STIMULATION BY FATTY ACIDS OF AMINO ACID ACCUMULATION IN PANTOTHENIC ACID DEPLETED LACTOBACILLUS PLANTARUM

Joseph T. Holden and James M. Bunch

Division of Neurosciences, City of Hope National Medical Center, Duarte, California 91010

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SUMMARY

Defective amino acid uptake and accumulation induced by a pantothenic acid deficiency in Lactobacillus plantarum are permanently restored to normal by incubating cells with long chain fatty acids in the absence of the vitamin. Both saturated and unsaturated (C12 to C20) fatty acids increased the accumulation level. The highest activities were observed with monounsaturated acids including oleic, cis-vaccenic and elaidic acids. Various fatty acid esters were moderately active, but diglycerides and sterols had little stimulatory activity. These findings indicate that subnormal amino acid accumulation by pantothenate-deficient cells is caused by a curtailment in lipid biosynthesis which prevents the formation of a membrane having normal retentive properties, and that the membrane lipid content can be increased subsequently in non-growing cells with the restoration of normal permeability properties.

Pantothenic acid and biotin deficiencies markedly decrease the steady state amino acid accumulation level in Lactobacillus plantarum without reducing the initial rate of transport (1-3). This defect can be corrected in two ways: a) by increasing the osmotic strength of the extracellular buffer, b) by supplementing the buffer used in transport experiments with the respective vitamin and acetate (3). The first procedure promotes a provisional reversal of the accumulation defect; the large pool is lost instantaneously if the external osmotic pressure is reduced. The second procedure produces a permanent reversal of the defect. Subsequently, both vitamin deficient cell types were shown to contain less than normal amounts of lipid, and incubation of non-growing cells with the vitamin and acetate was found to raise lipid levels into the normal range (4). The adverse effect of these vitamin deficiencies on transport, therefore, appears to result from the

inability of such cells to synthesize sufficient lipid to form a membrane capable of retaining transported amino acids within the cell. In further support of this interpretation, we show here that defective uptake and retention of amino acids by pantothenate-deficient cells can be corrected in the absence of the vitamin by exposing non-dividing cells to preformed long chain fatty acids.

METHODS AND MATERIALS: Procedures for the cultivation of L. plantarum (ATCC 8014) and its use in studying amino acid transport have been described (3, 5-7). All uptake experiments were carried out using ^{14}C-L-glutamic acid (UL) as substrate. Incubations were carried out and terminated by Millipore filtration as described previously for Streptococcus faecalis (8) except that a buffer 0.12 M in phosphate was used. Fatty acids and related lipids were dissolved in 95% ethanol. Concentrations of these solutions were adjusted so that the final ethanol concentration in the uptake buffer was 1%. At this level no inhibitory effects on uptake were observed under various incubation conditions. Fatty acids and most related lipids were obtained from Sigma Chemical Co.

RESULTS: The time-course of glutamic acid uptake by pantothenate-deficient L. plantarum is illustrated in Fig. 1. As reported previously (3), the exceptionally low steady-state accumulation could be raised to levels observed with nutritionally normal cells by supplementing the buffer with pantothenic acid and acetate. In the absence of the vitamin, fatty acids, including cis-vaccenic acid, promoted comparable accumulation.

Figure 2 illustrates dose-response curves for several fatty acids in stimulating accumulation, again, in the absence of pantothenic acid. Very low fatty acid concentrations (ca 1 µg/ml) produced significant improvements in accumulation capacity. Maximal improvement was observed with 5 to 15 µg/ml depending on the compound supplied. Both saturated and unsaturated fatty acids were effective. Maximal stimulation, however, was most often

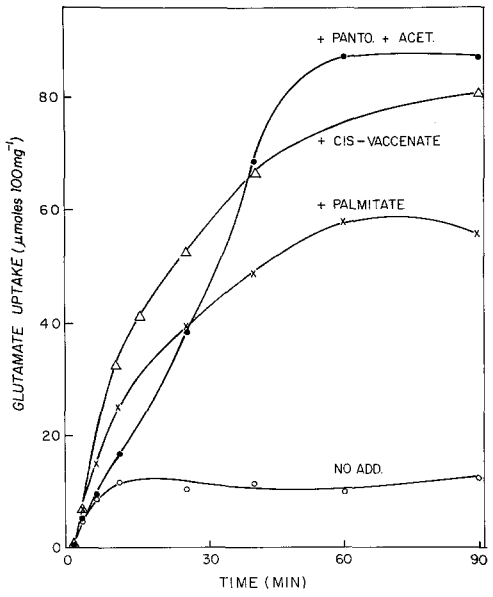

Fig. 1. Time-course of ^{14}C-L-glutamic acid uptake by pantothenate-depleted L. plantarum. Cells were grown for 19 hours in a medium containing 3.5 μg/l calcium pantothenate. ^{14}C-L-Glutamic acid at 2 mM in uptake buffer as described under Methods. The uptake buffer was supplemented as follows: O——O, no additions; ●——●, calcium pantothenate (1 μg/ml) and K acetate (5.8 mM); Δ——Δ, cis-vaccenic acid (10 μg/ml); X——X, palmitic acid (10 μg/ml).

obtained with unsaturated fatty acids. Except for the shorter chain (C12-C14) acids, saturated acids were less inhibitory when their concentrations were raised above the levels sufficient to stimulate uptake.

Table I summarizes the activity of various fatty acids and related substances. In addition to supporting the observations cited above, these data show that cholesterol and a fatty alcohol had little stimulatory activity, whereas various fatty acid esters including lysolecithin were moderately stimulatory. In contrast dipalmitin had little or no activity.

Additional experiments have shown that subnormal accumulation by biotin-deficient cells also was corrected by fatty acids, as was the reduced uptake of alanine, proline, cycloleucine and asparagine in pantothenate-deficient cells.

DISCUSSION: These experiments support earlier conclusions (3, 4) implicating a relatively "leaky" lipid-deficient membrane as the primary cause of the

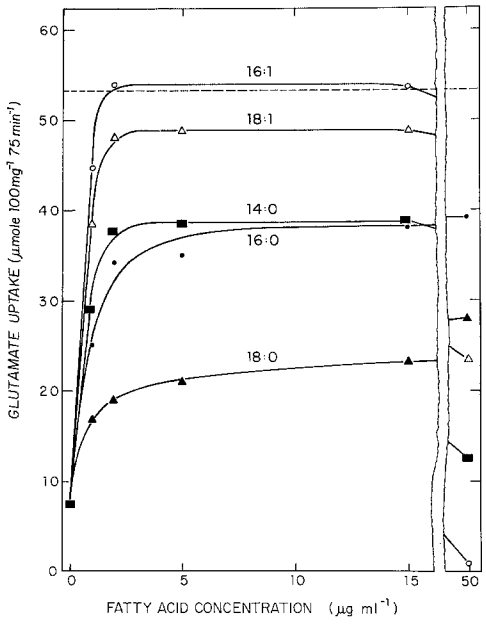

Fig. 2. Effect of fatty acid concentration on ^{14}C-L-glutamic acid uptake by pantothenic acid-depleted L. plantarum. Growth and uptake conditions as in Fig. 1. The numbers on the curves identify the following fatty acids: 14:0,myristic acid; 16:0, palmitic acid; 16:1, palmitoleic acid; 18:0, stearic acid; 18:1, oleic acid. Incubation time 75 min at 37°. The dashed line indicates uptake attained by cells incubated in uptake buffer supplemented with calcium pantothenate (1 μg/ml) and K acetate (5.8 mM).

markedly reduced steady state amino acid accumulation levels characteristic of pantothenate- (and biotin-) deficient L. plantarum. Since the initial rates of transport are not reduced in these deficient cells, it is likely that the stimulatory effect of fatty acids on accumulation level is more directly related to the permeability of the barrier membrane rather than to the operation of the transport catalysts which promote entry of the amino acids. The earlier finding that pretreatment of pantothenate- depleted cells with this vitamin restored both amino acid accumulation and cellular lipids to normal levels (4) strongly implied that the two changes were functionally related, but left open the possibility that the vitamin might have produced other cell changes which were primarily concerned in correcting the accumulation defect. The observations summarized here that several fatty acids were at least as (and occasionally more) effective than the vitamin in this

Table I. Effect of Fatty Acids and Related Lipids on Amino Acid Accumulation in Pantothenate-Deficient *L. plantarum*

Addition to Uptake Buffer	Amount for Maximal Stimulation	Glutamate Accumulation*	% of Maximal Uptake**
	$\mu g\ ml^{-1}$	$\mu moles\ 100\ mg^{-1}$	
None	-	12.0	15
Ca Pantothenate + K acetate	1.0 570	82.8	100
Tetradecanoic acid	5	52.6	64
Pentadecanoic acid	5	60.4	73
Hexadecanoic acid	15	50.7	61
Octadecanoic acid	50	38.0	46
Eicosanoic acid	50	19.5	24
cis-9-Octadecenoic acid	5	64.6	78
trans-9-Octadecenoic acid	50	65.8	80
cis-11-Octadecenoic acid	15	72.1	87
cis-9,cis-12 Octadecadienoic acid	5	51.2	62
DL-2-OH-Octadecanoic acid	15	18.5	22
1-Octadecanol	50	17.0	21
Cholesterol	15	18.2	22
Monostearin	15	43.2	52
Lysolecithin	15	45.5	55
Polyoxyethylene sorbitan mono-oleate	50	43.9	53

*Growth and uptake conditions as in Fig. 1. Incubation time 75 min at 37°.
**Uptake with pantothenate and acetate arbitrarily set at 100.

regard argues strongly that the previously demonstrated increase in membrane lipids is primarily responsible for the improved retention of transported amino acid by pantothenate supplemented cells.

These studies also support our earlier conclusions (3) that fully active amino acid transport catalysts can be deployed in the membrane of this organism at a time when the cell is not manufacturing sufficient lipid to allow the membrane to function normally in retaining low molecular weight intracellular solutes. Catalysts involved in sugar transport in *Escherichia coli* and *Staphylococcus aureus*, on the other hand, seem to be functionally

impaired when they are inserted into the membrane during a period of markedly reduced lipid biosynthesis (9, 10).

Gale and Llewellin (11, 12) recently have demonstrated that amino acid uptake in water-washed or osmotically shocked S. aureus is stimulated by unsaturated fatty acids. Saturated fatty acids were much less effective. Several groups studying glutamate excretion by biotin-limited bacteria have encountered fatty acid effects suggesting that increased unsaturation of membrane fatty acids enhances intracellular retention of amino acids (13, 14). In contrast, we have consistently observed significant stimulatory activity both with saturated and unsaturated fatty acids. Several possibilities might account for these apparently discrepant findings including (a) that this organism may rapidly convert saturated to unsaturated fatty acids, or (b) that the level of lipid depletion is so much more severe in this case, that even a comparatively inefficient substance such as a saturated fatty acid might still promote a sizeable improvement in pool retention.

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