THE CONTROL OF FATTY ACID SYNTHESIS IN LACTOBACILLUS PLANTARUM[1]

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There is, at present, little information available concerning control mechanisms in bacterial lipid metabolism, particularly in regard to fatty acid synthesis. Hofmann, et al., (1959), found that Lactobacillus delbrueckii failed to synthesize 16 or 18 carbon monoenoic acids when grown with lactobacillic acid substituted for biotin. Goldfine and Bloch (1961) reported that incorporation of acetate-1-C^{14} into cellular lipids of Clostridium butyricum was decreased by 85 per cent when biotin was replaced by oleic acid. In both of these instances, however, the absence of biotin made it impossible to assess the effect of the fatty acids upon synthesis. We have found recently that the synthesis of long-chain fatty acids by Lactobacillus plantarum in a complex medium---with biotin---is inhibited by low levels (10^{-5}M) of long-chain unsaturated or cyclopropanoid acids, while saturated acids have little effect. We conclude that these findings show the presence of a negative feedback control mechanism. Evidence in support of these conclusions is presented in this report.

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MATERIALS AND METHODS

L. plantarum ATCC 8014 was grown in stationary culture in air, in a complex medium which contained per liter: 10 g glucose, 7.5 g yeast extract, 12.5 g trypticase, 6.5 g sodium acetate ($NaC_2H_3O_2.3H_2O$), 0.1 mg thiamine.HCl, 5 g K_2HPO, 0.8 g $MgSO_4$, 0.14 g $MnCl_2$, and 0.04 g $FeSO_4.7H_2O$. Unless otherwise specified, fatty acids were added as Triton X 100 emulsions to give a concentration of 250 mg Triton and 50 mg fatty acid per liter of medium. Cellular fatty acids were analyzed by gas chromatography and assayed for radioactivity as previously described (Henderson et al., 1965). All radioactive counts have been corrected for any slight differences in growth.

RESULTS AND DISCUSSION

In a series of experiments, L. plantarum was grown in the presence of palmitic, stearic, oleic, elaidic, or dihydrosterculic acids, each acid being added singularly. Control cultures, containing 1) no fatty acids or Triton, and 2) no fatty acids plus Triton, were also grown. The most striking feature of these experiments is shown in Figure 1. When dihydrosterculic acid is added to the growth medium, only traces of other fatty acids are found in the cell lipids, showing almost complete inhibition of fatty acid synthesis. Both oleic and cis-vaccenic acids have similar effects, except for the presence of the C_{19} cyclopropanoid acids derived from them. Elaidic acid, the trans-isomer of oleic, behaves in like fashion, but much less of the cyclopropane derivative is formed. On the other hand, saturated fatty acids, while apparently being incorporated directly into cell lipid, have little effect on the synthesis of fatty acids in general. Cells grown in the presence of Triton with no fatty acids added, have a fatty acid composition almost identical to the untreated control culture. The above results are summarized in Table 1.

We then carried out a study of the effect of fatty acids added to the medium, upon the rate of incorporation of acetate-1-C^{14} into long-chain

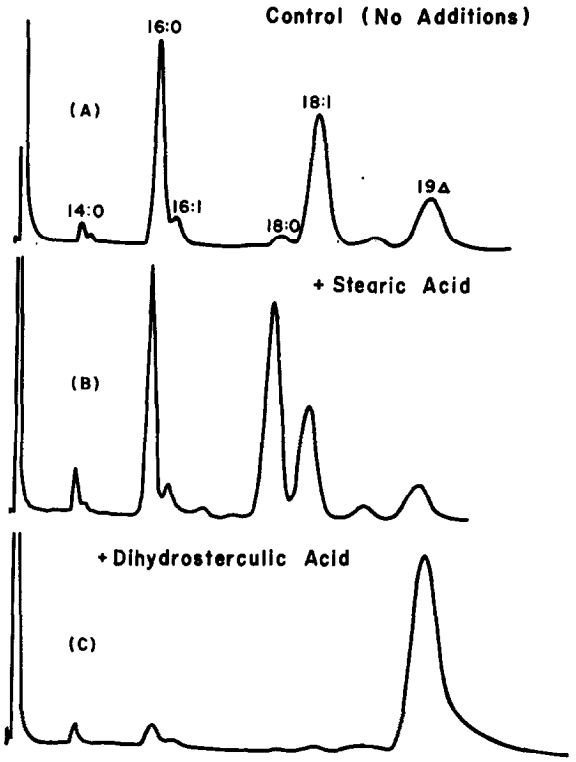

Figure 1: Chromatograms of the fatty acids of L. plantarum, showing the effect of long-chain fatty acids added to the medium. The number to the left of the colon indicates the number of carbon atoms in the fatty acid chain, while the number to the right indicates the number of double bonds; Δ represents a cyclopropane ring.

fatty acids by growing cultures. Sodium acetate-1-C^{14} was added to the 16-hour old cultures of L. plantarum. One hour later, fatty acids were added. Samples were taken quickly thereafter at regular intervals, and the radioactivity of the total fatty acid fraction determined. The results of this experiment, Figure 2, demonstrate that the addition of either oleic acid or dihydrosterculic acid causes an immediate decrease in the differential rate of acetate-1-C^{14} incorporation. This decrease is about twice that observed with stearic acid, while palmitic acid or Triton caused little change in the incorporation rate.

We then attempted to determine more specifically those reactions in fatty acid synthesis that are inhibited by unsaturated and cyclopropanoid

fatty acids. Previous experiments in our laboratory indicated that the L. plantarum fatty acid synthetase system is similar to the system found in Escherichia coli (Goldman and Vagelos, 1962). Bortz and Lynen (1963) had demonstrated that rat liver acetyl-CoA carboxylase is inhibited in vitro by the long-chain acyl-CoA esters of either palmitic, stearic, or oleic acids. It was reasoned, therefore, that if the acetyl-CoA carboxylase enzyme is the primary site of metabolic control in L. plantarum, then addition of malonic acid could have two possible effects. First, if malonate undergoes direct activation to the Coenzyme A ester, the site of inhibition would be by-passed. Secondly, since malonate is known to be

Table 1. Effect of Exogenous Fatty Acids on the Fatty Acid Composition of Lactobacillus plantarum

	FATTY ACID ADDITIONS TO MEDIUM[2]						
Fatty Acid[1]	None	Triton Control	16:0	18:0	18:1[3] cis	18:1 trans	19Δ
	Cellular Fatty Acid Composition (%)						
14:0	1.4	3.2	2.6	2.3	4.2	1.2	1.8
14:1	0.7	1.0	0.3	0.8	____	____	0.4
16:0	29.2	30.0	51.7	23.1	4.1	3.7	4.3
16:1	4.9	6.4	2.8	4.0	1.3	2.5	2.0
18:0	2.8	2.1	7.2	35.1	____	____	0.3
18:1	38.0	38.1	20.5	21.0	62.4	80.7	1.7
19Δ	22.4	19.1	14.9	12.5	27.9	11.9	89.5

[1]The number to the left of the colon indicates the number of carbon atoms, while the number to the right indicates the number of double bonds; Δ indicates a cyclopropane acid.

[2]Where indicated, 250 mg Triton and 50 mg fatty acid present per liter.

[3]Results were similar for both oleic and cis-vaccenic acids.

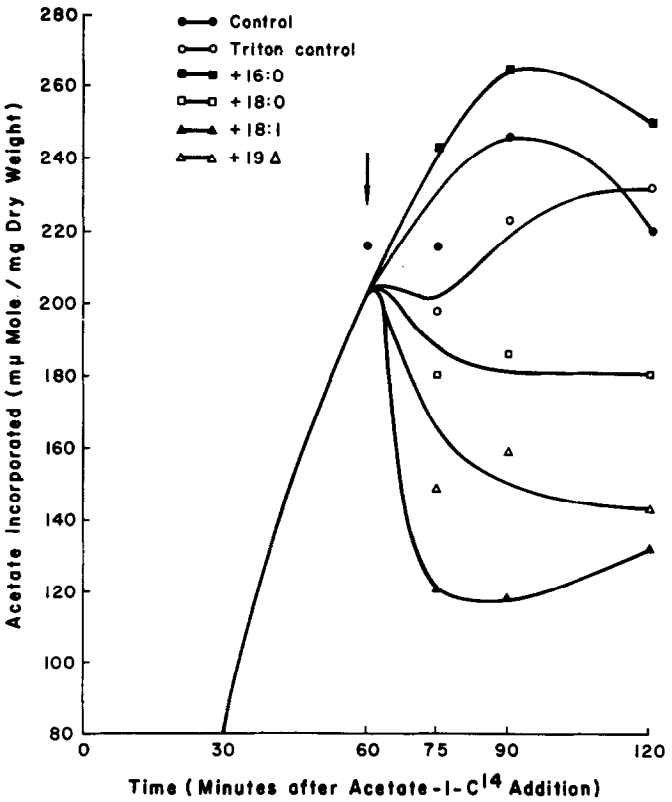

Figure 2: Differential plot showing effects of added fatty acids on acetate-1-C^{14} incorporation into the total fatty acid fraction of L. plantarum. Fatty acids added at 60 min (↓).

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an allosteric activator of acetyl-CoA carboxylase, then stimulation of enzyme activity might occur. In either case, reversal of inhibition could be the result.

In view of this, we studied the effect of malonic acid on acetate-1-C^{14} incorporation in the presence of dihydrosterculic acid. Tubes containing 10 ml of medium were inoculated and incubated at 33° for 64 hours. Each tube contained 476 μmoles (1.0μC) of sodium acetate-1-C^{14} and various combinations of dihydrosterculic acid and malonic acid (476 μmoles, when present).

Table 2 presents the results of this experiment. In the presence of dihydrosterculic acid, there was only 18% as much acetate-1-C^{14}

Table 2. Malonic Acid Reversal of Dihydrosterculate Inhibition of Acetate-1-C^{14} Incorporation into Fatty Acids.

Additions to Medium[1]	Acetate-1-C^{14} Incorporation	
	DPM	Relative (%)
Control	3730	100
Malonate	3870	104
Dihydrosterculate	680	18
Dihydrosterculate + Malonate	1510	40

[1] All tubes contained 0.25 mg Triton per ml. Where indicated, 169 μM dihydrosterculic acid and 47.6 μM malonic acid were added per ml of medium.

incorporated into fatty acids as was found with the control. When malonate was present also, dihydrosterculic acid inhibition was reversed so that there occurred 40% of the incorporation observed with the control, i.e., a twofold release of inhibition. These results indicate that acetyl-CoA carboxylase is a possible site of control.

We then studied the incorporation of acetate-1-C^{14} into cellular lipids, as a function of oleic acid concentration, and also determined the amount of oleic acid-1-C^{14} incorporated into the cells at various concentrations of oleic acid in the medium. Inoculated tubes contained 10 ml of medium and either 1μC of sodium acetate plus increasing levels of oleic acid, or increasing levels of oleic acid-1-C^{14} (specific activity 1μC/mg) with unlabeled acetate. All tubes contained 0.25 mg Triton per ml and were incubated for this experiment at 33° for 64 hours.

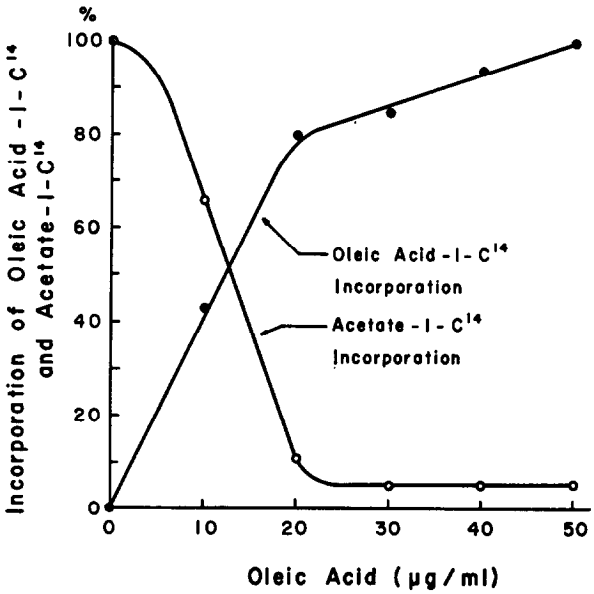

Figure 3: Incorporation of oleic acid-1-C^{14} and acetate-1-C^{14} into L. plantarum lipids as a function of oleic acid concentration in medium. Acetate-1-C^{14} incorporated in absence of oleic acid taken as 100% (= 1170 dpm/mg dry wt.). Oleic acid-1-C^{14} incorporation at highest oleic acid concentration, 50 µg/ml (= 36,350 dpm/mg dry wt.), also 100%. Relatively low counts from acetate-1-C^{14} due to dilution by cold acetate in medium.

The results of these experiments, presented in Figure 3, show an inverse relation between the uptake of oleic acid from the medium and the incorporation of C^{14}-acetate into long-chain fatty acids by growing cultures of L. plantarum. The amount of oleic acid-1-C^{14} incorporated is directly proportional to oleic acid concentration at lower levels ($\leq$ 20 µg/ml), with a loss of direct proportionality and decreased relative rate of incorporation at higher concentrations. On the other hand, the incorporation of acetate-1-C^{14} is inversely proportional to oleic acid concentration at lower concentrations, with nearly maximal inhibition occurring in the presence of 20 µg of oleic acid per ml.

On the basis of these observations, we conclude that the synthesis of long-chain fatty acids by growing cultures of L. plantarum is inhibited by the presence of long-chain unsaturated and cyclopropane fatty acids,

with saturated 16- and 18-carbon acids having little inhibitory effect. The nature of the inhibition is not completely established at this time. However, the rapidity with which the inhibition occurs (Figure 2) demonstrates the operation of a negative feedback mechanism. The reversal of dihydrosterculic acid inhibition by malonic acid indicates that at least one site of inhibition is the acetyl-CoA carboxylase enzyme.

It is also possible that unsaturated and cyclopropane fatty acids are inhibiting fatty acid synthesis by competing with acetate for Coenzyme A or acyl carrier protein sites. However, such an explanation is not consistent with effects observed with saturated acids, or with the reversal of inhibition by malonate. The ability of both trans-and cis-unsaturated acids and cyclopropanoid acids, to inhibit de novo synthesis of fatty acids, indicates the occurrence of enzyme inhibition, with an interruption of the aliphatic carbon chain being a stereochemical requirement for effective metabolic control. The low concentration of oleic acid (7.25×10^{-5}M), that produces nearly complete inhibition, likewise indicates direct enzyme inhibition.

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