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Physical mechanism for regulation of proton solute symport in *Escherichia coli*

(redox potential/coupled solute transport/dithiol–disulfide interchange/electrochemical proton gradient/ligand affinity)

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ABSTRACT **The activity of the *Escherichia coli* transport proteins for lactose and proline can be altered by changing the redox state of the dithiols in these carriers. A series of lipophilic oxidizing agents has been shown to inhibit and subsequent addition of dithiothreitol to restore full activity. Both systems are irreversibly inhibited by *N*-ethylmaleimide, but prior addition of oxidizing agents protects against this inhibition. These data, as well as studies on the inhibitory effect of the dithiol-specific reagent phenylarsine oxide, show that the redox-sensitive step is the conversion of a dithiol to a disulfide. Measurement of the initial rate as a function of the lactose and L-proline concentrations shows that the oxidation of a dithiol to a disulfide increases the K_m of the carriers for lactose and L-proline. The reduced (dithiol) form of the carrier has a low K_m and the oxidized (disulfide) form has a high K_m for its substrate. The changes in K_m brought about by reduction and oxidation are the same as those that accompany the generation and dissipation, respectively, of an electrochemical proton gradient ($\Delta\tilde{\mu}_{H^+}$). These results support a mechanism in which an $\Delta\tilde{\mu}_{H^+}$ or one of its components alters the ligand affinities of the carrier during a single transport cycle through conversion from the reduced to the oxidized form.**

Changes in the K_m values of transport proteins in response to the electrochemical proton gradient ($\Delta\tilde{\mu}_{H^+}$) across the membrane or in response to formation of phosphorylated protein intermediates in the case of primary transport systems are well documented. Observations have been made, for example, for the mitochondrial ADP/ATP translocator (1), the *Escherichia coli* lactose transport system (2), the Ca^{2+}-ATPase from sarcoplasmic reticulum (3), and a number of other systems. In each case, changes in affinities of one or two orders of magnitude have been reported. It is assumed that these affinity changes have a functional significance in the mechanism of solute transport but the precise physical mechanism behind them has remained a mystery.

The *E. coli* phospho*enol*pyruvate (*P-e*Prv)-dependent hexose transport system is also sensitive to the presence of an $\Delta\tilde{\mu}_{H^+}$ (4, 5). The $\Delta\tilde{\mu}_{H^+}$ inhibits transport. Recently, we investigated the mechanism by which this inhibition occurs and showed that the membrane-bound transport enzyme, enzyme II, can exist in two different states, a high-affinity and a low-affinity state, for solute binding. The physical mechanism controlling the change in affinity is a redox process involving a dithiol–disulfide interchange. The reduced (dithiol) form has a high affinity for solute and the oxidized (disulfide) form has a low affinity (6). The same changes in affinity can be generated by artificially changing the redox potential with oxidizing and reducing agents or by establishing an $\Delta\tilde{\mu}_{H^+}$ across the membrane.

Since the transmembrane electrical potential ($\Delta\psi$) can alter the redox state of different functional groups in the membrane (for review, see ref. 7) we proposed that the $\Delta\tilde{\mu}_{H^+}$ controls the activity of the *P-e*Prv-dependent hexose transport system by altering the redox state and thereby the affinity of the transport protein for its solute.

The *E. coli* lactose transport system also experiences changes in K_m on (de)energization. In this case, the K_m decreases by a factor of 1/100 on energization of membrane vesicles (2). In addition, this system is sensitive to sulfhydryl reagents (8,9) and the sensitivity can be altered by establishing an $\Delta\tilde{\mu}_{H^+}$ across the membrane (10). In this report, we present evidence for the involvement of dithiol–disulfide interchange in the function of the active transport systems for proline and lactose in *E. coli* and show that this oxidation–reduction process controls the affinities of these transport systems.

MATERIALS AND METHODS

Growth of Cells and Isolation of Membrane Vesicles. *E. coli* ML 308-225 ($i^-z^-y^+a^-$) was grown on minimal medium A (11)/1.0% sodium succinate/0.1% yeast extract. Membrane vesicles were prepared as described by Kaback (12).

Transport Assays. Uptake of [D-*glucose*-1-^{14}C]lactose (68 μM; specific activity, 59 mCi/mmol; 1 Ci = 3.7×10^{10} becquerels), L-[U-^{14}C]proline (3.5 μM; specific activity, 285 mCi/mmol), and tetraphenylphosphonium ion ([^{3}H]Ph_4P^+; 0.8 μM; 2.5 Ci/mmol) was studied under aerobic conditions by using 10 mM ascorbate/100 μM phenazine methosulfate as electron donor as described (13, 14). Uptake of L-proline driven by valinomycin-induced potassium diffusion potentials was carried out as described (2). Membrane vesicles (about 9 mg of membrane protein/ml) in 50 mM K phosphate, pH 8.0/10 mM $MgSO_4$ (buffer A) were incubated at room temperature for 30 min with or without 0.5 mM 5-hydroxy-2-methyl-1,4-naphthoquinone (plumbagin), washed with buffer A, and then incubated for 45 min with or without 5 mM dithiothreitol. Subsequently, the vesicles were incubated for 30 min with 10 μM valinomycin and concentrated to a protein concentration of 35 mg/ml, and 1 μl of the concentrated membrane vesicle suspension was diluted into 100 μl of buffer A/3.5 μM L-[^{14}C]proline or into 100 μl of 50 mM Tris maleate, pH 8/10 mM $MgSO_4$/3.5 μM L-[^{14}C]proline.

The effect of redox reagents on the uptake of lactose, L-proline, and Ph_4P^+ was studied by adding the redox reagents to the incubation mixture 5 min prior to the addition of ascorbate/phenazine methosulfate.

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Abbreviations: $\Delta\tilde{\mu}_{H^+}$, electrochemical proton gradient; MalNEt, *N*-ethylmaleimide; Ph_4P^+, tetraphenylphosphonium ion; *P-e*Prv, phospho*enol*pyruvate.

The apparent K_m of lactose transport was determined by measurement of the initial rate of transport (uptake after 1 min of incubation in the presence of ascorbate/phenazine methosulfate) at [^{14}C]lactose concentrations between 10 and 200 μM in the absence of plumbagin and between 1 and 10 mM in the presence of 0.5 mM plumbagin.

Effect of SH Reagents on Ascorbate/Phenazine Methosulfate-Driven Solute Transport. Membrane vesicles (7–10 mg of membrane protein/ml) were incubated with the redox reagents for 30 min at room temperature. Then, *N*-ethylmaleimide (MalNEt), freshly dissolved in H_2O, was added to a final concentration of 0.5 mM and incubation was continued for 30 min. The membrane vesicles were pelleted by centrifugation (15 min in a Hettich Microfuge). The membrane pellet was suspended in 0.1 M K phosphate, pH 6.6/10 mM dithiothreitol, and the suspension was incubated for 45 min at room temperature. The membrane vesicles were pelleted and suspended in 0.1 M K phosphate (pH 6.6) to a protein concentration of 5–7 mg of membrane protein/ml. Uptake of lactose and L-proline driven by ascorbate/phenazine methosulfate oxidization was studied as described above.

For control experiments, membrane vesicles were treated in the same way except that the redox reagents, MalNEt, dithiothreitol, or combinations of these reagents, were omitted from the incubation mixture. For other control experiments, the sequence of incubation steps was altered.

The redox reagents were dissolved in ethanol, dimethyl sulfoxide, or water. The final concentrations of ethanol and dimethyl sulfoxide never exceeded 1%.

Phenylarsine oxide treatment of the membrane vesicles was carried out by adding 20 μM phenylarsine oxide to the transport experiment reaction mixture 5 min before adding ascorbate/phenazine methosulfate. The effect of dithiothreitol (10 mM) on phenylarsine oxide inhibition was studied by adding it just prior to the addition of ascorbate/phenazine methosulfate. The reaction mixture (0.1 ml) contained 50–70 μg of membrane protein in 0.1 M K phosphate, pH 6.6/10 mM $MgSO_4$. Additions to this mixture were made as indicated.

Counterflow. For counterflow experiments, membrane vesicles (7–10 mg of membrane protein/ml) were incubated at about 20°C with 10 mM lactose/0.05 μM 3,5-di-*tert*-butyl-4-hydroxybenzylidemalononitrite (SF 6847) for 3 hr, redox reagents were added, and incubation was continued for 30 min. The membrane vesicles were concentrated in this medium to 30 mg of membrane protein/ml. The counterflow experiment was carried out at 25°C by diluting 3 μl of this membrane vesicle suspension into 0.3 ml of 0.1 M K phosphate, pH 6.6/10 mM $MgSO_4$, supplemented with 68 μM [^{14}C]lactose and, where indicated, 10 mM dithiothreitol. For control experiments, membrane vesicles were processed in the same way except that the redox reagents were omitted. The counterflow was stopped as indicated for the uptake experiments.

Efflux. Membrane vesicles were allowed to accumulate [^{14}C]lactose or [^{14}C]proline aerobically in the presence of ascorbate/phenazine methosulfate to steady-state levels as described for the transport assays. After 12 min of incubation, uptake was stopped and efflux was initiated by the addition of 0.5 μM SF 6847. The amount of lactose or proline retained in the vesicles was followed as described for the transport assays. The effects of redox reagents on this efflux process were studied by adding the redox reagents to the incubation mixture just prior to adding the uncoupler (SF 6847).

Materials. Radioactive substrates were purchased from the Radiochemical Centre, Amersham, England. [^{3}H]Ph_4P^+ was obtained from the Nuclear Research Centre (Beer-Sheva, Israel). All other chemicals were reagent grade.

RESULTS

Effects of Oxidants on Active Solute Transport. Membrane vesicles of *E. coli* ML 308-225 accumulate L-proline at a high rate under aerobic conditions in the presence of the electron donor system ascorbate/phenazine methosulfate. The uptake of proline was significantly decreased in membrane vesicles that had been treated with the lipophilic oxidant plumbagin (Fig. 1). The inhibition increased with increasing plumbagin concentration until, at 0.5 mM plumbagin, uptake was inhibited to the level obtained under nonenergized conditions. Similar effects were observed with other lipophilic oxidants (e.g., for phenazine methosulfate, menadione, and 1,2-naphthoquinone, maximal inhibition occurred at 5 mM, 0.6 mM, and 0.3 mM, respectively). The uptake of lactose by *E. coli* membrane vesicles was inhibited to a similar extent by these lipophilic oxidants (data not shown). The inhibition of proline and lactose transport by the oxidants does not result from a decreased $\Delta\tilde{\mu}_{H^+}$. In the presence of nigericin (2 μM), the ascorbate/phenazine methosulfate-generated steady-state potential as determined from the accumulation of Ph_4P^+ was −90 mV in the presence of 0.5 mM plumbagin and −86 mV in its absence. If dithiothreitol was added to the vesicle suspension after incubation with plumbagin, full transport activity was restored.

Similar effects of plumbagin and dithiothreitol were observed with L-proline uptake driven by a valinomycin-induced potassium diffusion potential (Fig. 2). In the presence of 0.5 mM plumbagin, L-proline uptake was inhibited to the level observed in the absence of a potassium gradient and this inhibition was reversed by addition of dithiothreitol.

Protection by Oxidants Against MalNEt Inhibition. The above data suggest that proline transport can be regulated by a redox-sensitive process in which the system is active in the reduced state and inactive in the oxidized state. To determine whether the redox process involved the conversion of dithiol

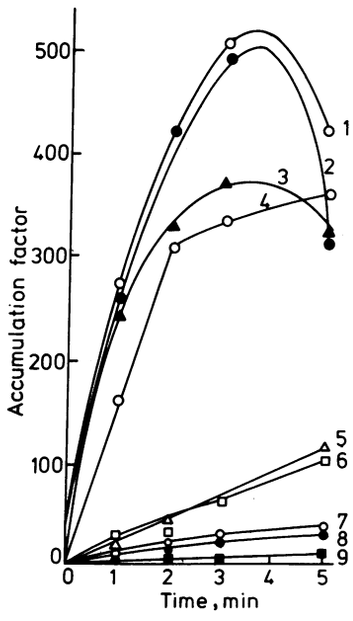

FIG. 1. Effects of 0.5 mM plumbagin and 10 mM dithiothreitol on uptake of L-proline and of 10 mM dithiothreitol on the inhibition by 0.5 mM MalNEt, 0.5 mM plumbagin, or both of L-proline uptake by membrane vesicles energized by ascorbate/phenazine methosulfate oxidation. Curves: 1, no additions; 2, plumbagin followed by dithiothreitol; 3, dithiothreitol; 4, plumbagin followed by MalNEt followed by dithiothreitol; 5, MalNEt; 6, MalNEt followed by dithiothreitol; 7, plumbagin; 8, plumbagin followed by MalNEt; 9, MalNEt followed by plumbagin.

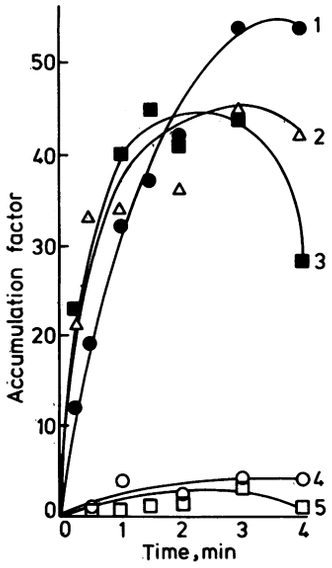

FIG. 2. Effects of 0.5 mM plumbagin and 10 mM dithiothreitol on uptake of L-proline by membrane vesicles energized by a valinomycin-induced potassium diffusion potential. Curves: 1, with a potassium gradient; 2, with a potassium gradient, incubation with 0.5 mM plumbagin and then with 10 mM dithiothreitol; 3, with a potassium gradient, incubation with 10 mM dithiothreitol; 4, no potassium gradient; 5, with a potassium gradient, incubation with 0.5 mM plumbagin.

to disulfide, similar to that observed for the *P-e*Prv-dependent glucose transport system in *E. coli* (6), the protection of proline transport activity against MalNEt inactivation was examined (Fig. 1). Previous treatment of the carriers with oxidants should convert the dithiols to disulfides, which cannot react with MalNEt, and subsequent addition of dithiothreitol will neutralize the MalNEt and regenerate an active carrier. Without previous treatment by oxidants, however, MalNEt will react with the dithiols and irreversibly inactivate the carrier. In Fig. 1, we show that MalNEt treatment of vesicles resulted in 80% inhibition of proline transport and that this inhibition was not reversed by dithiothreitol. The same concentration of dithiothreitol, however, was capable of restoring most of the transport activity of plumbagin-treated vesicles. Similar protection against MalNEt inhibition was also found with the lipophilic oxidants phenazine methosulfate, menadione, and 1,2-naphthoquinone, with maximal protection occurring at 5 mM, 0.6 mM, and 0.3 mM, respectively. Water-soluble reagents such as $CuCl_2$, 2,6-dichlorophenolindophenol, benzyl viologen, methyl viologen, and ferricyanide up to a concentration of 1 mM did not provide protection.

Lactose transport was measured in similar experiments and showed essentially the same pattern of reactivity as proline transport.

Effect of Phenylarsine Oxide on Lactose and Proline Transport. The data in Figs. 1 and 2 support the proposal that a redox reaction involving a dithiol–disulfide conversion can regulate the activity of the lactose and proline carriers. As a final test of this proposal, we monitored the response of these carriers to phenylarsine oxide, which complexes specifically with dithiols (15–17). Inhibition by this reagent is strong evidence for the presence of vicinal sulfhydryl groups. We observed that 20 μM phenylarsine oxide inhibited the ascorbate/phenazine methosulfate-driven proline uptake by 75–80% and that 85% of this activity was recovered by addition of dithiothreitol. These data confirm that dithiol–disulfide interchange can control the activity of the *E. coli* proline and lactose transport systems.

Effects of Lipophilic Oxidants on Efflux and Counterflow. To further characterize which elements of the transport process are affected by the redox potential, efflux and counterflow were examined in the presence of oxidants. Fig. 3 shows that lipophilic oxidants inhibit the efflux of accumulated solutes from membrane vesicles induced by the uncoupler SF 6847. When SF 6847 was added to membrane vesicles that had accumulated proline or lactose to steady-state levels with ascorbate/phenazine methosulfate, rapid efflux of proline or lactose occurred. This efflux was significantly decreased by plumbagin, and the inhibition increased with increasing plumbagin concentrations. In this experiment, prior incubation with plumbagin could not be carried out, and therefore the inhibition observed is not the maximal possible level.

Counterflow is also inhibited by lipophilic oxidants and the inhibition is reversed by dithiothreitol. Inhibition has been observed with menadione, phenazine methosulfate, plumbagin, and 1,2-naphthoquinone. The inhibition increased with increasing concentration of oxidant, as shown for plumbagin in Fig. 4*A*. When counterflow was carried out by diluting plumbagin-treated membrane vesicles into buffer containing dithiothreitol, the level of inhibition was significantly decreased (Fig. 4*B*). Hardly any effect of dithiothreitol was observed on counterflow from membrane vesicles that had not been treated with plumbagin.

Effects of Oxidants on Kinetics of Active Transport. The data in Figs. 1–4 show that proline and lactose carriers in *E. coli* can be regulated by altering the redox states of functional groups

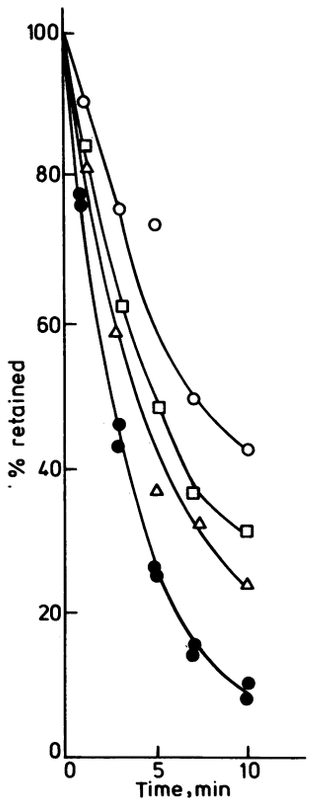

FIG. 3. Effect of plumbagin on SF 6847-induced efflux from lactose-loaded membrane vesicles. At the steady-state level of lactose accumulation (reached after 12 min with ascorbate/phenazine methosulfate), efflux was initiated by addition of 0.5 μM SF 6847. The effect of plumbagin on this efflux was studied by adding plumbagin just prior to the addition of SF 6847 to the incubation mixture. Efflux: ●, in the absence of plumbagin; △, with 0.5 mM plumbagin; □, with 1 mM plumbagin; ○, with 2 mM plumbagin.

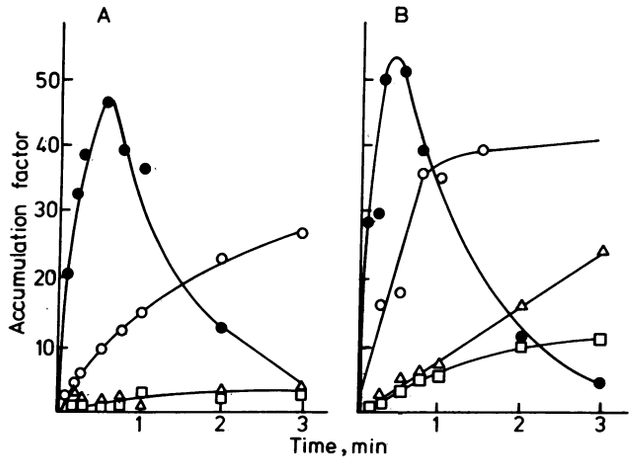

FIG. 4. Effects of plumbagin and dithiothreitol on lactose counterflow. Counterflow from lactose-loaded membrane vesicles (*A*) previously incubated without plumbagin (●) or with plumbagin at 100 μM (○), 300 μM (△), or 500 μM (□) was determined. (*B*) Lactose-loaded vesicles were incubated as in *A* and then treated with 10 mM dithiothreitol before determining counterflow.

in the carriers. The step in the mechanism that affects the V_{max} of transport or the K_m can be determined by following the kinetics of lactose and proline transport in the presence of oxidants. The initial rates of ascorbate/phenazine methosulfate-driven lactose and proline uptakes were measured in untreated membrane vesicles and in vesicles treated with 0.5 mM plumbagin. The data shown in Fig. 5*A* give K_m = 0.2 mM and V_{max} = 44 (nmol/min)/mg of protein for lactose transport in untreated membrane vesicles and K_m = 20 mM and V_{max} = 18 (nmol/min)/mg of protein for vesicles previously incubated with 0.5 mM plumbagin. These data are comparable with those for membrane vesicles in the presence of an $\Delta\tilde{\mu}_{H^+}$ (K_m = 0.17 mM) and for vesicles in the absence of an $\Delta\tilde{\mu}_{H^+}$ (K_m = 18.9 mM) for the facilitated diffusion process (10). Similar results are given in Fig. 5*B* for proline transport [untreated vesicles, K_m = 1 μM and V_{max} = 2.5 (nmol/min)/mg of protein; plumbagin-treated vesicles, K_m = 45 μM and V_{max} = 0.83 (nmol/min)/mg of protein].

We conclude from these data that the lactose and proline carriers can exist in two forms, a reduced high-affinity form and an oxidized low-affinity form.

DISCUSSION

The data presented above show that the *E. coli* lactose and proline transport proteins, like those of the *P-e*Prv-dependent hexose transport system, are physically regulated by their redox state. The reduced state of the carrier is the low K_m form and the oxidized state is the high K_m form. Just as in the hexose transport system (6), the changes observed on altering the redox state are the same as the changes in K_m on establishing an $\Delta\tilde{\mu}_{H^+}$ across the membrane (2). Thus, it is reasonable to conclude that the $\Delta\tilde{\mu}_{H^+}$ changes the K_m of a carrier by altering the redox state of the carrier in the membrane.

The K_m can be equated with either the real equilibrium binding constant K_S or with K_T, the half-saturation constant for transport, which includes both the binding constant and the rate constant for the transport step. At present, there is no evidence that the K_m for transport in various systems is not equal to the K_S. Therefore, we speak in the following section of changes in K_m as indicative of changes in affinity states.

Role of Affinity Changes in $\Delta\tilde{\mu}_{H^+}$-Driven Transport. The information collected on all three systems, hexose transport via the *E. coli P-e*Prv-dependent system (6) and proline and lactose transport in *E. coli*, supports a general role for dithiol–disulfide interchange in the mechanism of transport processes. Details of this mechanism for various types of transport and energy-transducing systems will be presented elsewhere (18). For the sake of clarity, we will discuss here only those elements pertinent to secondary solute transport. Transport proteins must be able to bind substrates with high affinity and subsequently release them. We propose that the release mechanism involves

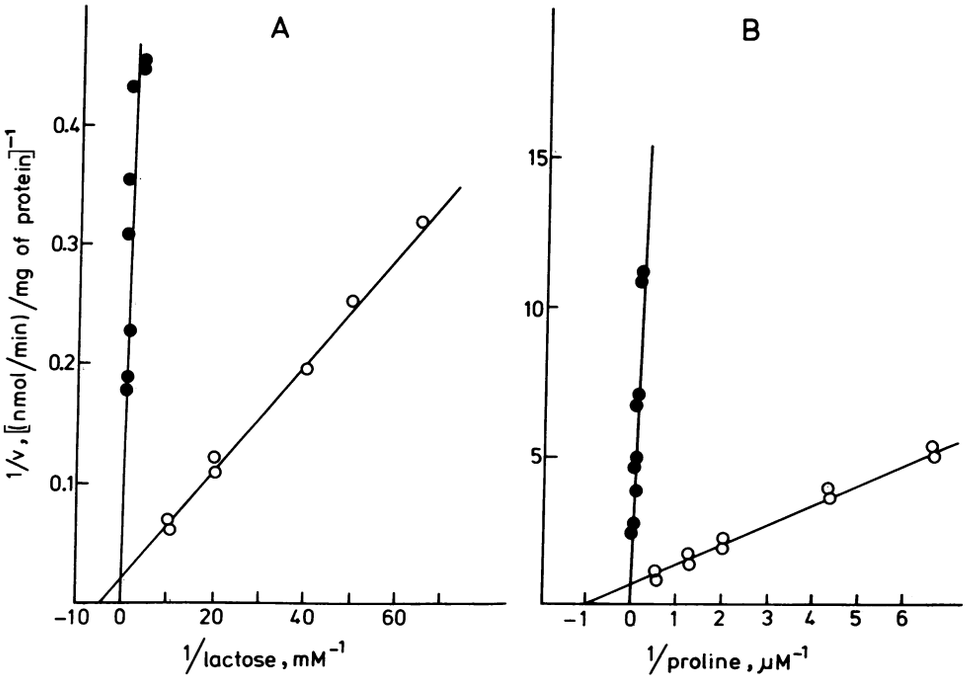

FIG. 5. Lineweaver–Burk plots of ascorbate/phenazine methosulfate-driven lactose (*A*) and L-proline (*B*) uptake by membrane vesicles in the presence (●) and the absence (○) of 0.5 mM plumbagin.

a switch in the oxidation state of the dithiol. Consider, for example, constructing a carrier in the form of a channel having one binding site at the outer end and one at the inner end of the channel (19). A pump mechanism can be created if the affinities of the two sites are coupled such that, when one is high affinity, the other is low affinity. This can be achieved by using a dithiol at each site to control the affinities. The redox state at each site in the channel will be related to the transmembrane electrical potential, $\Delta\psi$, by $\Delta\psi = \psi^2 - \psi^1 = E^1 - E^2$, where ψ and E are the electrical and redox potentials, respectively, at a given site (7). When $\Delta\psi$ is negative interior, the electrical potential at the outer site will be higher than at the inner site and the corresponding redox potential at the outer site will be lower relative to the inner site. The sites can be linked by two separate pathways, one for hydrogen conduction to move hydrogen ions toward the interior and one for electron conduction to bring the electrons back to the outer side after the proton is released on the interior. A cycle of proton solute symport could proceed as follows. Substrate binds to the outer site initially in the high-affinity form. Binding of a H^+ causes the carrier to switch to the oxidized form due to a shift in the apparent pK of the redox couple. Hydrogen moves down the hydrogen-conducting pathway reducing the inner site and the ligand is drawn to the inner high-affinity site. Proton and solute are released into the medium when the inner site is oxidized and the electron is carried back to the outer site by an electron-conducting pathway. The nature of the hydrogen- and electron-conducting pathways are as yet undefined. Hydrogen conduction could, for instance, occur via a series of sulfhydryl groups and electron conduction could occur via an organometallic or other suitable conductor of unhydrated electrons that migrate through the nonaqueous osmotic carrier domain of the protein via a π-bond system or by tunneling.

Accessibility of the Redox Sites. Many studies monitoring the effects of sulfhydryl reagents on carrier activities have shown that the presence of an $\Delta\tilde{\mu}_{H^+}$ sensitizes the carrier to inactivation (for review, see refs. 18 and 20). This has also been shown recently for the *E. coli* lactose and proline transport systems (10). The rate of inactivation of lactose transport in *E. coli* membrane vesicles by a variety of permeable and nonpermeable maleimides was 2.5 times faster in the presence of an ascorbate/phenazine methosulfate-generated $\Delta\tilde{\mu}_{H^+}$ than in its absence. The difference was a factor of 30 in the case of proline transport when glutathione hexane maleimide was used. These results are in agreement with the data presented here. In the presence of an $\Delta\tilde{\mu}_{H^+}$, interior negative and alkaline, the binding sites are reduced and capable of reacting more rapidly, even with nonpenetrating reagents.

Published data show that different systems respond in different ways to a given sulfhydryl reagent. In some cases, only nonpolar reagents inactivate the system and, in other cases, both polar and nonpolar reagents inactivate it. Presumably, the accessibility of these sites differs for each system. In studies such as those presented here, care should be taken to use oxidants and sulfhydryl reagents of various lipophilicities and sizes.

The proposed mechanism for solute transport systems has a number of features in common with a model for solute transport presented by Kaback and Barnes (21) in 1971. Those investigators proposed that the activity of the carrier is the result of a reversible interconversion of one dithiol to disulfide. According to their model, the carrier had a high-affinity site exposed to the outside in the oxidized state and a low-affinity site exposed to the inner surface in the reduced state. An important feature of their model was that the carrier was depicted as an essential intermediate of the electron-transfer system. Experimental evidence has since been presented that contradicts this latter feature and consequently their model was dismissed. It should be stipulated that the experimental evidence in favor of SH involvement—i.e., the inhibition of transport and efflux by MalNEt and *p*-chloromercuribenzenesulfonate and the reversal of the benzenesulfonate inhibition—still holds. In addition, we have provided evidence that dithiol–disulfide interchange is an essential element of the catalytic activity of these systems.

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1. Vignais, P. V. (1976) *Biochim. Biophys. Acta* **456**, 1–38.
2. Kaczorowski, G. T., Robertson, D. E. & Kaback, H. R. (1979) *Biochemistry* **18**, 3697–3704.
3. de Meis, L. & Vianna, A. L. (1979) *Annu. Rev. Biochem.* **48**, 275–292.
4. Hernandez-Asensio, M., Ramirez, J. M. & del Campo, F. F. (1975) *Arch. Microbiol.* **103**, 155–162.
5. Reider, E., Wagner, E. F. & Schweiger, M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5529–5533.
6. Robillard, G. T. & Konings, W. N. (1981) *Biochemistry* **20**, 5025–5032.
7. Walz, D. (1979) *Biochim. Biophys. Acta* **505**, 279–353.
8. Kaback, H. R. & Hong, J. S. (1973) *Crit. Rev. Microbiol.* **2**, 333–376.
9. Kaback, H. R. & Patel, L. (1978) *Biochemistry* **17**, 1640–1646.
10. Cohen, D. E., Kaczorowski, G. T. & Kaback, H. R. (1981) *Biochemistry* **20**, 3308–3313.
11. Davis, B. D. & Mingioli, E. S. (1959) *J. Bacteriol.* **60**, 17–28.
12. Kaback, H. R. (1971) *Methods Enzymol.* **22**, 99–120.
13. Konings, W. N., Barnes, E. M. & Kaback, H. R. (1971) *J. Biol. Chem.* **246**, 5857–5861.
14. Hellingwerf, K. J., Bolscher, J. G. M. & Konings, W. N. (1981) *Eur. J. Biochem.* **113**, 369–374.
15. Stocken, L. A. & Thompson, R. H. S. (1946) *Biochem. J.* **40**, 529–535.
16. Stevenson, K. J., Hale, J. & Perhem, R. N. (1978) *Biochemistry* **17**, 2189–2192.
17. Webb, J. L. (1964) in *Enzymes and Metabolic Inhibitors* (Academic, New York), Vol. 3, pp. 599–602.
18. Robillard, G. T. & Konings, W. M. (1982) *Eur. J. Biochem.*, in press.
19. Lo, T. C. Y. (1979) *Can. J. Biochem.* **57**, 289–296.
20. Fonyo, A. (1978) *J. Bioenerg. Biomembr.* **10**, 171–194.
21. Kaback, H. R. & Barnes, E. M. (1971) *J. Biol. Chem.* **246**, 5523–5531.