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Mechanism of Energy Coupling For Transport of D-Ribose in *Escherichia coli*

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In *Escherichia coli* ML 308-225, D-ribose is transported into the cell by a constitutive active transport system of high activity. The activity of this transport system is severely reduced in cells subjected to osmotic shock, and the system is not present in membrane vesicles. The mechanism by which metabolic energy is coupled to transport of ribose was investigated. Substrates which generate adenosine 5'-triphosphate primarily through oxidative phosphorylation are poor energy sources for ribose uptake in DL-54, a mutant of ML 308-225 which lacks activity for the membrane-bound Ca^{2+}, Mg^{2+}-dependent adenosine triphosphatase required for oxidative phosphorylation. Arsenate severely inhibits ribose uptake, whereas, under the same conditions, uptake of L-proline is relatively insensitive to arsenate. Anaerobiosis does not significantly inhibit ribose uptake in ML 308-225 or DL-54 when glucose is the energy source. A significant amount of ribose uptake is resistant to uncouplers of oxidative phosphorylation such as 2,4-dinitrophenol. These results indicate that the phosphate bond energy of adenosine 5'-triphosphate, rather than an energized membrane state, couples energy to ribose transport in ML 308-225.

In the active transport of substrates into cells of *Escherichia coli*, metabolic energy must be coupled to transport to allow accumulation of the substrate against a concentration gradient. It has been proposed that an energized membrane state, generated by respiration and from adenosine 5'-triphosphate (ATP), couples energy to active transport in whole cells of *E. coli* (20, 26, 28, 29). Recently, Berger (4) and Berger and Heppel (E. A. Berger and L. A. Heppel, J. Biol. Chem., in press) have found evidence indicating that both the energized membrane state and ATP itself can couple energy to the active transport of amino acids. They found that amino acid transport systems in *E. coli* can be classified into two groups based on the mechanism of energy coupling. The two groups also differ in their sensitivity to the osmotic shock treatment of Neu and Heppel (25) and in their activity in membrane vesicles.

In the case of one group of transport systems, which includes those for L-proline and L-serine, energy is coupled to transport through the energized membrane state. These systems are relatively insensitive to osmotic shock and are present in membrane vesicles. The other group of systems, which includes those for L-glutamine and L-ornithine, require the phosphate bond energy of ATP but not the energized membrane state for energy coupling. The activity of these systems is severely reduced by osmotic shock and is usually not present in membrane vesicles. In this paper, I report the results of an investigation into the mechanism of energy coupling for a shock-sensitive active transport system for D-ribose in the *E. coli* strain ML 308-225.

Detailed investigation of ribose transport in *E. coli* has only recently begun. In 1959, Eggleston and Krebs (10) obtained initial evidence for an inducible ribose transport system in *E. coli* 8571. This evidence indicated that whole cells of this strain could not ferment ribose unless they had been grown in the presence of ribose, even though enzymes for the fermentation of ribose were produced constitutively. David and Wiesmeyer (9) have found that both a constitutive and an inducible ribose transport system exist in *E. coli* X289. Both permeases are active transport systems based on their ability to accumulate ribose against a concentration gradient, and on the inhibition of this accumulation by sodium azide. David and Wiesmeyer found that ribokinase activity is not required for accumulation of ribose in *E. coli* X289. Ribose transport has also been investigated in *E. coli* B/r (1) and in *E. coli* K-12 (3). The results of these studies also indicate that accumulation

of ribose in these strains does not require ribokinase activity. Recently, interest in ribose transport has been stimulated by the isolation of ribose binding proteins from osmotic shock fluid of *Salmonella typhimurium* (2) and *E. coli* (14, 31). These binding proteins may be components necessary for ribose transport in *S. typhimurium* and *E. coli*.

The highly specific constitutive ribose transport system of ML 308-225 provides an ideal system for the study of the mechanism of energy coupling for a shock-sensitive active transport system for a sugar. The ability of various substrates such as D-glucose and D-lactate to provide energy for ribose transport in ML 308-225, and in a mutant of this strain, DL-54, which lacks activity for the Ca^{2+}, Mg^{2+}-adenosine triphosphatase (ATPase), was determined. The Ca^{2+}, Mg^{2+}-ATPase is believed to be necessary for oxidative phosphorylation, catalyzing generation of ATP from the energized membrane state produced by respiration (7, 19, 28, 29), and the reverse reaction (8, 13, 18, 28). The effect of inhibitors of active transport on ribose transport in ML 308-225 and DL-54 was also investigated. The results of these studies indicate that energy is coupled to ribose transport through the phosphate bond energy of ATP and not through the energized membrane state.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* strain ML 308-225 and its mutant DL-54 (29) were obtained from R. D. Simoni.

ML 308-225 and DL-54 were grown in a minimal phosphate-buffered medium, Medium A (30), supplemented with a carbon source. Glycerol was added to Medium A at a concentration of 10 g/liter and D-ribose was added at a concentration of 5 g/liter. For most experiments described in this paper, bacteria were inoculated into glycerol minimal medium to an optical density of 0.300 as measured at 600 nm in a Gilford model 240 spectrophotometer. The culture was incubated at 37 C in a New Brunswick gyrotory shaker until it had grown to an optical density at 600 nm of 0.800, corresponding to a cell density of approximately 7×10^8 cells per ml. Cells were harvested by centrifugation and washed twice with Medium A at room temperature. Washed cells were suspended in Medium A to a density of 1 g of cells (wet weight) to 20 ml of Medium A.

Starvation of cells. Cells of ML 308-225 and DL-54 were starved by the procedure of Berger (4). Bacterial cells, at a density of 1 g of cells per 200 ml of medium, were incubated in Medium A containing 5 mM 2,4-dinitrophenol (DNP) at 37 C in a gyrotory shaker. ML 308-225 cells were incubated with DNP for 10 h, and DL-54 cells were incubated for 1 h. After incubation with DNP, the cells were harvested by centrifugation and washed three times with 30 ml of Medium A at room temperature. The washed cells were then suspended in Medium A to a density of 1 g of cells per 20 ml of medium.

In certain cases, cells were starved by vigorous aeration (Berger and Heppel, in press) instead of by incubation with DNP. A cell suspension in Medium A without a carbon source with a density of 1 g of cells per 80 ml of medium was vigorously aerated at 37 C on a gyrotory shaker for 3 h. The cell suspension was then refrigerated at 3 C for 16 h and aerated again for 3 h at 37 C. The starved cells were then washed twice with Medium A and suspended to a density of 1 g of cells per 20 ml of Medium A.

Transport assays. Cells were added to a 50-ml flask containing Medium A, 80 μg of chloramphenicol per ml, and an energy source. The concentrations of the energy sources in the assay mixture were D-glucose or D-lactate (0.02 M), phenazine methosulfate (PMS; 20 μg/ml), and ascorbic acid (0.02 M). The reaction mixture containing the cells was incubated at 37 C for 5 min. After the incubation period, the assay mixture was allowed to cool to room temperature, and then the transport assay was initiated by the addition of a radioactive substrate. The final assay volume was 0.5 ml, and the amount of cells in the assay was equivalent to 150 μg of cell protein per ml. D-[1-^{14}C]ribose (10 μCi/μmol) was added to the assay mixture to a concentration of 20 μM, and L-[U-^{14}C]proline or L-[U-^{14}C]glutamine (20 to 25 μCi/μmol) was added to the assay mixture to a concentration of 10 μM. A 200-μliter sample of the assay mixture was removed from the flask 15 and 30 s after addition of the radioactive substrate. The 200-μliter sample was filtered on a 25-mm nitrocellulose filter (0.45-μm pore size, Matheson-Higgins) and washed with 10 ml of a solution containing 0.01 M Tris-hydrochloride (pH 7.3), 0.15 M NaCl, and 0.5 mM $MgCl_2$ at room temperature. The filters were dried and then placed in 7.5 ml of a scintillation fluid prepared as described previously (4). Radioactivity on the filters was measured in a Beckman LS-100C scintillation counter. The uptake at 15 s in the assays was used to calculate the initial rate of uptake. Uptake activity was expressed as nmoles of substrate accumulated per minute per milligram of cell protein. As a control, ribose uptake was measured at 4 C in the presence of 0.1 mM carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP). In the uptake assays less than 10% of the radioactive substrate was accumulated in the bacterial cells for the 30-s period during which uptake was measured.

To determine the effect of an inhibitor on the transport of a substrate, cells were first incubated with an inhibitor in Medium A containing chloramphenicol for 5 min at 37 C. Then an energy source was added to the assay mixture, and the mixture was incubated for 5 min more at 37 C. The cells were then assayed for transport as described above. Measurement of uptake rates in the presence of arsenate was performed with cells washed three times in 25 mM Tris-hydrochloride, pH 7.3, at room temperature, and in the uptake reaction mixture Medium A was replaced by Medium B (0.1 M Tris-hydrochloride, 15

mM KCl, 7.5 mM $(NH_4)_2SO_4$, and 0.4 mM $MgSO_4$, pH 7.3).

Anaerobic transport assays. Nitrogen was bubbled through 0.5 ml of a bacterial cell suspension containing 0.02 M glucose or containing no carbon source for 5 min at 37 C. An assay mixture containing Medium A, 80 μg of chloramphenicol per ml, and radioactive substrate was also equilibrated with N_2 for 5 min at 23 C. To start the uptake assay, 25 μliters of the N_2-equilibrated bacterial cell suspension was delivered from a syringe to the assay mixture. Nitrogen was bubbled through the mixture during the assay. The final volume of the assay mixture was 0.5 ml, and the cell protein concentration was 250 μg/ml. Samples of the assay mixture were removed, and the radioactivity of the samples was counted in the same manner as for the aerobic transport assays described above.

Osmotic shock of cells. Osmotic shock of ML 308-225 cells was performed using a modification of the procedure of Neu and Heppel (25). ML 308-225 cells in the exponential phase of growth were washed three times with 0.01 M Tris-hydrochloride, pH 7.3, containing 0.03 M NaCl, at 4 C. Washed cells were harvested by centrifugation and suspended in room temperature 0.033 M Tris-hydrochloride, pH 7.3, to a density of 1 g of cells to 40 ml of Tris-hydrochloride. An equal volume of 0.033 M Tris-hydrochloride buffer containing 40% sucrose and 0.2 mM ethylenediaminetetraacetic acid was added to the cell suspension, and the cell suspension was swirled gently for 10 min. The cell suspension was then centrifuged at 4 C. The cell pellet obtained was rapidly suspended in 0.5 mM $MgCl_2$ at 4 C to a cell density of 1 g of cells per 80 ml. This suspension was then swirled in ice for 5 min. The shocked cells were then harvested by centrifugation and suspended in Medium A to a density of 1 g of cells per 20 ml.

To prepare concentrated osmotic shock fluid, stationary phase cells of ML 308-225 were osmotically shocked. The procedure followed was the same as that described above, except that washed cells were suspended in 20 volumes of 0.033 M Tris-hydrochloride, pH 7.3, and then an equal volume of 0.033 M Tris-hydrochloride containing 40% sucrose and 4.0 mM ethylenediaminetetraacetic acid was added to the cell suspension. Also in the final step of the shock procedure the cells were rapidly suspended in 40 volumes of distilled water at 4 C. The shocked cells were harvested by centrifugation at 4 C, and the supernatant obtained, the shock fluid, was concentrated in an Amicon model 401 ultrafiltration cell with a UM-10 membrane. The concentrated shock fluid was dialyzed against 0.03 M sodium phosphate buffer, pH 7.0, containing 10 mM NaCl.

Binding assays. Ribose binding activity in concentrated shock fluid from ML 308-225 cells was measured by equilibrium dialysis by using the procedure of Furlong et al. (12). Plexiglas cells consisting of two chambers separated by dialysis tubing were used for the binding assays. One chamber contained 100 μliters of concentrated shock fluid, and the other chamber contained 100 μliters of D-[5-^{3}H]ribose (58 μCi/μmol) in 0.03 M sodium phosphate buffer, pH 7.0, containing 10 mM NaCl and a trace of chloroform. The Plexiglas cell was rotated 16 h at 4 C. Solution (50 μliters) from each chamber of a cell was placed in 5 ml of Bray scintillation fluid (6), and the radioactivity of the solution was measured in a Beckman scintillation counter.

Protein assays. Protein concentrations were determined by the method of Lowry et al. (23).

Chemicals. D-[1-^{14}C]ribose, L-[U-^{14}C]proline, and L-[U-^{14}C]glutamine were purchased from New England Nuclear Corp. D-[5-^{3}H]ribose was obtained from Amersham/Searle Co. D-Ribose and DNP were obtained from Sigma Chemical Company.

RESULTS

Specificity and sensitivity to osmotic shock of the ribose transport system. ML 308-225 has a constitutive ribose transport system of high activity. Unlike other *E. coli* strains (9, 10), ML 308-225 does not have an inducible ribose permease. The ribose uptake activity of ML 308-225 cells grown on ribose was not significantly greater than the activity of cells grown on glycerol (Table 1). The ribose transport system in ML 308-225 is of high specificity with an apparent K_m of 2.3 μM and a V_{max} value of 74.1 nmol per min per mg of cell protein, as calculated from the plot of 1/V versus 1/S in Fig. 1. The high specificity of the system is also indicated by the fact that neither D-xylose, 2-deoxy-D-ribose, nor D-glucose can serve as a substrate for the ribose permease based on the inability of these compounds to inhibit ribose transport in ML 308-225 (Table 2).

The sensitivity of ribose transport in ML 308-225 to osmotic shock is indicated by the data in Table 3. Cells of ML 308-225 subjected to osmotic shock retained only 7% of the ribose uptake activity of nonshocked cells. Proline and serine transport are both relatively insensitive to osmotic shock, and thus, osmotically shocked

TABLE 1. *Lack of induction of the ribose transport system in ML 308-225*

Growth medium	Initial rate of uptake[a]	
	Ribose (4 μM)	Ribose (20 μM)
Glycerol (1%)	39.9	46.2
Ribose (0.5%)	32.7	59.8

[a] Uptake assays were performed with cells of ML 308-225 harvested in the exponential phase of growth. Ribose uptake was assayed in the presence of glucose as described. Uptake activity is expressed as nmoles of substrate accumulated per minute per milligram of cell protein.

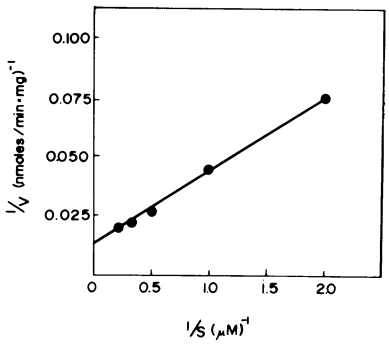

FIG. 1. *Double-reciprocal plot (22) of ribose uptake (V) as a function of ribose concentration (S) in ML 308-225. Uptake of ribose was measured in cells of ML 308-225 harvested in the exponential phase of growth.*

TABLE 2. *Lack of inhibition of ribose transport by other sugars in ML 308-225*

Competitor	Initial rate of ribose uptake[a]
None	22.8
D-Xylose	23.5
2-Deoxy-D-ribose	24.2
Glucose	20.8

[a] Ribose concentration was 1 μM in the uptake assays, and competitors were present in the assays at a concentration of 0.01 M. Uptake rates were measured in assay mixtures containing 0.02 M glycerol. Competitors were added to the assay reaction mixture first, and then radioactive ribose was immediately added to initiate the assay. Uptake activity is expressed the same as in Table 1.

TABLE 3. *Effect of osmotic shock on ribose transport in ML 308-225*

Permease	Initial rate of uptake[a]	
	Nonshocked	Shocked
Ribose	43.6	2.9
Proline	10.4	5.6
Serine	16.8	9.9

[a] Log-phase cells of ML 308-225 were subjected to osmotic shock as described. Uptake was measured in shocked and nonshocked cells in assay mixtures containing glucose. Uptake activity is expressed the same as in Table 1.

cells of ML 308-225 retained 54% of the proline uptake and 59% of the serine uptake of nonshocked cells.

Concentrated shock fluid obtained from ML 308-225 cells contained ribose binding activity. From equilibrium dialysis assays, it was determined that the shock fluid contained 5.8 U of ribose binding protein per mg of protein, a unit being equal to 1 nmol of ribose bound. The initial rate of ribose uptake in vesicles prepared by the method of Kaback (16) was extremely low, 1.7 nmol per min per mg of vesicle protein, compared to the uptake rate in whole cells. The ribose transport system therefore can clearly be classified as one of the shock-sensitive transport systems.

Energy sources for ribose transport in ML 308-225 and DL-54. The mechanism of energy coupling for ribose transport can be investigated by determining the ability of various substrates to provide energy for transport in ML 308-225 and a mutant of this strain, DL-54, which lacks membrane bound Ca^{2+}, Mg^{2+}-ATPase activity. Experiments were performed with starved cells in which the endogenous sources of energy were very low. Thus, energy for transport was obtained from the metabolism of the exogenously supplied substrate. The starvation procedure did not adversely affect ribose uptake, since incubation of starved cells with the energy sources indicated restored ribose uptake in the cells to the level in unstarved cells (Table 4).

In starved cells of ML 308-225, glucose and lactate were equally effective energy sources for ribose transport (Table 4). PMS plus ascorbate-driven ribose uptake was 33% of glucose-driven uptake, indicating that PMS plus ascorbate was a less effective energy source for ribose transport in ML 308-225. When glucose was the energy

TABLE 4. *Energy sources for ribose transport in ML 308-225 and DL-54*

Strain	Initial rate of ribose uptake[a]			
	None[b]	Glucose	Lactate	PMS plus ascorbate
ML 308-225				
Unstarved	43.2	46.6	49.7	18.0
Starved	7.4	55.8	56.0	18.3
DL-54				
Unstarved	3.6	40.6	18.7	1.7
Starved	2.2[c]	49.0	14.2	1.8

[a] Uptake assays were performed as described. ML 308-225 and DL-54 cells were starved by incubation with DNP. Uptake activity is expressed the same as in Table 1.

[b] Energy sources.

[c] Corresponding values for proline uptake in starved cells of DL-54 with different energy sources are: none, 0.15; glucose, 5.5; PMS plus ascorbate, 4.2. This result indicates that PMS plus ascorbate is an energy source for proline uptake in DL-54. Values for PMS plus ascorbate-driven proline uptake in DL-54 vary and can be as low as 50% of glucose-driven proline uptake.

source, ribose transport activity in DL-54 was not significantly different from the activity in ML 308-225. Lactate-driven ribose uptake in DL-54 was only 29% of the glucose-driven uptake of this strain. When PMS plus ascorbate was the energy source, ribose uptake was the same as the uptake measured when no exogenous energy source was supplied to the cells. Thus, PMS plus ascorbate can not serve as an energy source for ribose uptake in DL-54.

Arsenate inhibition of ribose transport. In DNP-starved cells of ML 308-225, 0.04 mM arsenate reduced both lactate- and glucose-driven ribose uptake approximately 90% or greater (Table 5). Lactate-driven proline uptake in ML 308-225 by comparison was only inhibited approximately 30%, and glucose-driven proline uptake was not inhibited by 1 mM arsenate. The effect of arsenate on ribose and proline transport in aeration-starved and unstarved cells of ML 308-225 was the same as the effect in DNP-starved cells. In DL-54, lactate- and glucose-driven ribose uptake were also reduced greater than 90% by arsenate in DNP-starved cells (Table 5). Ribose transport in aeration-starved and unstarved cells of DL-54 was also inhibited at least 90% by arsenate. Arsenate severely reduces the ATP concentration in ML 308-225 (20). Thus, the inhibition of ribose uptake by arsenate suggests that ATP is required for ribose transport.

Inhibition of ribose transport by uncouplers of oxidative phosphorylation. Uncouplers of oxidative phosphorylation, such as DNP and FCCP, dissipate the energized membrane state and prevent formation of ATP from respiration (24). Thus, transport systems which require an energized membrane state to couple metabolic energy to transport, such as that for proline, should be severely inhibited by uncouplers. Transport systems such as that for glutamine (4), which require the phosphate bond energy of ATP for energy coupling, should be inhibited by uncouplers when the energy source for transport can generate ATP only through oxidative phosphorylation.

The effect of FCCP and DNP on ribose uptake was similar to the effect of these uncouplers on glutamine transport. In DL-54, lactate-driven ribose uptake was not inhibited by DNP, and 40% of glucose-driven ribose uptake was not inhibited by DNP (Fig. 2A). Approximately 50% of glucose-driven glutamine uptake was also resistant to 1.5 mM DNP (Fig. 2B). Both lactate- and glucose-driven ribose uptake in ML 308-225 were severely inhibited by DNP (Fig. 3). As will be explained below, the lack of a DNP-resistant component of glucose-driven ribose transport in ML 308-225 may indicate that DNP has some secondary effect on the production of glycolytic ATP in the strain.

TABLE 5. *Inhibition of ribose transport by arsenate*

Strain	Energy source	Arsenate concn (mM)	Initial rate of uptake[a]	
			Ribose	Proline
ML 308-225	Lactate	0	51.7	9.2
		0.04	6.7	ND
		1.0	ND	6.4
	Glucose	0	55.0	4.4
		0.04	4.1	ND
		1.0	ND	5.8
DL-54	Lactate	0	13.2	ND
		0.2	1.2	ND
	Glucose	0	25.7	ND
		0.2	1.5	ND

[a] Ribose and proline uptake were measured in cells starved by incubation with DNP. Uptake activity was measured with and without the presence of arsenate in the assay mixture as described and is expressed the same as in Table 1. ND, Not done.

Significant ribose uptake can occur in the presence of FCCP in both ML 308-225 and DL-54. In ML 308-225, at least 40% of ribose transport activity remained resistant to inhibition by up to 10 μM FCCP when either glucose or lactate was the energy souce (Fig. 4A). In DL-54, lactate-driven ribose transport was not significantly inhibited, and 33% of glucose-driven ribose transport was not inhibited by 10 μM FCCP (Fig. 4B). The effects of 10 μM FCCP on proline and glutamine transport in ML 308-225 and DL-54 were similar to those found by Berger (4; Table 6). Proline transport was eradicated by FCCP in both strains, whereas 62% of glucose-driven glutamine transport was resistant to FCCP in DL-54. Lactate-driven glutamine uptake in DL-54 was extremely low since oxidative phosphorylation does not occur in the strain, and thus the effect of FCCP on this uptake was not readily interpretable. Since a substantial amount of ribose uptake occurs in the presence of FCCP and DNP, this suggests that ribose transport does not require an energized membrane state.

Effect of anaerobiosis on ribose transport. When glucose was the energy source, neither ribose nor proline uptake in ML 308-225 was reduced by anaerobiosis (Table 7). Anaerobiosis inhibited glucose-driven ribose uptake 20%, but

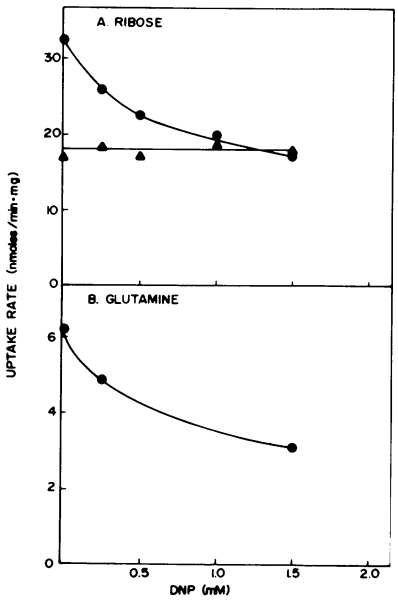

FIG. 2. *Effect of DNP on ribose and glutamine uptake in DL-54. Ribose and glutamine uptake were measured with and without the presence of DNP in the assay mixture as described. Cells were starved by incubation with DNP. Symbols: ▲, uptake with lactate as the energy source; ●, uptake with glucose as the energy source.*

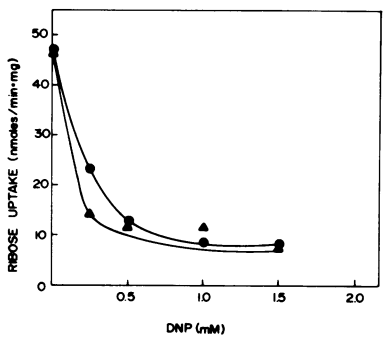

FIG. 3. *Effect of DNP on ribose uptake in ML 308-225. Ribose uptake was measured as described in Fig. 2. Symbols: ▲, uptake with lactate as the energy source; ●, uptake with glucose as the energy source.*

it inhibited proline uptake 86% in DL-54. Under anaerobic conditions, the oxidation chain did not function, and thus ATP was generated from glucose only through glycolysis. The energized membrane state could be generated only from glycolytic ATP by ATPase under anaerobic conditions. The effect of anaerobiosis on ribose transport thus indicated that glycolytic ATP alone could couple energy to ribose transport, since anaerobiosis did not significantly inhibit ribose transport in DL-54, which lacks ATPase activity. In agreement with the findings of Berger and Heppel (in press), glucose-driven anaerobic proline uptake occurred in ML 308-225 but not in DL-54. In DL-54, glycolytic ATP could not generate the energized membrane state required for proline transport.

DISCUSSION

The data presented provide strong evidence that the phosphate bond energy of ATP, rather than the energized membrane state, is required for coupling energy to transport of ribose in ML 308-225. Substrates such as glucose and PMS plus ascorbate can provide energy for ribose transport only when they can generate ATP. Glucose was as effective an energy souce for ribose transport in DL-54 as it was in ML 308-225. This is due to the fact that ATP could be generated directly from glycolysis of glucose in DL-54. Lactate and reduced PMS (from PMS plus ascorbate) were oxidized in reactions

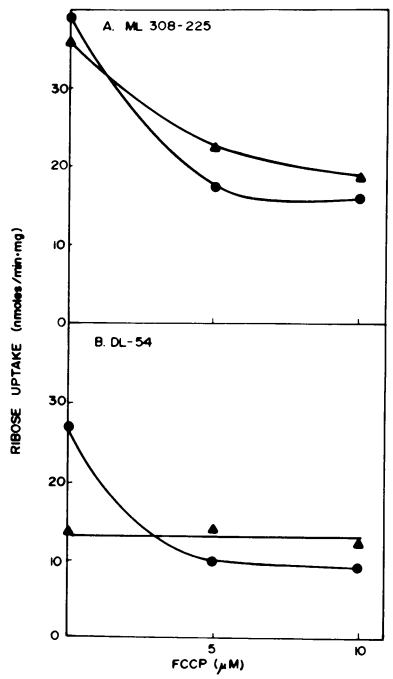

FIG. 4. *Effect of FCCP on ribose uptake in ML 308-225 and DL-54. Ribose uptake was measured with and without the presence of FCCP in the assay mixture, as described, in cells starved by incubation with DNP. A solution of FCCP containing methanol was used in the assays. The concentration of methanol present in assay mixtures containing FCCP did not affect ribose uptake. Symbols: ▲, uptake with lactate as the energy source; ●, uptake with glucose as the energy source.*

TABLE 6. *Effect of FCCP on proline and glutamine transport in ML 308-225 and DL-54*

Strain	Energy source	FCCP concn[a] (μM)	Initial rate of uptake[b]	
			Proline	Glutamine
ML 308-225	Lactate	0	4.0	3.1
		10	0.15	0.27
	Glucose	0	4.9	6.2
		10	0.11	0.86
DL-54	Lactate	0	3.5	0.78
		10	0.16	1.00
	Glucose	0	3.7	4.3
		10	0.14	2.6

[a] The FCCP solution contained methanol. In uptake assay mixtures containing FCCP, the methanol concentration present, 2%, did not affect glutamine or proline uptake.

[b] Uptake of proline and glutamine was measured with and without FCCP in the uptake assay mixture in cells of ML 308-225 and DL-54 starved by incubation with DNP as described. Uptake activity is expressed the same as in Table 1.

TABLE 7. *Effect of anaerobiosis on ribose transport in ML 308-225 and DL-54*[a]

Strain	Initial rate of ribose uptake		Initial rate of proline uptake	
	Aerobic	Anaerobic	Aerobic	Anaerobic
ML 308-225 ...	28.0	33.8	4.4	5.4
DL-54	32.5	25.8	6.8	0.94

[a] Cells assayed for ribose and proline uptake were starved by incubation with DNP. Aerobic and anaerobic uptake rates were measured as described. Uptake activity is expressed the same as in Table 1. Glucose was the energy source in the uptake assays.

that were coupled to electron transport. Generation of ATP from these sources occurred through oxidative phosphorylation, which requires the Ca^{2+}, Mg^{2+}-ATPase. Thus, in DL-54, which lacks ATPase activity, oxidation of reduced PMS could not generate ATP and, therefore, could not drive ribose uptake. Some residual metabolism of lactate apparently generated enough ATP to allow only a low level of ribose uptake in DL-54. This ATP was not generated from oxidative phosphorylation since lactate-driven ribose uptake in DL-54 was not inhibited by the uncouplers of oxidative phosphorylation, DNP and FCCP. The lack of inhibition of lactate-driven ribose uptake by uncouplers in DL-54 also indicates that this uptake did not require an energized membrane state.

ATP may be generated from lactate in DL-54 by some other mechanism such as the phosphoroclastic reaction. D-Lactate can generate a small amount of ATP in DL-54 (Berger and Heppel, in press). Apparently, this level of ATP was sufficient to allow significant ribose uptake, but was not sufficient to allow substantial glutamine uptake in DL-54 (4). Further investigation is required to determine the exact nature of the pathway which could generate ATP from metabolism of lactate in DL-54.

The severe inhibition of ribose uptake in ML 308-225 and DL-54 by arsenate, compared to the relative insensitivity of proline uptake to arsenate, indicates that ATP was required to couple energy to ribose transport. Ribose uptake could not occur in the presence of arsenate because the ATP concentrations of the cells was severely reduced (20). As suggested by Klein and Boyer (20) and Berger (4), the relative insensitivity to arsenate of proline transport in ML 308-225 indicates that energy is coupled to proline transport through the energized membrane state rather than through ATP.

Under anaerobic conditions where ATP, but not the energized membrane state, could be generated, ribose uptake in DL-54 was not significantly inhibited, but proline uptake was severely inhibited. This result is possibly the clearest evidence indicating that ATP rather than the energized membrane state couples energy to ribose transport.

The effect of the uncouplers of oxidative phosphorylation, FCCP and DNP, on ribose transport suggests that an energized state is not required to couple energy to ribose transport. In DL-54, a substantial amount of ribose uptake occurred in the presence of DNP. Lactate-driven ribose uptake was not inhibited by DNP in DL-54, presumably because ATP is generated from lactate in the strain through some pathway other than oxidative phosphorylation. Glucose-driven ribose uptake in DL-54 was also relatively resistant to DNP, since energy was coupled to this uptake by glycolytic ATP. In ML 308-225, DNP severely inhibited lactate-driven ribose uptake, as expected, since generation of ATP from lactate occurred primarily through oxidative phosphorylation in this case.

It would be expected that a substantial amount of glucose-driven ribose transport in ML 308-225 would be resistant to DNP. The severe inhibition of glucose-driven ribose up-

take in the wild strain by DNP may indicate that glycolytic ATP is extensively hydrolyzed by ATPase in the presence of uncouplers. Such enhanced hydrolytic activity of ATPase has been observed in mitochondria (21).

In both ML 308-225 and DL-54, a substantial amount of ribose uptake occurred in the presence of FCCP, whereas proline uptake was almost completely abolished. The inhibition of proline uptake in both strains indicates that generation of an energized membrane state is severely inhibited by FCCP (4). These results provide good evidence that ribose uptake can occur even when the energized membrane state has been dissipated. Glutamine transport was much more sensitive to FCCP than ribose transport in ML 308-225. One possible explanation is that lactate and glucose can generate sufficient ATP in the presence of FCCP to allow ribose uptake, but this level of ATP is not sufficient for significant glutamine uptake.

The properties of DL-54 are apparently different from those of other ATPase mutants. Although membrane vesicles of ATPase mutants, such as AN120 (7), can couple respiration to transport of amino acids and to other energy-requiring functions (27), membrane vesicles of DL-54 can not (29). This defect is in general not found in whole cells of DL-54, although Berger and Heppel (in press) found that PMS plus ascorbate-driven proline uptake in cells of the mutant was lower than in cells of the parent strain. Bragg and Hou (5) have suggested that the ATPase mutation of DL-54 alters the structure of the enzyme such that it cannot stabilize the energized membrane state in vesicles. Altendorf et al. (K. Altendorf, F. M. Harold, and R. D. Simoni, J. Biol. Chem., in press) have found evidence indicating that the ATPase mutation of DL-54 prevents formation of the proton gradient across the vesicle membrane, which is believed to be necessary to generate the energized membrane state from respiration (24). The results of these studies argue strongly against the proposal that DL-54 contains a mutation in addition to that affecting the catalytic activity of ATPase in oxidative phosphorylation (15). They indicate that the ATPase mutation also significantly alters the structure of the enzyme as well as affecting its catalytic activity.

Addition of *N,N'*-dicyclohexylcarbodiimide (DCCD) to vesicles of DL-54 has been found to restore their ability to couple respiration to amino acid transport and other functions (5). DCCD can not replace ATPase in its role in oxidative phosphorylation. Berger and Heppel (in press) have found that DCCD can increase PMS plus ascorbate-driven proline uptake in whole cells of DL-54. This suggests that DCCD can stabilize the energized membrane state generated from PMS plus ascorbate in DL-54. However, ribose uptake driven by PMS plus ascorbate was not increased by DCCD in DL-54 (data not shown) since DCCD can not restore oxidative phosphorylation in the mutant.

Berger has suggested that the different mechanisms of energy coupling for shock-sensitive and shock-insensitive amino acid transport systems in *E. coli* are a result of the different structural arrangements of the two classes of systems in the bacterial cell membrane (4). The results of the investigation of ribose transport in ML 308-225 suggest that there may be a similar relationship between the mechanism of energy coupling and the structural arrangement of active transport systems for sugars. Evidence obtained by others also supports this idea. Wilson has found that a shock-sensitive transport system for β-methylgalactosides also requires ATP to couple energy to transport in *E. coli* (D. B. Wilson, J. Bacteriol., submitted for publication). The β-galactoside transport system of *E. coli* may be an example of a shock-insensitive sugar transport system which requires an energized membrane state to couple metabolic energy to transport. The β-galactoside permease is present in membrane vesicles prepared by the method of Kaback (16), and the carrier component of the permease, the M protein isolated by Fox and Kennedy (11), is tightly bound to the bacterial cell membrane. Although an alternative hypothesis has been proposed by Kaback and his co-workers (17), investigations by others indicate that an energized membrane state couples metabolic energy to β-galactoside transport in *E. coli* (26, 28). Hence, it is probable that, in general, an energized membrane state is required to couple metabolic energy to active transport for shock-insensitive sugar transport systems such as the β-galactoside transport system, whereas the phosphate bond energy of ATP is required to couple energy to active transport for shock-sensitive transport systems such as the ribose transport system.

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