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Transport of 3,4-Dihydroxybutyl-1-Phosphonate, an Analogue of *sn*-Glycerol 3-Phosphate

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3,4-Dihydroxybutyl-1-phosphonate (DHBP), an analogue of glycerol 3-phosphate, is actively transported by the *sn*-glycerol 3-phosphate transport system of *Escherichia coli* strain 8. The K_m for the transport of DHBP is 200 μM.

3,4-Dihydroxybutyl-1-phosphonate (DHBP) is an analogue of *sn*-glycerol 3-phosphate, in which a methylene group has been substituted for the esteric oxygen (6). It inhibits the growth of *Escherichia coli* (14, 16) and *Bacillus subtilis* (9) and has been shown to have a marked effect upon phosphoglyceride metabolism in these microorganisms (2, 9, 15, 16, 18, 19).

In an earlier report we noted that DHBP inhibited the transport of glycerol 3-phosphate by *E. coli* strain 8 (14). This observation suggested that the glycerol 3-phosphate transport system, which has been carefully studied by Lin and his collaborators (3, 7, 8, 10), is responsible for the transport of DHBP. As part of our ongoing investigation of DHBP metabolism (1, 2, 6, 9, 14-16, 18-20), it was of interest to determine unambiguously the mode of transport of DHBP, the kinetic parameters involved, and the relation of analogue uptake to the transport of the natural substrate.

E. coli strain 8 was incubated in the presence of 3,4-dihydroxy[3-^{3}H]butyl-1-phosphonate until the pool was saturated. After 5 min of incubation, sodium azide or dinitrophenol was added to a final concentration of 10 or 1 mM, respectively. The addition of these compounds, which interfere with cellular energy metabolism, resulted in the immediate and rapid loss of the internal pool (Fig. 1). This experiment shows that DHBP, like the natural substrate glycerol 3-phosphate, is actively transported into the cell.

The pool formed during these short assay times does not represent incorporation. Greater than 90% of the accumulated labeled material is released by washing the cells with distilled water. Even after much longer incubation times the labeled intracellular pool was demonstrated to consist almost exclusively of intact DHBP (19). Figure 2 shows that transport is reversible since the pool of labeled DHBP can be reduced by incubation in the presence of cold DHBP. The addition of cold glycerol 3-phosphate also results in a decrease in the intracellular pool of labeled DHPB, indicating that the two compounds probably occupy the same pool (Fig. 2). DHBP also causes the release of intracellular glycerol 3-phosphate (unpublished data). This exchangeability of DHBP and glycerol 3-phosphate could cause a depletion of intracellular glycerol 3-phosphate in cells cultured in the presence of DHBP. The resultant glycerol 3-phosphate starvation might explain the drop in the rate of phosphatidylethanolamine formation previously reported (15) and may also be related to the inhibition of cell growth.

The intracellular DHBP concentration increases with increasing external concentrations, plateauing when the latter reaches 0.2 to 0.3 mM (Fig. 3). A qualitatively similar observation has been made between DHBP concentrations in a culture of strain 8 and growth inhibition (16; R. Salamon and B. E. Tropp, unpublished data). Maximum growth inhibition is achieved when DHBP concentrations are in the 0.2- to 0.3-mM range in the external medium. The intracellular DHBP concentration was calculated by using the internal water values given by Pizer et al. (12). When DHBP is present in the medium at a concentration of 0.3 mM, there is a 177-fold concentration effect. Even greater concentration effects are observed at lower external concentrations of DHBP. The intracellular concentrations reported here are in good agreement with those calculated from the data reported by Tyhach et al., who used a completely different procedure to monitor intracellular content (19). Enzymes not ordinarily involved in glycerol 3-phosphate metabolism may interact with the analogue at elevated intracellular concentrations. The in vivo degradation of DHBP previously reported (19) may be due to such abnormal enzyme-substrate interactions.

The K_m for the transport of the DHBP stereo-

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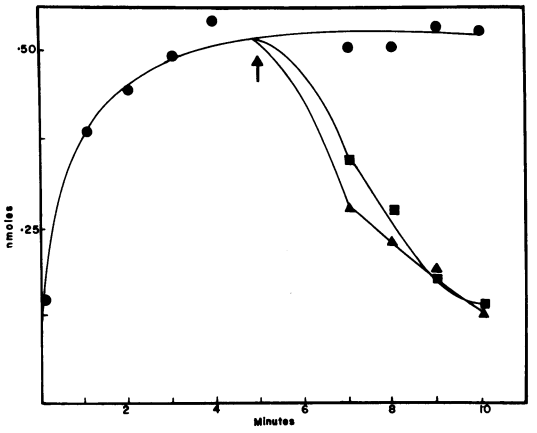

FIG. 1. *Effect of sodium azide and dinitrophenol upon the intracellular pool of DHBP. E. coli strain 8 [HfrC, glpD3, glpR°2, phoA8, tonA22, T2, rel-1 (λ)], isolated by Hayashi et al. (7), was cultured in Garen and Levinthal medium (5) containing 0.5% sodium succinate as the sole carbon source (pH 7.4). Cultures were incubated at 37°C, and growth was monitored as previously described (14, 15). Transport was assayed by a modification of the procedure of Freedberg and Lin (4). A 10-ml sample of a culture at 110 Klett units was centrifuged for 10 min at 755 × g at room temperature in the presence of 40 μg of chloramphenicol per ml. The pellet was suspended in 2 ml of 10 mM potassium phosphate, pH 7.0, containing 0.85% sodium chloride and 40 μg of chloramphenicol (CM-cell suspension). The transport assay was initiated by adding 1 volume of CM-cell suspension to a vial containing 5 volumes of 10 mM potassium phosphate (pH 7.0), 0.85% sodium chloride, and 0.25 mM DL-3,4-dihydroxy[3-³H]butyl-1-phosphonate (specific activity, 10 μCi/μmol) (6). The mixture, containing approximately 4 × 10⁸ cells/ml, was incubated at 30°C, and at the indicated times 50-μl samples were placed on a membrane filter (0.45-μm pore size and 25-mm diameter; Milipore Corp., Bedford, Mass.), washed with 8 drops of 0.85% sodium chloride, dried, and counted as previously described (14). At 5 min, 20 μl of 30 mM dinitrophenol or 300 mM sodium azide was added in a final volume of 0.6 ml. Symbols: sodium azide, ■; dinitrophenol, ▲; and untreated, ●.*

isomer corresponding to *sn*-glycerol 3-phosphate is 200 μM (Fig. 4). Hayashi et al. previously reported the K_m of *sn*-glycerol 3-phosphate to be 12 μM (7). Our value of 16 μM (Fig. 4) is in agreement with this. Using our data, the K_m for DHBP is approximately 12 times greater than that for glycerol 3-phosphate. The V_{max} calculated from the data in Fig. 4 is approximately 160 nmol/min per mg of protein for DHBP and 55 nmol/min per mg of protein for glycerol 3-phosphate.

Several lines of evidence suggest that the glycerol 3-phosphate transport system is responsible for the uptake of DHBP. This conclusion is supported by the isolation of a class of DHBP-resistant mutants which simultaneously lost the ability to transport DHBP and glycerol 3-phosphate. Results with three typical

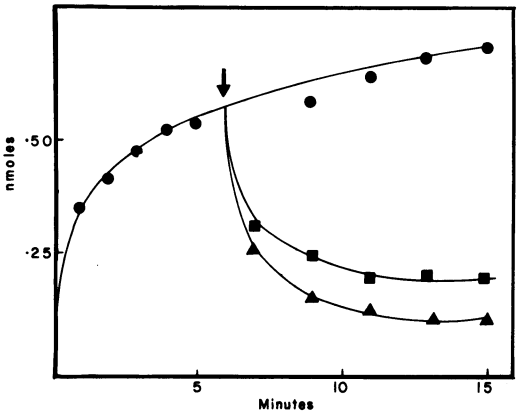

FIG. 2. *Effect of the addition of cold DHBP and glycerol 3-phosphate on the intracellular DHBP pool. The transport assay was performed as described in the legend of Fig. 1. At 6 min (arrow), unlabeled DHBP or glycerol 3-phosphate was added to give a final concentration of 0.86 mM (biologically active stereoisomer). Symbols: glycerol 3-phosphate, ▲; DHBP, ■; and untreated, ●.*

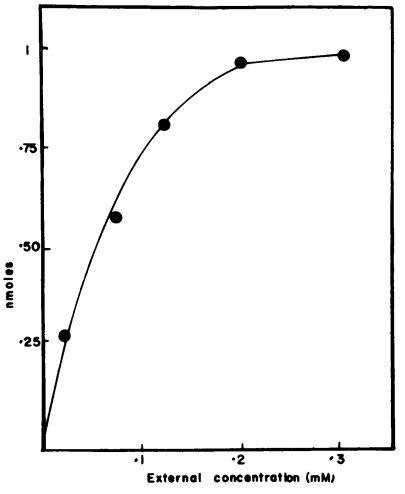

FIG. 3. *Effect of increasing external DHBP concentrations on the intracellular pool size. The standard transport assay described in Fig. 1 was performed in the presence of various concentrations of DL-3,4-dihydroxy[3-³H]butyl-1-phosphonate for 9 min. This was sufficient time to permit the intracellular pool to reach saturation (unpublished data). The intracellular saturation levels at each external concentration of biologically active isomer are plotted.*

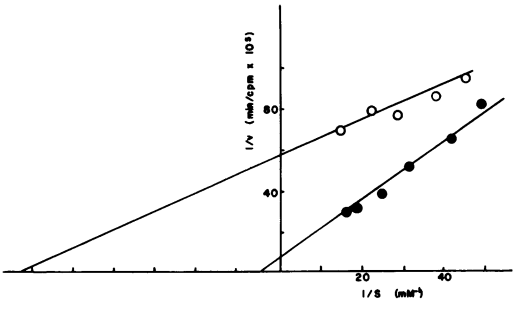

FIG. 4. *Determination of the kinetic parameters for DHBP and glycerol 3-phosphate transport. The standard transport assay described in Fig. 1 was modified in the following fashion: the CM-cell suspension (see Fig. 1 legend) was prepared from cells cultured at 25°C and the transport assay was performed at 20°C. The final cell concentration was 5 × 10^8 cells/ml for DHBP (specific activity, 75 μCi/μmol) and 2 × 10^8 cells/ml for glycerol 3-phosphate transport (specific activity, 24 μCi/μmol). Samples were removed at 20-s intervals, filtered, washed, and counted. The initial rates of transport were determined. These data were used to construct the above reciprocal plot from which the K_m and V_{max} were obtained. Symbols: ○, glycerol 3-phosphate; ●, DHBP.*

TABLE 1. *Transport mutants*[a]

Strain	Uptake (nmol)	
	DHBP	Glycerol 3-phosphate
8	1.16	1.92
Mutant		
3-3	0.039	0.003
4-9	0.064	0.012
5-6	0.076	0.000

[a] Mutagenesis of *E. coli* strain 8 was performed by the nitrosoguanidine procedure of Miller (11). Phenotypic segregation was carried out in Garen and Levinthal medium (5) containing potassium succinate as the sole carbon source. This medium plus 2.5 mM DHBP was used to prepare agar plates for the selection of DHBP-resistant mutants. Colonies appearing on these test plates were then assayed for transport ability as described in the legend of Fig. 1. The assay mixture contained either 0.25 mM DL-DHBP (specific activity, 10 μCi/μmol) or 0.11 mM DL-glycerol 3-phosphate (specific activity, 1.4 μCi/μmol). The assays were run for 4 min.

and independently isolated mutants of this type are presented in Table 1. Some antimicrobial agents, such as silver sulfadiazine, exert their antimicrobial activity without entering the cell (13). Our findings indicate that, as expected, DHBP transport into the cell is a prerequisite for the inhibition of cell growth.

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