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HETEROGENEOUS ELEVATION OF AMINO ACID TRANSPORT RATES IN PANTOTHENATE- AND LIPID-DEFICIENT *LACTOBACILLUS PLANTARUM*

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Summary

The effect of a pantothenic acid deficiency in *Lactobacillus plantarum* on the initial rate of amino acid transport was investigated. Although the steady-state accumulation capacity for all amino acids was markedly reduced in pantothenate-deficient cells, initial rates of uptake either were not changed (asparagine, alanine, lysine) or were increased (glutamic acid, aspartic acid, leucine). The findings suggest that a reduction in membrane lipid content heterogeneously affects the operation and/or synthesis of amino acid transport catalysts.

Previous reports from this laboratory have described the following transport and composition changes in pantothenate-deficient *Lactobacillus plantarum*: (1) premature cessation of amino acid uptake at markedly reduced steady-state accumulation levels [1—3]; (2) reversal of this defect by elevation of the extracellular osmotic pressure or by providing non-growing cells with pantothenate and acetate [3]; (3) a reduced lipid content which is restored almost to normal during incubation with pantothenate and acetate [4]; (4) stimulation of amino acid uptake by long-chain saturated and unsaturated fatty acids [5]. These observations suggest that a pantothenate deficiency curtails lipid synthesis, leading to the formation of a cell membrane which is incapable of retaining large pools of intracellular amino acids.

The early transport experiments conducted during this investigation utilized a relatively slow centrifugation procedure to terminate incubations. These studies indicated that in contrast to the large decline in accumulation capacity for all amino acids observed with pantothenate-deficient cells, the initial rates of uptake were not reduced [3]. We have recently repeated these experiments using a more rapid millipore filtration technique to terminate incubations and have found that while the uptake rates for some amino acids

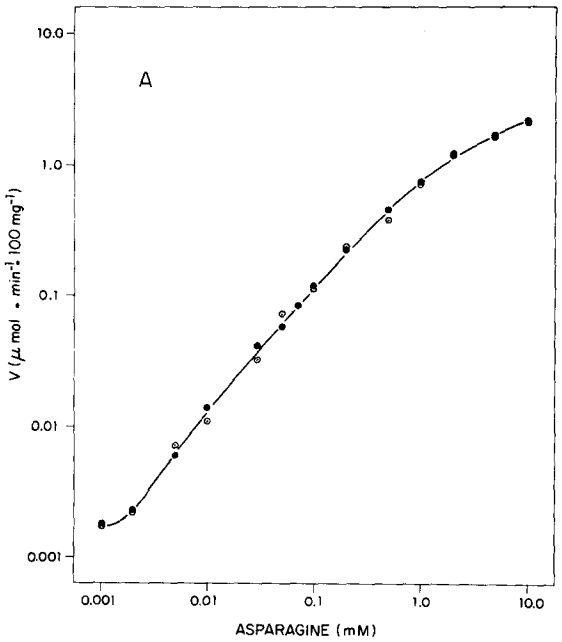
were equal in pantothenate-deprived and nutritionally normal control cells, the uptake rates of several other amino acids were substantially increased in pantothenate-deficient cells.

Pantothenate-deprived cells were grown as previously described [3]. In most experiments, the calcium pantothenate concentration in the growth medium was reduced from 400 μg/l to 3.5—4.0 μg/l. Cells were harvested when the growth rate was markedly reduced, usually after 18—22 h of incubation. Amino acid uptake was estimated by incubating washed cells in a glucose-supplemented phosphate buffer using previously described sampling, millipore filtration and isotope counting methods [6, 7]. Initial uptake rates were determined by taking five to seven accurately timed samples during the first 45—75 s of incubation, and estimating the rate from the slope of an uptake vs time plot.

As shown in Fig. 1A, initial rates of asparagine transport over a broad concentration range were not significantly different in pantothenate-deficient and pantothenate-sufficient cells. In contrast, glutamic acid initial uptake rates were substantially increased over a comparably broad range of concentrations (Fig. 1B). The heterogeneity in transport rate changes is further indicated in Table I which shows that the rates of aspartic acid and leucine uptake also were elevated in pantothenate-deficient cells, whereas those for alanine and lysine were equal in pantothenate-deficient and pantothenate-sufficient cells. Chromatographic examination of extracts prepared from cells incubated under these conditions revealed no significant differences in the formation of amino acid metabolites by pantothenate-deficient and -sufficient cells. A parallel set of experiments carried out with biotin-deficient cells, which also contain reduced amounts of lipid [4] have revealed corresponding elevations in initial uptake rates for glutamic acid, aspartic acid and leucine.

It should be noted that the initial rate changes and differences described here cannot be accounted for by differential membrane leakage properties of pantothenate-deficient and -sufficient cells. Membrane hyperpermeability in pantothenate-deficient cells appears to be a latent property which does not conspicuously express itself until several minutes after the period in which initial uptake rates are estimated when the cells have taken up sizeable amounts of amino acid. Apparently, the resultant increase in intracellular osmotic pressure promotes a non-specific leak which affects the retention of elevated amounts of all amino acids, not only those whose transport rates are modified.

The observations reported here are consistent with a number of studies indicating that membrane fatty acid hydrocarbon chains affect the operation of sugar and amino acid transport catalysts [8—10]. For example, the nature of the fatty acid used to support the growth of *Escherichia coli* fatty acid auxotrophs can markedly alter the temperature at which sugar and amino acid transport processes undergo a change from a relatively high to a lower activation energy. The relative position of the transition temperature in Arrhenius plots has been correlated by several groups with the relative temperature at which the membrane fatty acids undergo a change from a predominantly ordered to a predominantly fluid state. Such findings strongly imply that the degree of fluidity of the membrane lipid fatty acid hydrocarbon chains affects



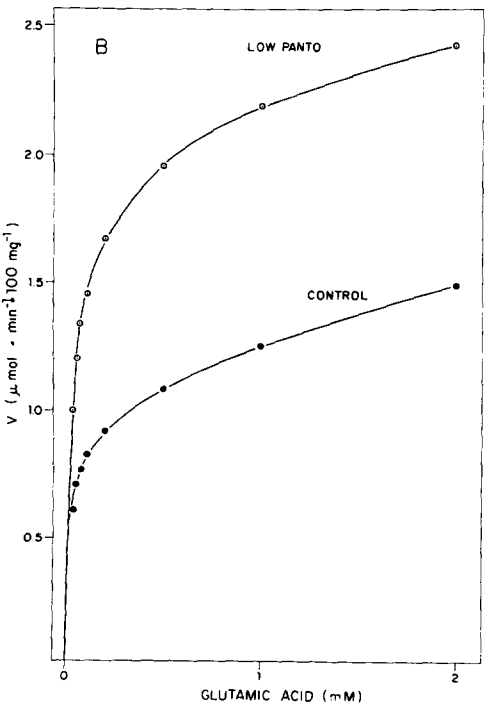


Fig. 1. Comparative initial rates of amino acid uptake by pantothenate-deficient (○) and -sufficient (●) cells. (A) L-Asparagine; (B) L-glutamic acid.

TABLE I

AMINO ACID TRANSPORT RATES IN PANTOTHENATE-DEFICIENT AND PANTOTHENATE-SUFFICIENT *L. PLANTARUM*

Amino acid	Concn (mM)	No. of expts	Transport rate (μmol · min^{-1} · 100 mg^{-1})		Rate ratio (deficient/sufficient)
			Deficient cells	Sufficient cells	
Glutamic acid	0.20	10	1.26	0.57	2.21
Aspartic acid	0.20	2	1.77	0.88	2.01
Leucine	0.05	9	0.31	0.19	1.63
Asparagine	0.10	6	0.17	0.18	0.94
Alanine	0.20	5	0.60	0.65	0.92
Lysine	0.10	3	0.16	0.16	1.00

the reaction rates of the resident transport catalysts. The findings described here, that some, but not all, amino acid transport catalysts in *L. plantarum* operate at a more rapid rate when the membrane lipid content is reduced, also indicate that at least some transport catalysts in this organism interact with and are affected by neighboring membrane lipid components. An alternative interpretation of these findings is that there is a heterogeneous increase in the synthesis and/or implantation of some amino acid transport systems when membrane lipid synthesis is reduced.

The heterogeneous alteration of amino acid transport rates in a series of *E. coli* mutants which overproduce various fatty acids and lipids [11] supports the present findings indicating that membrane-localized transport catalysts may respond independently and diversely to a given change in membrane lipid composition.

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