Mechanisms of Active Transport in Isolated Membrane Vesicles

II. THE MECHANISM OF ENERGY COUPLING BETWEEN D-LACTIC DEHYDROGENASE AND β-GALACTOSIDE TRANSPORT IN MEMBRANE PREPARATIONS FROM ESCHERICHIA COLI

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SUMMARY

The results presented in this paper provide preliminary evidence for the concept that the "carriers" of the D-lactic dehydrogenase-coupled transport systems in isolated membrane vesicles from Escherichia coli may be electron transfer intermediates.

Initial rates of lactose transport and D-lactic dehydrogenase activity respond identically to temperature and both processes have the same activation energy of 8400 cal per mole. The steady state levels of lactose accumulation at a variety of temperatures represent equilibrium states in which there is a balance between influx and efflux. This balance can be easily influenced by raising or lowering the temperature. Temperature-induced efflux is a saturable process with an apparent affinity constant that is approximately 60 times higher than the affinity constant for influx determined under the same experimental conditions. The apparent maximum velocity of temperature-induced efflux, on the other hand, is the same as that of influx. Potassium cyanide also induces a saturable efflux phenomenon which has an apparent $K_m$ that is much higher than that of the influx process.

$p$-Chloromercuribenzoate inhibits D-lactic dehydrogenase-coupled transport of lactose, galactose, arabinose, glucuronate, glucose-6-P, proline, glutamic acid, serine, alanine, tyrosine, lysine, and tryptophan, and inhibition of each system by $p$-chloromercuribenzoate is reversed by dithiothreitol. Furthermore, $p$-chloromercuribenzoate inhibits temperature-induced efflux of intramembranal lactose, exchange of external lactose with $[^{14}C]$lactose in the intramembranal pool, and lactose efflux induced by 2,4-dinitrophenol. Inhibition of these experimental parameters and of D-lactic dehydrogenase by $p$-chloromercuribenzoate is reversed by dithiothreitol.

Reduction of the respiratory chain between D-lactic dehydrogenase and cytochrome $b_1$ is responsible for carrier-mediated efflux of lactose. Anaerobiosis, cyanide, and 2-heptyl-4-hydroxyquinoline-N-oxide, each of which inhibits electron transfer after cytochrome $b_1$, cause marked efflux. Amytal causes slow efflux, and oxamate and $p$-chloromercuri-

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The data presented in the first paper in this series (2) indicate that the site of energy coupling of D-lactic dehydrogenase to active transport lies between the primary dehydrogenase and cytochrome $b_1$, the first cytochrome in the respiratory chain of Escherichia coli. In addition, the possibility that the "carriers" may be electron transfer intermediates between D-lactic dehydrogenase and cytochrome $b_1$ was suggested.

The experiments presented in this paper provide further preliminary evidence for a possible electron transfer nature of the transport-specific components of the D-lactic dehydrogenase-coupled transport systems.

METHODS

Membrane Preparations—E. coli ML 308-2251 ($i^{-},z^{-},y^{+},a^{+}$) was grown on Medium A (3) containing 1% disodium succinate (hexahydrate); E. coli ML 301 ($i^{+},z^{+},y^{+},a^{+}$) on Medium A containing 0.5% gluconic acid or arabinose as indicated; E. coli ML 31 ($i^{+},z^{+},y^{-},a^{-}$) on Medium A containing 0.5% galactose; and E. coli GN-22 ($i^{-},z^{+},y^{+},a^{+}$; enzyme I-) on Medium 63 (4) containing 0.2% glucose-6-P. Membrane vesicles were prepared from these cells as described previously (5-7).

Transport Studies—Assays for lactose and amino acid uptake were carried out as reported previously (7-9). Glucuronate, arabinose, galactose, and glucose-6-P uptake studies were performed exactly as described for lactose and amino acid uptake with final concentrations of glucuronate, arabinose, galactose, and glucose-6-P of 0.1, 0.3, 0.2, and 0.02 mM, respectively.

1 These strains were obtained from Dr. T. H. Wilson.

2 This mutant was reisolated from a culture provided by Dr. L. Heppel by selecting a glucose-negative colony from a MacConkey plate (Difco Manual, Difco Laboratories, Detroit, 1953, p. 131).
Fig. 1. Effect of temperature on the time course of lactose uptake. Aliquots (25 μl) of membranes prepared from E. coli ML308-225 containing 0.158 mg of membrane protein were diluted to a final volume of 50 μl, containing, in final concentrations, 50 mM potassium phosphate (pH 6.6) and 10 mM magnesium sulfate. Lithium D-lactate and [1-14C]lactose (14.9 mCi per mmole) at final concentrations of 20 and 0.4 mM, respectively, were added to the reaction mixtures at 0° and they were immediately transferred to water baths at the temperatures shown. The incubations were carried out for the times indicated, the reactions were terminated, and the samples were assayed as described previously (8, 9). Each experimental point was corrected for a control sample obtained as described previously (5). Incubations were carried out at the following temperatures: 0° (○), 10° (▲), 18° (○), 25° (■), 35° (▲), 45° (▽), and 53° (□).

D-Lactic Dehydrogenase Assays—The chromatographic assay utilizing D-[14C]lactate has been reported (8).

Oxygen Uptake Measurements—Rates of d-lactate-dependent oxygen uptake were measured with a Clark oxygen electrode as described previously (2).

Materials—In addition to [1-14C]lactose and the [14C]amino acids used, the sources of which have been reported (8, 9), potassium D-[U-14C]glucuronate (25 mCi per mmole) and D-[U-14C]glucose-6-P (127 mCi per mmole) were obtained from Amersham-Searle, and D-[U-14C]galactose (10 mCi per mmole) and D-[U-14C]arabinose (25 mCi per mmole) were obtained from New England Nuclear.

All other materials used in these experiments were of reagent grade and were obtained from commercial sources.

RESULTS

Effect of Temperature on D-Lactic Dehydrogenase Activity and β-Galactoside Transport—Fig. 1 represents time courses of lactose uptake by ML 308-225 membrane vesicles at a variety of temperatures. As shown, initial rates of uptake increase with temperature up to 53°, whereas the steady state level of lactose accumulation at 15 min increases from 0°-18° and then decreases above 15°. At 53°, membranes take up lactose very rapidly for 15 sec and then lose radioactivity such that, by 1 min, approximately 50% of the radioactive lactose that had been accumulated in 15 sec is lost. After 1 min, loss of radioactivity continues, but at a much slower rate. These data are similar to studies carried out with whole cells (10).

The initial rate of D-lactic dehydrogenase activity increases very slightly as the temperature is raised from 0° to approximately 15°, and then increases more markedly above 15° (Fig. 2A). From 15° to approximately 50°, the reaction rate increases essentially linearly, and then decreases abruptly at temperatures exceeding 55°. In Fig. 2B, initial rates of lactose transport (15 SEC.) and steady state levels of lactose accumulation (15 MIN.) in the presence of D-lactate are plotted as a function of temperature. The steady state level of lactose accumulated in 15-min incubations exhibits a broad peak from 15-35° with a maximum at approximately 18°. The initial rate of lactose uptake (15 SEC) is optimal at 50-55°, and the initial rates of lactose trans-
FIG. 3 (left). Arrhenius plots (11) of the initial rates of lactose transport and $\delta$-lactic dehydrogenase ($D$-LDH) activities. The data shown were obtained from Fig. 2, A and B. TRANSPORT ($C$--$O$) corresponds to 15 SEC ($O$--$O$) in Fig. 2B, and $D$-LDH ($O$--$O$) to $D$-LDH ($O$--$O$) in Fig. 2A.

FIG. 4 (right). Effect of temperature shifts on the steady state intramembranal level of lactose accumulation. Reaction mixtures containing ML 308-225 membrane vesicles were prepared as described in the legend to Fig. 1. The samples were incubated at 45°C in the presence of lithium $\delta$-lactate and [1-14C]lactose (14.9 mCi per mmole) at final concentrations of 20 and 0.4 mM, respectively. Samples were assayed at the times shown by methods described previously (8, 9). At 3 min (indicated by arrow), samples were transferred to a water bath at 25°C, and assayed at the times shown. At 18 min (indicated by arrow), the remaining samples were transferred back to the 45°C water bath and assayed at the times shown. $C$--$O$, initial uptake at 45°C; $C$--$O$, uptake at 25°C after incubation at 45°C; $A$--$A$, uptake at 45°C after final shift to 45°C.

transport and $\delta$-lactic dehydrogenase activity have similar, if not identical, temperature profiles. Membranes incubated in the absence of $\delta$-lactate (20 SEC: NO ADD.) have almost negligible rates of uptake.

The similarity of the effect of temperature on both $\delta$-lactic dehydrogenase activity and the initial rate of lactose transport in the presence of $\delta$-lactate is further emphasized in Fig. 3. Clearly, the initial rates of transport and $\delta$-lactic dehydrogenase activity respond almost identically to temperature. Increments in the log10 of the velocities of both activities from approximately 20-45°C are identical, yielding an activation energy of 8400 cal per mole. Furthermore, there are discontinuities in both activities at 10-15°C and at 45-50°C. The small discrepancies in the temperatures at which the discontinuities occur (from 3-5°C) are within experimental error.

Data given in Fig. 4 show that the steady state levels of lactose accumulated at 25°C or 45°C represent equilibrium states which can be shifted by changing temperature. At 45°C, [14C]lactose is taken up very rapidly by the vesicles which achieve a steady state level of lactose accumulation of approximately 12 nmoles per mg of membrane protein (corresponding to an intramembranal concentration of about 5.5 mM) within about 3 min. When the temperature is lowered to 25°C, the membranes accumulate lactose to a steady state level of 31 to 34 nmoles per mg of membrane protein (an intramembranal concentration of 14 to 16 mM) approximately 5 min after the temperature shift. If the temperature is again raised to 45°C, the vesicles re-equilibrate at the same steady state level as that observed during the initial incubation at 45°C within 2 min.

The temperature dependence of efflux is shown in Fig. 5.

FIG. 5. Rate of lactose efflux as a function of temperature. Reaction mixtures containing ML 308-225 membrane vesicles were prepared as described in the legend to Fig. 1. The samples were incubated at 18°C in the presence of lithium $\delta$-lactate and [1-14C]lactose (14.9 mCi per mmole) at final concentrations of 20 and 1.0 mM, respectively. After 15 min, a number of control samples were assayed (60.1 nmoles per mg of membrane protein; average of five determinations), and the remaining samples were transferred to water baths at the temperatures given. Incubations were continued for 1 min, and the samples were assayed as described previously (8, 9). The differences between the control samples and the samples incubated at the temperatures given (expressed as nanomoles per mg of membrane protein) are presented as a function of temperature.

FIG. 6. Effect of external lactose concentration on the rates of lactose uptake at 45°C. A, reaction mixtures containing ML 308-225 membrane vesicles prepared as described in the legend to Fig. 1 were incubated at 45°C in the presence of lithium $\delta$-lactate at 20 mM final concentration and [1-14C]lactose (14.9 mCi per mmole) in the concentrations indicated. The reactions were terminated at the times shown and the samples were assayed as described previously (8, 9). 15 SEC ($O$--$O$), samples assayed at 15 sec; 30 SEC ($O$--$O$), samples assayed at 30 sec; 45 SEC ($A$--$A$), samples assayed at 45 sec. B, data from A plotted by the method of Lineweaver and Burk (12).

Membranes were first loaded with [14C]lactose by incubation at 18°C for 15 min in the presence of $\delta$-lactate. The reaction mixtures were then shifted to the temperatures shown, and loss of radioactivity in 1 min was measured. Initial rates of efflux are...
The rate of lactose efflux at 45°C also exhibits saturation kinetics when measured at 15, 30, and 45 sec as a function of external lactose concentration. In Fig. 6B, the data have been plotted by the method of Lineweaver and Burk (12). Each function yields a $K_m$ of 0.4 mM. With the data obtained from experiments carried out for 15 sec, a $V_{max}$ of 50 nmol per mg of membrane protein per min is obtained. This value is in good agreement with previous kinetic studies carried out at 25°C (9). The $V_{max}$ at 45°C is higher than that obtained at 25°C (approximately 20 nmol per mg of membrane protein per min at 25°C (9)).

The rate of lactose efflux at 45°C also exhibits saturation kinetics when studied as a function of intramembranous lactose concentration (Fig. 7A). Vesicles were first loaded with lactose by incubation at 20°C in the presence of $\beta$-lactate and various concentrations of [14C]lactose (1.9 mCi per mmole) at 20 and 0.4 mM final concentrations, respectively, after a 3-min preliminary incubation with the concentrations of PCMB shown.

Fig. 9 (right). Effect of dithiothreitol on PCMB inhibition of lactose transport. Three aliquots (0.5 ml) of ML 308-225 membranes containing 4.2 mg of membrane protein were diluted to a final volume of 1 ml containing, in final concentrations, 50 mM potassium phosphate (pH 6.6) and 10 mM magnesium sulfate. PCMB (8.3 × 10^{-4} M, final concentration) was added to two of the samples. After a 6-min incubation at room temperature (approximately 20°C), the samples were centrifuged in the cold for 15 min at approximately 20,000 × g. The supernatants were discarded, and the pellets were washed twice in the following solutions: control (O-O), sample incubated in the absence of PCMB, washed in 0.1 M potassium phosphate (pH 6.6); PCMB-DTT WASHED (Δ-Δ), sample incubated with PCMB, washed in 0.1 M potassium phosphate (pH 6.6) containing 1 mM dithiothreitol; PCMB (○-○), sample incubated with PCMB, washed in 0.1 M potassium phosphate (pH 6.6). After the second wash, each pellet was resuspended to 0.5 ml in 0.1 M potassium phosphate (pH 6.0) and 25 μl aliquots were assayed at 20°C for the times given. The reactions were carried out as described previously (8, 9) in the presence of lithium $\beta$-lactate and [1-14C]-lactose (14.9 mCi per mmole) at final concentrations of 20 and 0.4 mM, respectively. The sample treated with PCMB and washed in the absence of PCMB was also assayed in the presence of 1 mM dithiothreitol (final concentration) (PCMB+DTT [1 mM], □-□).

Addition of energy poisons to either membrane vesicles (8) or...
whole cells (10, 13, 14) which have been loaded first with lactose causes rapid efflux. In the experiment described in Fig. 7B, membranes were first loaded with "[\textsuperscript{\textdagger}]lactose at 25\textdegree\), and potassium cyanide was then added. Rapid efflux is induced (see Fig. 13 in addition to Fig. 7B), and, furthermore, the rate of efflux exhibits saturation kinetics when plotted as a function of intracellular lactose concentration. The data yield a reciprocal plot (see inset) from which a $K_m$ of 25 nm is obtained. The $V_{max}$ of cyanide-induced efflux at 25\textdegree\ is 20 nmoles per mg of membrane protein per min.

**Effect of Sulphydryl Reagents on Carrier-mediated Transport—**

The initial rate of lactose transport by ML 308-225 membrane vesicles in the presence of n-lactate is sensitive to PCMB\textsuperscript{2} (Fig. 8), and decreases markedly with PCMB concentrations up to approximately 0.05 mM. NEM is also an effective inhibitor of galactoside transport by membrane vesicles (9) and PCMB inhibits β-galactoside uptake into whole cells (15).

The inhibitory effect of PCMB on n-lactate-dependent concentrative uptake of lactate is reversed by dithiothreitol. As described in Fig. 9, membranes were treated with PCMB and then washed with solutions to which dithiothreitol either had or had not been added. The time course of lactose uptake by membranes which had been treated with PCMB and subsequently with dithiothreitol is insignificantly different from the control preparation. On the other hand, the sample which had been treated with PCMB but not dithiothreitol shows marked inhibition of the initial rate of uptake and significant inhibition of the steady state level of lactose accumulation. On addition of dithiothreitol, the rate and extent of lactose uptake by this preparation are almost doubled.

The inhibitory effect of PCMB on β-galactoside transport is general for all n-lactic dehydrogenase-coupled transport systems, and, in each case, PCMB inhibition is virtually completely reversed by treating vesicles with dithiothreitol (Table I). Initial rates of transport of lactose, galactose, arabinose, glucuronate, glucose-6-P, proline, glutamic acid, serine, alanine, lysine, tyrosine, and tryptophan are inhibited by PCMB to varying extents. As shown in the last column of Table I, after membrane preparations are washed in phosphate buffer containing dithiothreitol, the rate and extent of lactose uptake by this preparation are almost doubled.

The data presented in Table II represent measurements of n-lactate oxidation by the same membrane preparations used for most of the transport studies presented in Table I. PCMB produces approximately 70% inhibition of n-lactate-induced respiration; this inhibition is completely reversed by subsequent treatment with dithiothreitol. Moreover, when dithiothreitol is added to membranes which had been treated with PCMB but not

### TABLE I

**Inhibition of sugar and amino acid transport by PCMB and reversal by dithiothreitol**

Membranes prepared from *E. coli* ML 308-225 were assayed for the uptake of lactose, proline, glutamic acid, serine, alanine, tyrosine, lysine, and tryptophan. For glucuronate and arabinose uptake, membranes were prepared from *E. coli* ML 30 grown on glucuronic acid or arabinose, respectively, as described under "Methods." In order to avoid complications due to galactose transport via the β-galactoside transport system, membranes prepared from *E. coli* ML 3 grown on galactose were then added. Rapid efflux is induced (see Fig. 7B), and, furthermore, the rate of efflux exhibits saturation kinetics when plotted as a function of intracellular lactose concentration. The data yield a reciprocal plot (see inset) from which a $K_m$ of 25 nm is obtained. The $V_{max}$ of cyanide-induced efflux at 25° is 20 nmoles per mg of membrane protein per min.

**TABLE II**

**Inhibition of n-lactate oxidation by ML 308-225 membranes and reversal by dithiothreitol**

The samples of membrane vesicles prepared from *E. coli* ML 308-225 used in Table I for the transport of lactose, proline, glutamic acid, serine, alanine, tyrosine, lysine, and tryptophan were assayed for n-lactate oxidation with the oxygen electrode as described previously (2).

| Sample and treatment | n-Lactate oxidation |
|----------------------|---------------------|
| Sample and treatment | mg atom O/min. mg membrane protein | % |

| Sample and treatment | Control |
|----------------------|---------|
| I. Control........... | 183     |
| II. 8.3 X 10\textsuperscript{-5} m PCMB | 57.7 |
| Washed with KPO<sub>4</sub> | 118.5 |
| III. +1 mM DTT\textsuperscript{a} | 217   |
| Washed with KPO<sub>4</sub> + DTT | 115   |
| III +1 nm DTT | 217   |

\textsuperscript{a} DTT, dithiothreitol.

The abbreviations used are: PCMB, p-chloromercuribenzoate; NEM, N-ethylmaleimide.

F. J. Lombardi and H. R. Kaback, manuscripts in preparation.
Effect of diithiothreitol on PCMB inhibition of temperature-induced lactose efflux. Reaction mixtures containing ML 308-225 membrane vesicles were prepared as described in the legend to Fig. 1. The samples were incubated at 20° in the presence of lithium β-lactate and [1-14C]lactose (14.9 mCi per mmole) at final concentrations of 20 and 0.4 mM, respectively. After 10 min, 8.3 x 10−5 m PCMB (final concentration) was added to the samples labeled PCMB (●—●) and PCMB−DTT (△—△), and the incubations were continued for another 5 min at 20°. At this time (t = 0 time on the figure), 1 mM (final concentration) dithiothreitol was added to one set of samples to which PCMB had been added (PCMB−DTT) (△—△), and the reactions were transferred to a water bath at 45°.

CONTROL (●—●), neither PCMB nor dithiothreitol added to the samples.

Effect of diithiothreitol on PCMB inhibition of lactose exchange. Reaction mixtures containing ML 308-225 membrane vesicles were prepared as described in the legend to Fig. 1. The samples were incubated at 20° in the presence of lithium β-lactate and [1-14C]lactose (14.9 mCi per mmole) at final concentrations of 20 and 0.4 mM, respectively. After 10 min, 8.3 x 10−5 m PCMB (final concentration) was added to the samples labeled PCMB (●—●) and PCMB−DTT (○—○), and the incubations were continued for another 5 min at 20°. Nonradioactive lactose was then added to all of the samples at a final concentration of 2 mM (Arrow 1), and the incubations were continued at 20° for the times shown. After 5 min, 1 mM dithiothreitol (final concentration) was added to the appropriate samples containing PCMB (Arrow 2). Reactions were terminated and samples were assayed as described previously (8, 9). "L-ACT" (○—○), [1-14C]lactose added at zero time (Arrow 1) in the absence of either PCMB or dithiothreitol. The data are presented as a percentage of the control samples after prior loading for 15 min.

dithiothreitol, there is complete restoration of activity. Each transport system studied in Table I and β-lactate oxidation is also sensitive to NEM; however, with this sulfhydryl reagent, the effects are not reversed with dithiothreitol.

Addition of PCMB to membrane vesicles first loaded at 20° causes marked inhibition of lactose efflux at 45° (Fig. 10). When dithiothreitol is added just before the temperature shift, however, the rate of efflux is indistinguishable from the control.

PCMB also inhibits exchange of external lactose with [14C]lactose present in the intramembranal pool (Fig. 11) and lactose efflux induced by the addition of 2,4-dinitrophenol to previously loaded membrane vesicles (Fig. 12). Moreover, PCMB inhibition of both of these phenomena is reversed by addition of dithiothreitol (Figs. 11 and 12).

β-Lactate-stimulated concentrative uptake of lactose and the loss of radioactive lactose evoked by raising the temperature or adding [14C]lactose or 2,4-dinitrophenol are also inhibited by PCMB and PCMB−DTT (Fig. 12). Moreover, PCMB inhibition of both of these phenomena is reversed by addition of dithiothreitol (Figs. 11 and 12).
tion mixtures, and, even at the lowest concentration, each amino acid was present at a saturating concentration for its transport system (8). It can be seen that, at either temperature, the presence of amino acids causes only mild inhibition of either the rate or extent of lactose uptake.

**DISCUSSION**

The experimental findings presented in this and the previous paper are consistent with the conceptual working model presented in Fig. 16. Detailed experiments with other n-lactic dehydrogenase-coupled transport systems which will be published at a later date show that each system behaves in a manner qualitatively similar to that shown here for the β-galactoside transport system. In the mechanism presented, the carriers (in this specific case, the M protein (17-20)) are depicted as electron transfer intermediates which undergo reversible oxidation-reduction. As shown, in the oxidized state, the carrier has a high affinity site for ligand which it binds on the exterior surface of the membrane. Electrons coming ultimately from n-lactate through one or possibly more flavoproteins reduce a critical disulfide in the membrane. This conformational change, the affinity of the carrier for its ligand is markedly decreased and ligand is released on the interior surface of the carrier molecule resulting in a conformational change. With this conformational change, the affinity of the carrier for its ligand is markedly decreased and ligand is released on the interior surface of the membrane. The reduced “sulfhydryl” form of the carrier is oxidized by cytochrome b₁ and electrons then flow through the remainder of the cytochrome chain to reduce molecular oxygen to water. The reduced form of the carrier can also “vibrate” and catalyze a low affinity, carrier-mediated, non-energy-dependent transport of ligand across the membrane.

Although no direct evidence has been presented which shows unequivocally that the carriers are electron transfer intermediates or that they are the only sulfhydryl-containing components of the respiratory chain between n-lactic dehydrogenase and cytochrome b₁, this formulation is consistent with all of the experimental observations presented and is the simplest conception possible.

The concept that the carriers may be electron transfer intermediates is supported primarily by experiments in which the

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**Fig. 14.** Effect of various electron transport inhibitors on the time course of lactose uptake. Reaction mixtures containing ML 208-225 membranes were prepared as described in the legend to Fig. 1. In addition, where indicated, the reaction mixtures contained each of the following amino acids (A.A.) in the final individual concentrations given: proline, glutamic acid, aspartic acid, lysine, serine, glycine, alanine, threonine, tryptophan, leucine, isoleucine, valine, histidine, tyrosine, phenylalanine, and cysteine. The control samples (NO ADD., O—O) contained no amino acids. At the times shown, the reactions were terminated and the samples were assayed by methods described previously (8, 9). A, lactose uptake at 20°; B, lactose uptake at 45°.

**Fig. 15.** Effect of amino acids on the time course of lactose uptake. Reaction mixtures containing ML 208-225 membranes were prepared as described in the legend to Fig. 1. In addition, where indicated, the reaction mixtures contained each of the following amino acids (A.A.) in the final individual concentrations given: proline, glutamic acid, aspartic acid, lysine, serine, glycine, alanine, threonine, tryptophan, leucine, isoleucine, valine, histidine, tyrosine, phenylalanine, and cysteine. The control samples (NO ADD., O—O) contained no amino acids. At the times shown, the reactions were terminated and the samples were assayed by methods described previously (8, 9). A, lactose uptake at 20°; B, lactose uptake at 45°.
Mechanism of Energy Coupling for β-Galactoside Transport

Fig. 16. Conceptual working model for β-lactic dehydrogenase-coupled transport systems. D-LAC, β-lactate; PYR, pyruvate; fp, flavoprotein; Cyto b₁, cytochrome b₁; OX, oxidized; RED, reduced. OUT signifies the outside surface of the membrane; IN signifies the inside surface. The hemispheres located between fp and cyto b₁ represent the "carrier": (▲), a high affinity binding site and (▼), a low affinity binding site. The remainder of the cytochrome chain from cytochrome b₁ to oxygen has been omitted.

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