REPAIR OF MEMBRANE HYPERPERMEABILITY IN L. plantarum DURING PARTIAL REVERSAL OF LIPID DEFICIENCY

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Received October 15,1974

SUMMARY - Previous studies suggest that the reduced amino acid accumulation capacity of pantothenate-deficient L. plantarum is caused by a lipid deficiency which results in membrane hyperpermeability. The accumulation defect can be reversed by supplying such cells with saturated or unsaturated fatty acids which are incorporated into the major lipid constituents. Simultaneous measurement of ^{3}H-amino acid uptake and ^{14}C-fatty acid incorporation revealed that some unsaturated fatty acids promote an 80% reversal of the amino acid accumulation deficit when the cells have taken up only enough fatty acid to replace 12 to 20% of the lipid deficit. Apparently, only a small fraction of the absent lipid plays a decisive role in membrane permeability.

Cultivation of L. plantarum in a medium containing growth-limiting amounts of pantothenic acid yields cells with a distinctly reduced lipid content (1). These cells also have a markedly reduced amino acid accumulation capacity (1-3). Initial rates of uptake either are not reduced or are increased for some amino acids.* Several procedures reverse the adverse effects of the vitamin deficiency on the steady-state accumulation levels including elevation of the extracellular osmotic pressure (3) and synthesis of additional lipid under conditions which preclude protein synthesis (1). More recently, restoration of normal pool forming capacity has been attained without relieving the pantothenate deficiency by providing cells with any one of several saturated or unsaturated fatty acids (4). These observations have suggested that curtailment of fatty acid biosynthesis in pantothenate-deprived cells leads to the formation of a lipid-deficient hyperpermeable membrane. Although the transport

*Holden et al. Presented at the First International Congress of Bacteriology, Jerusalem, 1973. In preparation for publication.

systems appear to move amino acids into the cell at a normal or even increased rate, the membrane lacks its normal retentive properties and, consequently, influx and efflux rates balance at abnormally low pool concentrations. We have now found that restoration of nearly normal amino acid accumulation levels can be attained during the incorporation of relatively small amounts of some unsaturated fatty acids, in most cases enough to restore only 12 to 20% of the lipid deficit. These findings suggest that only a relatively small fraction of the lipid absent in pantothenate-deficient cells normally plays a decisive role in determining the degree of membrane permeability to hydrophilic substances.

MATERIALS AND METHODS

Pantothenate-deficient and -sufficient cultures were grown as described previously (1,3,4). Methods for processing cells and carrying out amino acid transport studies have been described (1-3,5). Fatty acid uptake was measured using ^{14}C-labeled compounds (Applied Science Laboratory). Fatty acids were prepared in ethanol at 100 times their final concentrations. Inhibitory effects of high fatty acid concentrations were avoided by including serum albumin (essentially fatty acid-free) in the uptake buffer. ^{14}C-fatty acid and ^{3}H-amino acid uptake were determined by measuring the respective isotopes in cells removed from the incubation medium by millipore filtration. To determine the identity of ^{14}C-labeled lipids, cells separated from buffer by centrifugation were extracted with methanol at 60° for 30 min after which chloroform was added to yield a 2/1 (C/M) mixture and extraction was continued for 1 hour at room temperature with stirring. Although extraction of the total cell lipid was incomplete under these conditions, 90 to 95% of the cellular ^{14}C content was removed. Extracted lipids were freed of water soluble impurities by sephadex chromatography and analyzed by thin layer chromatography (1, 6). Separated lipids were aspirated from the plates and the isotope was measured by liquid scintillation spectrometry.

RESULTS

Figure 1 illustrates the time-course of fatty acid uptake by pantothenate-

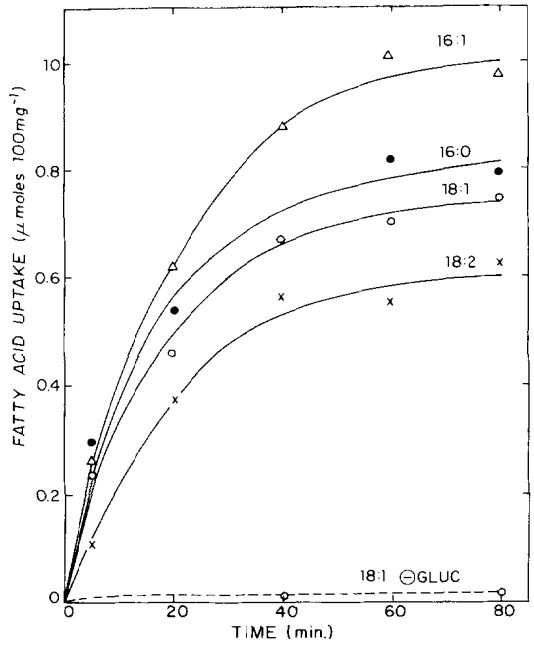

Fig. 1. Time-course of ^{14}C-fatty acid uptake by pantothenate-deficient L. plantarum. Cells were incubated at 37° in uptake buffer containing L-glutamic acid (2 mM) serum albumin (400 μg ml^{-1}) and the following fatty acids at 20 μM: △——△, palmitoleic acid; ●——●, palmitic acid; ⊙——⊙, oleic acid; x——x, linoleic acid; ⊙-----⊙, oleic acid, glucose omitted.

deficient cells. Uptake was dependent on glucose as an energy source and reached a plateau within 60 min. Generally, larger amounts of C16 than of C18 fatty acids were taken up. Analysis of the extracted lipids revealed the following distribution of isotope supplied as ^{14}C-oleic acid: phosphatidylglycerol (PG), 31.1%; diphosphatidylglycerol (DPG), 9.6%; (these two compounds comprise 82-86% of the phospholipids in this organism) diglycosyldiglyceride (DGDG), 11.8%; free fatty acid (FFA), 9.4%; neutral lipid (NL), 28.1%. These cells apparently contain sufficient pantothenic acid to activate preformed fatty acids, but an insufficient amount to synthesize fatty acids de novo at a rate rapid enough to maintain lipid synthesis.

Figure 2 illustrates the results of a double label experiment. The amounts of ^{3}H-L-glutamic acid and ^{14}C-oleic acid taken up by pantothenate-deficient cells incubated in the presence of various oleic acid concentrations

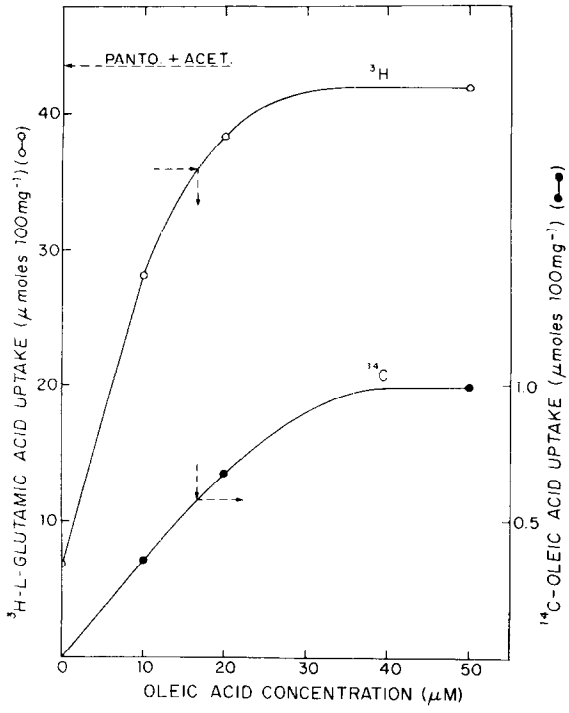

Fig. 2. Simultaneous uptake of ^{14}C-oleic acid and ^{3}H-glutamic acid by pantothenate-deficient L. plantarum. Uptakes are shown at various oleic acid concentrations after incubation for 60 min in the presence of 2 mM glutamic acid: o——o, ^{3}H-glutamate uptake, left ordinate; •——•, ^{14}C-oleate uptake, right ordinate. The upper dashed line indicates the glutamate accumulation observed in the absence of fatty acids by cells incubated in uptake buffer containing pantothenic acid and potassium acetate. Fatty acids were compared in their ability to produce 80% of the stimulation observed with pantothenate and acetate. The arrows on the two curves indicate the oleic acid concentration producing this level of amino acid uptake and the corresponding oleic acid uptake observed.

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were measured. Such experiments established the amount of fatty acid incorporation that was associated with various degrees of recovery of glutamate accumulation capacity. Since the distribution of ^{14}C-fatty acids among the major lipids was known, the maximal amount of lipid that could be synthesized from the incorporated fatty acids could be calculated. As indicated in Table I, several unsaturated fatty acids restored 80% of the glutamate accumulation deficit when sufficient fatty acid had been taken up to restore only 12 to 20% of the lipid deficit. The validity of this conclusion is supported by separate lipid isolation experiments showing that cells provided with

TABLE I

Increase in L. plantarum Fatty Acid and Lipid Contents During Glutamate Accumulation Increase*

Fatty Acid	^{14}C-Fatty Acid Uptake	Equivalent Lipid Mass**	% Lipid Deficit Restored***
	μmoles 100 mg^{-1}	mg 100 mg^{-1}	
Myristic acid	0.88	0.28	20
Palmitic acid	0.76+	0.26	19
Palmitoleic acid	0.74	0.26	19
Oleic acid	0.46	0.17	12
Linoleic acid	0.55	0.21	15
Linolelaidic acid	0.51	0.19	14

*Growth and uptake conditions as in Fig. 2. The second column shows the amounts of ^{14}C-fatty acid in cells whose glutamate uptake capacity had been restored to 80% of the maximal increase observed in cells incubated with pantothenate and acetate.

**The distribution of fatty acids among the major lipid classes (53% in PG + DPG + DGDG; 38% in FFA + NL) predicted the formation of the following amounts of lipid per mg of incorporated fatty acid: C18, 1.33 mg; C16, 1.37 mg; C14, 1.41 mg.

***The following average cell lipid contents have been observed: control, nutritionally normal, 4.6 mg 100 mg^{-1}; pantothenate-deficient, 3.2 mg 100 mg^{-1}.

+69% of maximal glutamate uptake increase.

larger amounts of fatty acid (50 μM) formed measurable though still relatively small amounts of additional lipid.

DISCUSSION

The reversal of the amino acid accumulation defect in pantothenate-deficient L. plantarum during the incorporation of relatively small amounts of fatty acid suggests that a large fraction of the lipid absent from these cells does not play a decisive role in membrane permeability. There are several possible explanations for such an observation. (1) A large portion of the absent lipid normally might be found in intracellular rather than in peripheral membrane. L. plantarum usually contains mesosomes (7). While we have observed them also in pantothenate-deficient cells, the relative amounts of mesosomal

membranes in normal and deficient cells have not been quantitatively evaluated. This explanation would require the preferential association of newly synthesized lipid with peripheral rather than with mesosomal membrane during the recovery process. (2) A substantial amount of lipid might be lost from the peripheral membrane without markedly altering its permeability to hydrophilic molecules. Eventually, the loss would reach a point at which small further reductions in lipid content would produce large permeability changes. Thus, a partial reversal of the lipid deficit could restore most of the lost accumulation capacity. Although we have observed an apparently progressive reduction in accumulation capacity as the cell lipid content was reduced, this possibility cannot yet be rigorously excluded. (3) A specific lipid is prominently involved in regulating membrane permeability and its synthesis is favored during the reactivation process. Pantothenate-deficient cells contain relatively less phospholipid than control cells. There is also a reduction in the PG to DPG ratio. Although all the major lipids were labeled during the recovery phase, there was a relative increase in phospholipids and larger amounts of PG than of DPG were formed. While this would shift the PG/DPG ratio closer to its normal value, the relevance of this relationship to the permeability changes has not yet been evaluated. (4) Leakage occurs primarily in special membrane regions. The membrane model proposed by Singer and Nicolson (8) in which some proteins are embedded in the lipid matrix and occasionally penetrate it entirely, raises the possibility that hydrophobic protein-lipid associations might be more easily disrupted during a lipid deficiency than the interaction between the fatty acid hydrocarbon side chains in the bulk of the lipid matrix. The osmotic swelling of the cytoplasm associated with amino acid pool accumulation, therefore, might produce fissures in the region of protein-lipid junctions in membranes having a reduced lipid content. The resealing of such sites during the synthesis of relatively small amounts of lipid might simply reflect the occurrence of many such leakage sites in the vicinity of membrane protein catalysts which are involved in lipid

synthesis. Thus, the newly formed lipid molecules would in many cases immediately be introduced into the region of the fissure thereby preventing the leakage of intracellular hydrophilic molecules.

ACKNOWLEDGEMENTS

This work was supported by Public Health Service Grant GM-20395.

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