final concentration) for 30 min at 25 °C (Cohn et al., 1981).

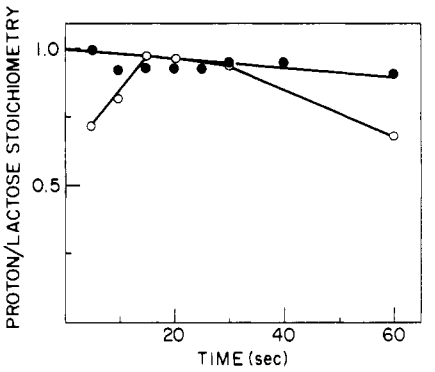

FIGURE 4: Stoichiometry of proton/lactose symport under nonenergized conditions. *E. coli* ML 308-225 membrane vesicles (●) were washed and resuspended in 150 mM KCl containing 10 mM $MgSO_4$ to appropriate protein concentrations, and valinomycin was added to a final cocentration of 1.0 μM. Lactose-induced proton influx was measured as described under Experimental Procedures and in Figure 1 after addition of lactose to a final concentration of 10 mM. Lactose-facilitated diffusion was measured as described in Figure 3 after addition of [1-^{14}C]lactose (1.7 mCi/mmol) to a final concentration of 10 mM, and the data were corrected for passive lactose permeability by performing an identical experiment with vesicles that were treated with NNM (0.1 mM final concentration) for 30 min at 25 °C (Kaczorowski et al., 1979; Cohn et al., 1981). Proton/lactose stoichiometry was determined from data taken over the first minute of each reaction. (○) Data obtained under identical conditions with T206 membrane vesicles, except that valinomycin was omitted from the reaction mixtures.

absence of exogenous energy sources under conditions similar to those used to measure lactose-induced proton influx. With ML 308-225 vesicles (panel A), influx is rapid during the first 15–20 s and reaches a steady state of about 20 nmol/mg of membrane protein in about 10 min (not shown) that is maintained for at least 3 h (○). Since the vesicles have an internal volume of about 2.2 μL/mg of membrane protein and the external lactose concentration used in the experiment was 10 mM, it is apparent that the steady state observed represents equilibration of external lactose with the intravesicular space within experimental error. The protonophore CCCP stimulates the rate of equilibration with no effect on the steady state (●), while treatment of the vesicles with NNM, which completely inactivates carrier-mediated β-galactoside transport (Cohn et al., 1981), severely inhibits the rate of influx (△). Eventually, however, the intravesicular space equilibrates with the external medium by means of passive diffusion. With vesicles prepared from T206, the amplified strain, similar findings are obtained (panel B), except that the initial rates of influx are higher in both the absence and the presence of CCCP, observations that are consistent with the increased level of *lac* carrier.

Stoichiometry of Lactose/Proton Symport under Nonenergized Conditions. Quantitative comparison of data presented in Figures 1 and 3 and other experiments allows an estimate of the stoichiometry between lactose and proton influx (Figure 4). Clearly, with ML 308-225 vesicles, the relationship approximates unity over the entire first minute of the influx reaction (●). With T206 vesicles, on the other hand, the ratio falls short of unity during the first 5–10 s of the time course but yields a value of 1 thereafter (○). In all likelihood, the deviation from unity over the earliest time points with T206 vesicles is related to difficulty in estimating the initial rates of lactose influx in these preparations (cf. Figure 3B). In any event, the stoichiometry of 1 observed here is in excellent agreement with previous estimates of lactose/proton stoichiometry in intact cells under similar conditions (West & Mitchell, 1972, 1973; Zilberstein et al., 1979; Booth et al., 1979).

Comparative Kinetics of Lactose-Induced Proton Influx and Lactose-Facilitated Diffusion. When initial rates of lactose-induced proton influx are measured after addition of lactose to various external concentrations and the data are plotted in double-reciprocal fashion, a linear function is observed, and values of 4.6 mM and 16.0 nmol of protons min^{-1} (mg of membrane protein)$^{-1}$ are obtained for the apparent K_m and V_{max}, respectively (data not shown). Importantly, initial rates of lactose influx as a function of lactose concentration yield a remarkably similar double-reciprocal plot with the same apparent K_m and V_{max} within experimental error. Although the kinetic parameters for lactose-facilitated diffusion obtained here are significantly different from those reported previously (Kaczorowski et al., 1979; Robertson et al., 1980), it should be emphasized that the respective experiments were carried out under different conditions (i.e., the experiments presented here were carried out in unbuffered 150 mM KCl, while the previous measurements were performed in 50 mM potassium phosphate).

Effect of DEPC Treatment on Lactose/Proton Symport. When ML 308-225 vesicles are treated with given concentrations of DEPC for 10 min, washed free of reagent, and assayed for lactose-induced proton influx, concentration-dependent inactiviation is observed, and a half-maximal effect is obtained at 1.2 mM DEPC (data not shown). At each DEPC concentration used, inactivation of lactose-induced alkalinization is time-dependent and reaches a maximum after 10 min of incubation. For further investigation of the nature of the functional group(s) involved in proton translocation under these conditions, preliminary experiments were performed in which vesicles were treated with 2.0 mM DEPC at different pHs for 1 min and assayed for lactose-induced alkalinization. Although the experiments were not carried out in comparable detail, the pH profile obtained is similar to that observed previously [cf. Figure 4 in Garcia et al. (1982)]. Inactivation of lactose-induced proton influx is pH dependent, increasing from pH 5.0 to 8.0 with an inflection between pH 6.0 and 6.5.

In contrast to its effects on lactose-induced alkalinization, DEPC produces a partial, but particularly interesting, effect on lactose-facilitated diffusion (Figure 5). As shown, vesicles treated with the acylating agent exhibit a time course of fa-

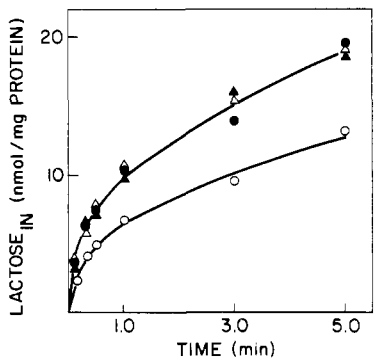

FIGURE 5: Effect of DEPC treatment on lactose-facilitated diffusion. Untreated *E. coli* ML 308-225 membrane vesicles and ML 308-225 vesicles that were treated with 2.0 mM DEPC for 10 min at room temperature as described under Experimental Procedures were suspended in 150 mM KCl and 10 mM $MgSO_4$ to a final concentration of 15 mg of protein/mL. Aliquots (100 μL) of the membrane suspensions were incubated at 25 °C, and [1-^{14}C]lactose (1.7 mCi/mmol) was added to a final concentration of 10 mM. Samples were assayed at given times, and the data were corrected for background radioactivity as described (Kaback, 1971, 1974b). (○) Untreated vesicles; (●) untreated vesicles assayed in the presence of 10 μM CCCP; (△) DEPC-treated vesicles; (▲) DEPC-treated vesicles assayed in the presence of 10 μM CCCP.

cilitated diffusion that is essentially identical with that observed in control preparations in the presence of CCCP, and addition of the protonophore has no effect whatsoever on the rate of influx. Thus, it seems likely that acylation of the *lac* carrier dissociates lactose influx from proton influx such that the modified protein is able to catalyze carrier-mediated diffusion, but the process no longer occurs in symport with protons.

Discussion

The experiments presented here document a long sought after phenomenon, the direct demonstration of lactose/proton symport in *E. coli* membrane vesicles under nonenergized conditions. Clearly, addition of lactose to vesicles containing functional *lac* carrier results in alkalinization of the external medium, and it seems likely that earlier attempts to elicit the effect probably failed because the vesicles were exposed to unbuffered media for relatively long periods of time. Although reasons for the loss of activity are not readily apparent, it is noteworthy that other activities catalyzed by vesicles containing the *lac* carrier (i.e., $\Delta\bar{\mu}_{H^+}$-driven lactose transport and counterflow) are also diminished under similar conditions.

Importantly, both lactose-induced proton uptake and lactose-facilitated diffusion are absolutely dependent upon the presence of functional *lac* carrier protein. Furthermore, the overall magnitude of the lactose-induced pH change and the initial rate of lactose-facilitated diffusion reflect roughly the amount of *lac* carrier in the membrane. Thus, vesicles derived from *E. coli* T206, which contains multiple copies of the *lac y* gene on a recombinant plasmid, exhibit significantly higher activities than vesicles from *E. coli* ML 308-225, which contains a single copy of the gene. On the other hand, it is apparent that neither phenomenon can be related quantitatively to the absolute amount of *lac* carrier present in the membrane. That is, although T206 vesicles contain 5–6 times more *lac* carrier protein than ML 308-225 vesicles,[2] the initial rates of lactose-induced proton influx and lactose-facilitated diffusion are only about 3-fold higher in T206 vesicles. Similar discrepancies have been observed for $\Delta\bar{\mu}_{H^+}$-driven translocaton (Overath et al., 1979; M. L. Garcia and H. R. Kaback, unpublished experiments).

In addition to the overall phenomenon itself, many other aspects of the data provide strong support for the contention that the pH change induced by lactose represents the coupled uptake of lactose and protons: (i) Lactose-induced proton influx is abolished by proton-conducting ionophores (i.e., nigericin and CCCP) but stimulated by valinomycin, and the initial rate of lactose-facilitated diffusion is enhanced in the presence of CCCP. In all likelihood, therefore, lactose influx occurs concomitantly with protons, leading to the generation of a $\Delta\bar{\mu}_{H^+}$ (interior positive and acid) which acts to slow the symport reaction. (ii) Comparison of time courses for lactose-induced alkalinization and lactose-facilitated diffusion (Figures 1 and 3) reveals that lactose and proton influx occur simultaneously during the first minute of the reaction. Subsequently, as the intravesicular lactose concentration approaches equilibrium, net proton influx slows and then ceases, followed by reversal of the pH change. This behavior is completely consistent with the putative mechanism. during the initial phase of the reaction, there is net influx of both lactose and protons, and as the intravesicular lactose concentration approaches steady state, the driving force on the protons decreases and the pH gradient across the membrane dissipates relatively slowly. (iii) The stoichiometry between lactose and proton influx approximates unity over the initial phase of the reaction. (iv) Kinetic studies of lactose-induced proton influx and lactose-facilitated diffusion yield remarkably similar kinetic parameters for both processes. That is, the effect of lactose on the kinetics of proton influx is essentially identical with the kinetics of lactose-facilitated diffusion.

Having demonstrated that lactose/proton symport can be measured directly in membrane vesicles, it was of considerable interest to study the effect of DEPC treatment on lactose-induced alkalinization. Interestingly, treatment of ML 308-225 vesicles with DEPC inhibits lactose-induced proton influx but stimulates the rate of lactose-facilitated diffusion in much the same manner as the protonophore CCCP without increasing the proton permeability of the membrane (Padan et al., 1979; Garcia et al., 1982). The results are consistent with the notion that acylation of a histidyl residue(s) in the *lac* carrier dissociates lactose influx from proton influx such that the altered protein is able to catalyze carrier-mediated diffusion without the simultaneous influx of protons. Furthermore, the findings provide a phenomenological explanation for the observation that DEPC treatment leads to biphasic kinetics for $\Delta\bar{\mu}_{H^+}$-driven lactose transport without dissipating $\Delta\bar{\mu}_{H^+}$ (Garcia et al., 1982). Although the precise mechanism of proton/β-galactoside symport is far from resolved, the results taken as a whole provide a strong indication that a histidyl residue(s) in the *lac* carrier protein is (are) important in the pathway of proton translocation.

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[2] The amount of *lac* carrier protein in the membrane was determined by photoaffinity labeling with *p*-nitro[2-^{3}H]phenyl α-D-galactopyranoside (Kaczorowski et al., 1980).

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