REDUCED LIPID CONTENT AS THE BASIS FOR DEFECTIVE AMINO ACID ACCUMULATION CAPACITY IN PANTOTHENATE- AND BIOTIN-DEFICIENT *LACTOBACILLUS PLANTARUM*

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SUMMARY: Pantothenic acid and biotin deficiencies markedly reduce the total amount of amino acid that can be taken up and retained intracellularly by *Lactobacillus plantarum*. The total lipid constituents of these deficient cells also are markedly reduced. Incubation of deficient cells with acetate and the respective vitamin promotes an increase in lipid content at the same time that the accumulation capacity is permanently restored to normal levels. A provisional increase in accumulation capacity stimulated osmotically by a high extracellular concentration of sucrose is not accompanied by lipid synthesis. These vitamin deficiency-associated transport defects appear to result from membrane lipid changes which render this structure incapable of retaining normal-sized pools.

Pantothenic acid and biotin deficiencies have been shown in this laboratory to cause a marked reduction in amino acid accumulation capacity in *Lactobacillus plantarum* without significantly decreasing the initial rate of uptake (1-3). Defective accumulation can be restored to normal either by increasing the extracellular osmotic pressure or by incubating non-dividing cells with acetate and the respective vitamin. A structural change, most likely membrane localized, was proposed as the cause of reduced accumulation capacity in these vitamin-depleted cells (3). Accordingly, the effect of growth in vitamin-deficient media on the lipid content and composition of this organism has been investigated.

METHODS AND MATERIALS: Procedures for the cultivation of *L. plantarum* (ATCC No. 8014) and its use in studying amino acid transport have been described

(3-6). Lipid constituents were extracted and fractionated using the quantitative procedures described by Rouser et al (7). The specific methods used will be described in detail separately (8). For extraction, frozen cells were suspended in solvent and sonicated in the cold with washed glass beads using successively the following solvent mixtures: chloroform/methanol (C/M) 2/1, 45 min; C/M, 2/1, 15 min; C/M, 2/1, 15 min; C/M, 1/2, 15 min; C/M, 7/1 saturated with NH_4OH, 15 min. The extracts were combined and evaporated nearly to dryness repeatedly after the addition of chloroform. The concentrated extract was redissolved in C/M, 19/1 and water-soluble nonlipid impurities were removed by sephadex column chromatography. The fraction eluted by C/M, 19/1 is referred to as total extractable lipids. After concentration by evaporation, the lipids were redissolved in C/M, 9/1, an aliquot weighed and the remainder stored at -20°. A small amount of protein measured colorimetrically (9) was encountered in all samples. Lipid weights have been corrected by subtracting this material. The total fatty acid contents of cell extracts and residues were measured after saponification in 3 N ethanolic KOH using a modification of the Novak procedure (10,11).

TABLE I. Effect of Pantothenate and Biotin Deficiencies on Glutamic Acid Uptake and Accumulation by L. plantarum*

	Buffer Supplement		
	None	0.8 M Sucrose	Acetate + Vitamin
	μmoles glutamate/100 mg cells		
Normal, Early exponential	62	60	-
Normal, Late exponential	74	65	-
Pantothenate-deficient	9	74	71
Biotin-deficient	20	73	77

*The amount of isotopically labeled L-glutamic acid accumulated is shown. Cells were incubated at 37° for 75 min with the indicated supplements to the standard uptake buffer. K acetate was used at 5.8mM, pantothenic acid at 0.27 μg/ml and biotin at 1.2 μg/ml. Growth conditions as described previously (3).

RESULTS: Table I summarizes the glutamic acid accumulation capacities of pantothenic acid- and biotin-deficient cells measured under a variety of conditions. The markedly reduced capacities of both vitamin-deficient cell types are restored essentially to control levels either by increasing the extracellular osmotic pressure using sucrose or by supplementing the uptake buffer with acetate and the respective vitamin.

The large pools accumulated by pantothenate- and biotin-deficient cells in the presence of sucrose are lost instantaneously when the extracellular osmotic pressure is reduced suggested that a structural defect limits accumulation capacity. In contrast, the large pools accumulated in the presence of acetate and the respective vitamin are retained as tenaciously as those in nutritionally-normal cells, suggesting that the structural defect has been corrected. The apparent absence of major cell wall changes in these deficient cells (12), and the involvement of both vitamins in fatty acid biosynthesis suggested that changes in membrane lipids might account for the defective amino acid accumulation.

As shown in Table II, both deficiencies markedly reduce the total amount of extractable lipid in L. plantarum. Normal cells contain between 3.4 and

TABLE II. Effect of Pantothenate and Biotin Deficiencies on Total Extractable Lipid Content of L. plantarum

Nutritional Type	Extractable Lipid*	% of Normal, late
	mg/100 mg cells	
Normal, Early exponential	3.40	89
Normal, Late exponential	3.82	100
Pantothenate-deficient	2.19	57
Biotin-deficient	2.65	69

*Weight of material eluted by C/M 19/1 from sephadex columns, corrected for protein content; average of 3 experiments.

3.8% extractable lipid. This amount is reduced 30 and 40% in biotin- and pantothenate-deficient cells respectively. Since a significant portion of the lipid in this organism is difficult to extract (8, 13), the possibility was considered that the decline in recovered lipid reflects a change in extractability rather than in the amount present. Measurement of the total fatty acid content of the cell extracts and the remaining cell residues revealed that while there was a significant increase in non-extractable lipid in the vitamin-deficient cell residues, the total amount of fatty acid in both deficient cell types was still approximately 35% lower than normal. Thus, the total amount of lipid and its association with other cell constituents seem to be altered by pantothenate and biotin deficiencies.

If these changes are functionally significant, incubation conditions which produce a permanent improvement in transport capacity should be accompanied by an increase in lipid content. The experiment described in Table III demonstrates that the lipid content of pantothenate-deficient cells is restored essentially to normal by supplements to the uptake buffer (acetate and pantothenic acid) which produce a permanent increase in accumulation

TABLE III. Effect of Incubation Conditions on Extractable Lipid Content of Pantothenate-Deficient Cells

Incubation Conditions*	Extractable Lipid mg/100 mg cells
1. Unwashed cells, control	2.43
2. Washed cells, control	2.21
3. Buffer	2.28
4. Buffer + glucose + glutamate	1.83
5. Buffer + glucose + glutamate + pantothenate + acetate	3.45
6. Buffer + glucose + glutamate + sucrose	2.13

*Control cells (1,2) were processed at 2° and not incubated. Incubation (3-6) was at 37° for 75 min using PO_4 buffer (0.12 M), glucose (28 mM), glutamic acid (3 mM), pantothenic acid (1 µg/ml), K acetate (5.8 mM) and sucrose (0.6 M). Following incubation centrifuged cells were frozen and extracted as described in Methods.

capacity. Cell numbers or protein and RNA contents do not change significantly under these conditions. Incubation in the presence of sucrose which produces only a provisional improvement in accumulation capacity does not increase lipid content. The latter control also demonstrated that increased uptake and retention of the amino acid by itself does not stimulate lipid synthesis. Comparable results were obtained in parallel studies with biotin-deficient cells.

DISCUSSION: The large, osmotically-reversible, decrease in amino acid accumulation capacity previously described in pantothenate- and biotin-deficient cells has been shown here to be associated with a marked reduction in cell, presumably membrane, lipid content. Incubation conditions which stimulate an increase in lipid content also increase the accumulation capacity. These findings suggest that a reduction in lipid content makes the cell membrane unusually susceptible to distortion by the elevated intracellular osmotic pressure associated with accumulation of large amino acid pools. As a result, the influx and efflux rates equilibrate prematurely at lower than normal pool levels. This defect can be reversed temporarily by preventing the influx of water osmotically, or permanently, by allowing the synthesis of lipid. Additional studies have shown that all the major lipid classes are reduced by these deficiencies and resynthesized under reactivation conditions (8). It remains to be established whether all or only a few specific membrane components must be synthesized for reversal of the accumulation defect. Several other investigators have encountered fatty acid or lipid changes in biotin (14-17) and pantothenate (18) deprived cells, and, in a few instances, a relation between biotin-deprivation and altered membrane permeability has been proposed (19-22). Comparable effects of a pantothenate-deficiency apparently have not been described, and the molecular basis of such permeability changes has not yet been definitively investigated in any case.

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