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# Reconstitution of D-Lactate-Dependent Transport in Membrane Vesicles from a D-Lactate Dehydrogenase Mutant of *Escherichia coli**

**(guanidine hydrochloride/chaotrope)**

JOHN P. REEVES, JEN-SHIANG HONG,† AND H. RONALD KABACK

The Roche Institute of Molecular Biology, Nutley, New Jersey 07110

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**ABSTRACT** **Membrane-bound, flavin-linked D-lactate dehydrogenase in membrane vesicles of *E. coli* ML 308-225 is solubilized by extraction with guanidine·HCl. When membrane vesicles prepared from a D-lactate dehydrogenase mutant are treated with this extract, they regain the capacity to catalyze D-lactate oxidation and D-lactate-dependent transport. Similar effects are obtained with wild-type membrane vesicles in which D-lactate oxidation and D-lactate-dependent transport have been inactivated by 2-hydroxy-3-butynoate. Although treatment of wild-type vesicles with the extract results in an increased capacity to catalyze D-lactate oxidation, no effect on transport is observed. Reconstituted transport activity is a saturable function of the amount of guanidine extract added. Moreover, the quantity of extract required to achieve maximum initial rates of transport varies with each transport system. On the other hand, reconstituted D-lactate oxidation increases linearly over a broader range of extract concentrations.**

**Oxamate, a competitive inhibitor of D-lactate dehydrogenase, and *p*-chloromercuribenzenesulfonate block both the initial rate of transport and the steady-state level of accumulation in reconstituted vesicles. Furthermore, these reagents induce efflux of transport substrates from preloaded, reconstituted vesicles. The same reagents inhibit the initial rate of uptake but not the steady-state level of accumulation in ML 308-225 vesicles, and do not induce efflux. These results suggest that, although reconstituted vesicles catalyze D-lactate oxidation and D-lactate-dependent transport, the system has not been reconstituted to its native state.**

Transport of various metabolites by membrane vesicles of *Escherichia coli* is coupled primarily to oxidation of D-lactate (1–12) or reduced phenazine methosulfate (13). D-Lactate oxidation is catalyzed by a membrane-bound, flavin-linked D-lactate dehydrogenase, which has been purified to homogeneity (26, 27). The energy-coupling site for transport lies within a segment of the respiratory chain between the dehydrogenase and cytochrome $b_1$, and recent evidence suggests that an electron-transfer-coupling component may mediate transfer of electrons between the respiratory chain and the "carriers" (14). Membrane vesicles from D-lactate dehydrogenase mutants (*dld*$^-$) of *E. coli* and *Salmonella typhimurium* do not catalyze D-lactate oxidation or D-lactate-dependent transport (14). Although *dld*$^-$ cells exhibit normal respiration-linked transport, the absence of a demonstrable defect is probably due to a compensatory increase in the coupling of succinic dehydrogenase to transport (14).

Abbreviations: *p*-CMBS, *p*-chloromercuribenzenesulfonate; HQNO, 2-heptyl-4-hydroxyquinoline-*N*-oxide.

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† Present address: Graduate Department of Biochemistry, Brandeis University, Waltham, Mass. 02154.

Chaotropic agents solubilize membrane-bound proteins (15), and such agents have been used to extract succinate dehydrogenase and NADH dehydrogenase from mitochondrial preparations (16, 17). This report describes the solubilization of D-lactate dehydrogenase activity from membrane vesicles of *E. coli* with guanidine·HCl. The extract restores D-lactate oxidation and D-lactate-dependent transport activities to membrane vesicles from a *dld* mutant of *E. coli* ML 308-225 and to wild-type vesicles in which the dehydrogenase has been inactivated by treatment with 2-hydroxy-3-butynoic acid (7). Reconstituted and native D-lactate-dependent transport are similar in many respects, but differ in their response to oxamic acid, a competitive inhibitor of D-lactate-dehydrogenase, and to *p*-chloromercuribenzene sulfonate (*p*-CMBS).

## METHODS

*Growth of Bacteria, Preparation of Membrane Vesicles, and Transport Assays. E. coli* ML 308-225 ($i^-z^-y^+a^+$) and ML 308-225*dld-3* were grown on minimal medium A containing succinate as a sole source of carbon (14). Membrane vesicles were prepared and transport was assayed (1–8, 13, 14, 18–20).

*Extraction of Vesicles with Guanidine·HCl.* Membrane vesicles suspended in 0.1 M potassium phosphate (pH 6.6) at about 8 mg of protein per ml were adjusted to a final concentration of 0.75 M guanidine·HCl by addition of an appropriate volume of 5 M guanidine·HCl. After a 15-min incubation at 0°, the samples were centrifuged at about 40,000 × *g* for 10 min, and the supernatant was carefully aspirated and stored at 0°.

*Reconstitution of D-Lactate Oxidation and D-Lactate-Dependent Transport.* The 0.75 M guanidine extract was diluted 1/20 into a membrane suspension (0.3–0.4 mg of extract protein per mg of membrane protein) in 0.1 M potassium phosphate buffer (pH 6.6). The sample was incubated for 15 min at 0° and centrifuged at about 40,000 × *g* for 15 min. The supernatant was carefully aspirated and discarded, and the pellet was resuspended in 0.1 M potassium phosphate buffer (pH 6.6) to a final concentration of 3–4 mg of protein per ml. For rubidium uptake studies, the procedure was identical except that extraction and reconstitution were done in 0.1 M sodium phosphate (pH 6.6).

*Miscellaneous Analytical Methods.* Oxygen uptake was measured at 25° with a YSI model 53 Oxygen Monitor (3). D-Lactate:phenazine methosulfate reductase activity was measured by oxygen uptake with 0.1 mM phenazine methosulfate as an electron carrier. The cytochrome content of the guanidine extract was measured with a Cary 15 recording spectrophotometer, with sodium dithionite as a reductant (3). The

TABLE 1. *Effect of inhibitors on solubilized* D-*lactate: phenazine methosulfate reductase activity and reconstituted* D-*lactate oxidation*

| Inhibitor | Concentration (mM) | Inhibition (%) D-Lactate: phenazine methosulfate reductase activity in guanidine extract | Inhibition (%) Reconstituted D-lactate oxidation in *dld-3* vesicles |
|---|---|---|---|
| Oxamate | 10 | 95 | 93 |
| *p*-CMBS | 0.4 | 95 | 93 |
| NEM | 1 | 64 | 75 |
| Amytal | 5 | 0 | 63 |
| HOQNO | 0.04 | 0 | 68 |
| KCN* | 10 | 0 | 95 |

Oxygen uptake was measured in a 0.75 M guanidine extract from ML 308-225 vesicles (control value = 750 ng atoms of oxygen per min per mg of protein) or in reconstituted *dld-3* vesicles (control value = 500 ng atoms of oxygen per min per mg of membrane protein). *p*-CMBS, *p*-chloromercuribenzenesulfonate; *NEM*, *N*-ethyl-maleimide; *HOQNO*, 2-heptyl-4-hydroxy quinidine-*N*-oxide.

* The effect of KCN was measured at pH 6.6. At pH 7.5, KCN inhibited D-lactate: phenazine methosulfate reductase activity by about 25%.

phospholipid content of the guanidine extract was determined as organic phosphorous (21) after extraction with chloroform—methanol and subsequent partition into chloroform (22). Protein was determined by the method of Lowry *et al.* (23).

## RESULTS

*Solubilization of Membrane Protein and* D-*Lactate: Phenazine Methosulfate Reductase Activity by Guanidine·HCl.* Treatment of membrane vesicles of *E. coli* ML 308-225 with 0.75 M guanidine·HCl solubilizes about 15% of the membrane protein and 40–50% of the membrane-bound D-lactate: phenazine methosulfate reductase activity. The specific activity of the extract is about 3-times higher than that of the intact membranes. Various experiments demonstrate that the solubilized D-lactate dehydrogenase activity in the guanidine extract is not due to unsedimented membrane vesicles: (*i*) The disc-gel electrophoretic pattern of the extract differs markedly from that of whole membranes (data not shown); several of the major bands present in the vesicles do not appear in the extract. (*ii*) Centrifugation of the guanidine extract at 140,000 × *g* for 1 hr does not sediment any D-lactate: phenazine methosulfate reductase activity. (*iii*) Neither phospholipid phosphorous nor cytochromes could be detected in the extract (data not shown).

Solubilized D-lactate: phenazine methosulfate reductase activity is markedly inhibited by oxamate and *p*-CMBS, and is partially inhibited by *N*-ethylmaleimide (Table 1). *p*-CMBS and *N*-ethylmaleimide do not inhibit the D-lactate: dichlorophenolindophenol reductase activity of D-lactate dehydrogenase, as measured in intact membrane vesicles (3) or in partially purified (3) or homogeneous preparations (26, 27) of this enzyme. These results suggest that the functional D-lactate: phenazine methosulfate reductase activity present in the guanidine extracts consists of more than one component, or possibly that the enzyme contains a sulfhydryl component necessary for reduction of phenazine methosulfate but not dichlorophenolindophenol. KCN, 2-heptyl-4-hydroxyquinoline-*N*-oxide (HOQNO), and amytal, agents that block D-lactate oxidation in intact vesicles (3), do not affect D-lactate: phenazine methosulfate reductase activity in the guanidine extract (Table 1). These inhibitors act on components of the electron transfer chain distal to the primary dehydrogenase (3, 24).

*Restoration of* D-*Lactate Oxidation in Membrane Vesicles.* When membrane vesicles prepared from a mutant of *E. coli* ML 308-225 lacking D-lactate dehydrogenase are treated with a guanidine extract from wild-type vesicles, the *dld*$^-$ vesicles regain the ability to oxidize D-lactate (Table 1). No D-lactate oxidation is observed with the guanidine extract alone (i.e., in the absence of phenazine methosulfate), with *dld-3* vesicles alone, or when *dld-3* vesicles are treated with a guanidine extract from *dld-3* vesicles. D-Lactate oxidation by reconstituted *dld-3* vesicles is blocked by oxamate, *p*-CMBS, *N*-ethylmaleimide, HOQNO, amytal, and KCN (Table 1), all of which block D-lactate oxidation in ML 308-225 vesicles (3). The inhibitory effects of KCN, HOQNO, and amytal on reconstituted D-lactate oxidation in *dld-3* vesicles strongly suggest that electrons are transferred from D-lactate to oxygen by means of the electron transfer chain in *dld-3* vesicles.

Reconstituted D-lactate oxidation catalyzed by *dld-3* vesicles increases linearly with the amount of extract added up to about 0.6 mg of extract protein per mg of *dld-3* membrane protein (Fig. 1). Above this concentration ratio, activity continues to increase linearly as a function of extract protein added, but the slope of this portion of the curve is only about 50% of that of the initial portion of the curve. This finding suggests that there may be at least two functionally distinct binding sites for D-lactate dehydrogenase. D-Lactate oxidation by ML 308-225 vesicles also increases when these vesicles

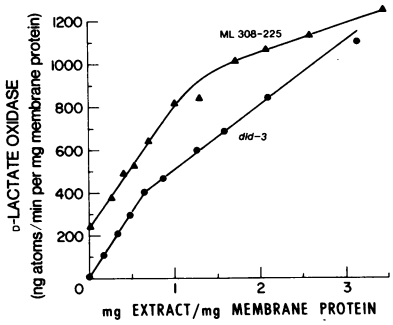


FIG. 1. Effect of a guanidine hydrochloride extract from membrane vesicles of *E. coli* ML 308-225 on D-lactate oxidation in ML 308-225 and ML 308-225 *dld-3* membrane vesicles. Various amounts of *dld-3* (●——●) or ML 308-225 (▲——▲) membrane vesicles containing 6–40 μg of membrane protein were placed in the sample chamber of the oxygen electrode with 0.1 M potassium phosphate (pH 6.6). Aliquots (5–20 μl) of the guanidine extract from ML 308-225 vesicles (1.0 mg of protein per ml) were added, and oxygen uptake was monitored in the presence of 10 mM lithium D-lactate. The specific activities for D-lactate oxidation were calculated on the basis of the membrane protein and not the total protein (membranes plus extract).

are treated with the guanidine extract (Fig. 1). In this case, the increase in D-lactate oxidation also exhibits two linear regions as a function of the amount of extract added, but the transition point occurs at higher ratios of extract to membrane protein than observed with *dld-3* vesicles. In addition, the slope of the curve at higher extract concentrations is less than that observed with *dld-3* vesicles. The apparent Michaelis constants for D-lactate oxidation in native ML 308-225 vesicles and reconstituted *dld-3* vesicles are about 1.4 and 0.67 mM, respectively (data not shown).

Guanidine extracts from *E. coli* ML 308-225 also reconstitute D-lactate oxidation in membrane vesicles from a *dld*$^-$ mutant of *S. typhimurium* LT-2 (14) to a degree similar to that shown for ML 308-225*dld-3* vesicles (Table 2). However, the extract is less effective in reconstituting D-lactate oxidation in membrane vesicles from *Micrococcus denitrificans*, *Aerobacter aerogenes*, *E. coli* AN 120, or *Serratia marcescans*, and completely ineffective with membrane vesicles from *Staphylococcus aureus* and *Proteus vulgaris* (Table 2).

*Reconstitution of D-Lactate-Dependent Transport in dld-3 Membrane Vesicles.* Membrane vesicles prepared from ML 308-225*dld-3* exhibit no D-lactate-dependent transport (14). However, when these vesicles are treated with guanidine extracts from ML 308-225 vesicles, they regain D-lactate-dependent transport activity towards several amino acids, lactose, and rubidium (in the presence of valinomycin) (Fig. 2). In most cases (i.e., with proline, serine, leucine, lactose, and rubidium transport), the initial rates and steady-state levels of accumulation are similar to that of ML 308-225 membrane vesicles. Glycine transport is reconstituted to the extent of about 50% of ML 308-225 vesicles. Similar results have been

TABLE 2. *Reconstitution of D-lactate oxidation in membrane vesicles prepared from various bacteria*

| Vesicles | D-Lactate oxidation (ng atoms of $O_2$/per min per mg of extract protein) |
|---|---|
| *Escherichia coli* ML 308-225*dld-3* | 778 |
| *Salmonella typhimurium* LT-2*dld-27* | 802 |
| *Escherichia coli* AN120 | 489 |
| *Aerobacter aerogenes* | 416 |
| *Micrococcus denitrificans* | 269 |
| *Seratia marcesans* | 186 |
| *Staphylococcus aureus* | 0 |
| *Proteus vulgaris* | 0 |

Membrane vesicles (about 40 μg of membrane protein) were placed in the sample chamber of the oxygen electrode containing 1.0 ml of 0.1 M potassium phosphate. 20 mM D-Lactate was added, and the rate of D-lactate oxidation was monitored (native). 20 μl (25 μg of protein) of a guanidine extract of *E. coli* ML 308-225 vesicles was then added and D-lactate oxidation was monitored for at least another 5 min. The values are the rates of D-lactate oxidation per mg of extract protein added, after subtraction of the native values. No endogenous D-lactate oxidation was observed with vesicles of *E. coli dld-3*, *S. typhimurium dld-27*, *P. vulgaris*, or *S. aureus*. For *E. coli* AN120, *M. denitrificans*, *S. marcesans*, and *A. aerogenes*, the native rates of D-lactate oxidation were 53, 30, 26, and 185 ng atoms of $O_2$ per min per mg of membrane protein, respectively.

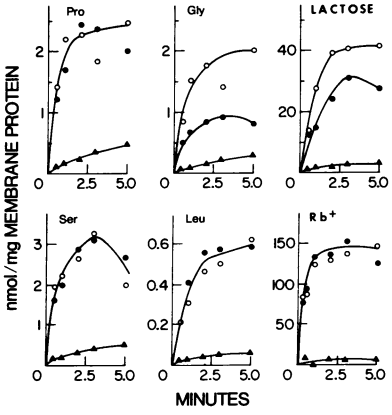


FIG. 2. Reconstitution of D-lactate-dependent transport in *dld-3* vesicles by a guanidine hydrochloride extract of ML 308-225 membrane vesicles. ML 308-225 membrane vesicles (○——○), reconstituted *dld-3* membrane vesicles (●——●), and untreated *dld-3* membrane vesicles (▲——▲) were assayed for transport of the substrate indicated in the presence of 20 mM D-lactate. Reconstitution of D-lactate-dependent transport was also observed for aspartate, phenylalanine, histidine, and lysine (data not shown). Valinomycin-induced rubidium uptake was assayed in sodium phosphate (19).

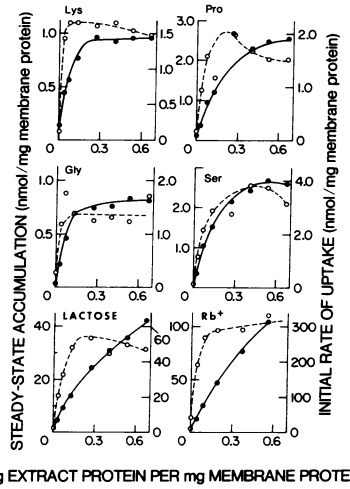


FIG. 3. Effect of increasing amounts of ML 308-225 guanidine extract on D-lactate-dependent transport by *dld-3* membrane vesicles. Aliquots of *dld-3* membrane vesicles (1.6 mg of protein) were added to separate tubes containing 25 ml of 0.1 M potassium phosphate (pH 6.6). Increasing amounts (0.06–1.1 mg of protein) of a guanidine hydrochloride extract of ML 308-225 membranes were added to the tubes. Guanidine hydrochloride was also added in appropriate amounts to adjust the final concentrations of guanidine hydrochloride in each tube to 30 mM. After a 15-min incubation at 0°, the membranes were centrifuged and suspended in 0.1 M potassium phosphate (pH 6.6) to about 2.7 mg of membrane protein per ml. Transport was assayed. For valinomycin-induced rubidium uptake, a similar procedure was followed except that the extraction and reconstitution steps were done in 0.1 M sodium phosphate (pH 6.6) (19). D-Lactate oxidation by the vesicles used in these experiments increased in a nearly linear fashion with the amount of extract added; the maximal D-lactate oxidation rate (at 0.67 mg of extract per mg of *dld-3* membrane protein) was 480 ng atoms per min per mg membrane protein. (○), Steady-state levels of accumulation measured after 10-min incubation in the presence of 20 mM D-lactate and the indicated transport substrate. (●), Initial rates of transport determined at 10 and 20 sec under the same conditions.

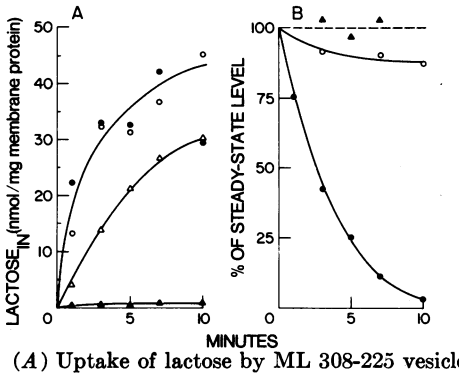


FIG. 4. (*A*) Uptake of lactose by ML 308-225 vesicles (*open symbols*) and reconstituted *dld-3* vesicles (*closed symbols*) with (△, ▲) and without (○, ●) 10 mM oxamate. (*B*) Effect of 10 mM oxamate on ML 308-225 (○) and reconstituted *dld-3* (●) vesicles preloaded with lactose. Vesicles were incubated for 10 min with [*1*-$^{14}$C]lactose and 20 mM D-lactate before addition of oxamate. Control (▲): preloaded reconstituted *dld-3* membrane vesicles in the absence of oxamate. IN, internal.

obtained with membrane vesicles prepared from penicillin-induced spheroplasts (data not shown).

Both the initial rates and steady-state levels of accumulation of lysine, proline, glycine, and serine approach an upper limit as increasing quantities of the guanidine extract are added to *dld-3* vesicles (Fig. 3). In each case, the steady-state accumulation, as opposed to initial rates of uptake, saturate at somewhat lower extract concentrations. With lactose and rubidium, the initial rates of uptake increase nearly linearly with increasing extract concentration, although the steady-state levels of accumulation attain a maximal value at relatively low extract concentrations.

D-Lactate-dependent transport by reconstituted *dld-3* vesicles is markedly inhibited by anoxia and by the electron transfer inhibitors, KCN, HOQNO, and amytal (data not shown). Moreover, these inhibitors all cause efflux from reconstituted *dld-3* vesicles preloaded with lactose (data not shown). In these respects, reconstituted D-lactate-dependent transport in *dld-3* membrane vesicles is similar to transport in ML 308-225 vesicles (4).

Reconstituted and native vesicles differ markedly, however, in the effects on transport of oxamate, a competitive inhibitor of D-lactate dehydrogenase. Oxamate strongly inhibits the initial rate of D-lactate-dependent lactose transport in ML 308-225 vesicles (Fig. 4*A*); however, lactose is accumulated to high internal concentrations if the incubation is continued for a prolonged time. Moreover, efflux of accumulated lactose does not occur when oxamate is added to previously loaded ML 308-225 vesicles (Fig. 4*B*). Similar results have been reported for lactose (4), various amino acids (6), and rubidium (20). In reconstituted *dld-3* vesicles, however, oxamate blocks D-lactate-dependent uptake of lactose, but does not allow significant accumulation to occur over extended incubation periods (Fig. 4*A*). Moreover, oxamate induces rapid efflux of lactose when added to preloaded, reconstituted *dld-3* vesicles (Fig. 4*B*). Oxamate also causes efflux of $Rb^+$ (Fig. 5) in preloaded reconstituted *dld-3* vesicles, but not in preloaded ML 308-225 vesicles. In a similar manner, *p*-CMBS, a potent inhibitor of D-lactate oxidation in reconstituted *dld-3* (Table 1) and wild-type vesicles (3), causes rapid efflux of $Rb^+$ in preloaded, reconstituted *dld-3* vesicles, but not in preloaded ML 308-225 vesicles (Fig. 5) (20).

D-Lactate oxidation by reconstituted *dld-3* vesicles exhibits a similar sensitivity to oxamate and *p*-CMBS as that observed in ML 308-225 vesicles. At 10 mM oxamate or 0.5 mM *p*-CMBS, the concentrations used in the experiments described above, D-lactate oxidation is inhibited by more than 90% in both the reconstituted *dld-3* and ML 308-225 vesicles. It seems unlikely, therefore, that the remarkable differences in the effects of oxamate and *p*-CMBS on these two membrane preparations could be due solely to the sensitivity of D-lactate dehydrogenase to these inhibitors. When reconstitution is

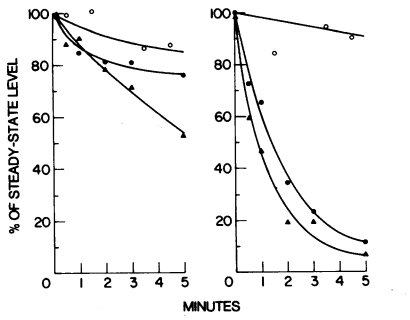


FIG. 5. Effect of oxamate and *p*-CMBS on ML 308-225 (*left*) and reconstituted *dld-3* (*right*) vesicles preloaded with $Rb^+$. Vesicles, supplemented with valinomycin (1 nmol/mg of membrane protein), were incubated for 5 min with 2 mM $^{86}$RbCl and 20 mM Li D-lactate before addition of 10 mM sodium oxamate (●) or 0.5 mM sodium *p*-CMBS (▲). Controls (○): no inhibitor added.

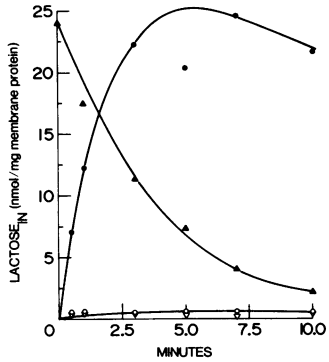


FIG. 6. Effect of guanidine extract on transport of lactose by ML 308-225 vesicles treated with 2-hydroxy-3-butynoate. Membrane vesicles of ML 308-225 were incubated for 30 min at 25° with 10 mM 2-hydroxy-3-butynoate in 50 mM potassium phosphate and 10 mM $MgSO_4$. The reaction was terminated by dilution into cold potassium phosphate buffer, and the vesicles were collected by centrifugation. A portion of the treated vesicles was assayed for lactose uptake (○) and the remainder was supplemented with a guanidine hydrochloride extract of untreated ML 308-225 vesicles as described for *dld-3* vesicles. Lactose uptake by reconstituted hydroxybutynoate-treated vesicles in the presence of 20 mM D-lactate (●); lactose uptake by reconstituted hydroxybutynoate-treated vesicles in the presence of 20 mM D-lactate and 10 mM sodium oxamate (▽); efflux of lactose from reconstituted hydroxybutynoate-treated vesicles preincubated for 10 min with [$^{14}$C]lactose and 20 mM D-lactate before addition of 10 mM sodium oxamate (▲). The rate of D-lactate oxidation for hydroxybutynoate-treated vesicles was essentially zero. After reconstitution, the rate of D-lactate oxidation was 200 ng atoms of oxygen per min per mg of protein.

done in the presence of 10 mM oxamate or 0.5 mM *p*-CMBS, D-lactate oxidation and D-lactate-dependent transport by *dld-3* vesicles is not diminished after removal of the oxamate or by washing the vesicles in dithiothreitol (data not shown). These observations indicate that oxamate and *p*-CMBS do not affect the ability of D-lactate dehydrogenase to bind to *dld-3* vesicles.

Addition of ML 308-225 guanidine extracts to ML 308–225 vesicles (i.e., to vesicles with normal amounts of D-lactate dehydrogenase) produces only small stimulation of the initial rate of lactose uptake and has no effect on the steady-state rate of accumulation. These vesicles behave like untreated ML 308-225 vesicles with respect to oxamate, i.e., oxamate inhibits the initial rate of uptake but not the steady-state rate of lactose accumulation, and does not cause efflux from preloaded vesicles. However, when the D-lactate dehydrogenase activity of ML 308-225 vesicles is inactivated with 2-hydroxy-3-butynoic acid (7), reconstitution with guanidine extract from untreated ML 308-225 vesicles restores high initial rates of lactose uptake (Fig. 6). Moreover, oxamate induces rapid efflux of lactose from reconstituted, hydroxybutynoate-treated ML 308-225 vesicles (Fig. 6).

## DISCUSSION

D-Lactate oxidation and D-lactate-dependent transport are restored to membrane vesicles prepared from a D-lactate dehydrogenase mutant by treatment with a guanidine extract from wild-type membranes. The results suggest that the D-lactate dehydrogenase extracted from wild-type vesicles binds to the mutant membranes in such a way as to transfer electrons from D-lactate to the electron transfer chain present in the vesicle membrane. Since transport is stimulated by D-lactate in these preparations, at least a portion of these electrons must enter the electron transfer chain at a point before the energy-coupling site for transport.

Initial rates of transport and steady-state levels of accumulation of proline, serine, glycine, and lysine approach an upper limit as increasing quantities of extract are added to *dld-3* membranes. In contrast, D-lactate oxidation increases linearly within the same range of extract concentrations. Moreover, with lactose and rubidium, the initial rates of uptake do not saturate at extract concentrations sufficient to saturate the other transport systems. These results suggest that the bound D-lactate dehydrogenase donates electrons to various transport systems with different degrees of efficiency. This interpretation is consistent with an earlier proposal (6) that transport involves a local interaction between the carriers and individual electron transfer chains.

In view of the profound differences in the effects of oxamate and *p*-CMBS on ML 308-225 and reconstituted *dld-3* membrane vesicles, the D-lactate-dependent transport systems in the latter membranes probably have not been restored to their native state. It appears instead that bound D-lactate dehydrogenase may be serving as an electron donor in a manner similar to ascorbate-phenazine methosulfate. A corollary of this interpretation is that D-lactate dehydrogenase in the native system does not merely funnel electrons into the energy-coupling site for transport. The fact that oxamate and *p*-CMBS cause efflux in reconstituted *dld-3* vesicles but not in ML 308-225 vesicles, suggests that D-lactate dehydrogenase in the native system interacts in some unknown way with the energy-coupling site for transport. Although the differential effects of oxamate and *p*-CMBS on the native and reconstituted systems cannot be fully explained, the results suggest the possibility of a control mechanism in the native system that allows the cell to "turn off" transport so as to maintain the steady-state rate of accumulation with little expenditure of energy.


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