REGULATION OF CYCLOPROPANE FATTY ACID BIOSYNTHESIS BY VARIATIONS IN ENZYME ACTIVITIES

L. A. Halper and S. J. Norton
Department of Chemistry
North Texas State University
Denton, Texas 76203

Received December 16,1974

It is proposed that cyclopropane fatty acid biosynthesis in Lactobacillus plantarum is regulated by in vivo variations in the activities of two enzymes acting sequentially. S-adenosylhomocysteine hydrolase relieves the end-product inhibition of cyclopropane synthetase by degrading a product (S-adenosylhomocysteine) of the latter enzyme activity. Both enzymes show an abrupt increase and subsequent decrease in activity at a time during the bacterial growth cycle which corresponds to the period of most rapid synthesis of cyclopropane fatty acid in vivo.

INTRODUCTION

Many bacterial species contain cyclopropane fatty acids in membrane phospholipids (1). Several workers have reported that these esterified acids reach maximal levels as the bacteria pass from logarithmic into stationary growth (2-4). The factors involved in regulating the rate of biosynthesis of the cyclopropane ring have not been reported.

We report here experiments performed with Lactobacillus plantarum which indicate a possible regulatory mechanism for the synthesis of cyclopropane fatty acids. Regulation is apparently effected by variations in the activities of two enzymes working sequentially. The first enzyme, cyclopropane synthetase, catalyzes the formation of cyclopropane fatty acid by the S-adenosylmethionine-dependent methylation of unsaturated fatty acids in the phospholipid molecule. The products are phospholipid cyclopropane fatty acids and S-adenosylhomocysteine. It

---

Abbreviations: SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine.

is significant that SAH has been shown to inhibit cyclopropane synthetase *in vitro* (5). The second enzyme in this sequence, S-adenosylhomocysteine hydrolase (SAH hydrolase), hydrolyzes SAH to form adenine and ribosylhomocysteine. The presence of SAH hydrolase in bacterial extracts was reported some years ago (5,6), but this paper presents the first evidence of involvement of the enzyme in regulation of biosynthesis of cyclopropane fatty acids.

METHODS AND MATERIALS

L. plantarum (ATCC 8014) was grown on hydrolyzed casein medium at 37°C. Cell growth was monitored by following turbidity at 540 nm, and samples were harvested at various times after the cells had reached logarithmic growth. The harvested cells were suspended in five times their volume of 0.05 M potassium phosphate buffer at pH 7.0. Cell-free extracts were prepared by disrupting the suspension in a Braun Model MSK mechanical cell homogenizer using glass beads of 0.10-0.11 mm diameter. After centrifugation at 20,000g, the supernatant solution was removed from the pellet. Membrane lipids were extracted from the pellet with chloroform:methanol (2:1,v,v). After esterification with boron trichloride-methanol (7), the fatty acid spectra were analyzed by gas chromatography using a Beckman GC 72-5 chromatograph. A portion of each cell-free extract was dialyzed against 0.05 M potassium phosphate buffer at pH 7.0 for 20 hours. Enzyme assays were performed on both the undialyzed and the dialyzed cell-free extracts.

Cyclopropane synthetase was assayed by the method of Goldfine (8) using [^{3}H]methyl-S-adenosylmethionine (New England Nuclear), and a sonicated dispersion of *L. plantarum* phospholipids as substrates. SAH hydrolase was assayed using the triphenyltetrazolium chloride test for reducing sugars (9) with commercial SAH (Sigma Chemical Company) as substrate. The NAD-independent D- and L-lactate dehydrogenases were assayed together by the method of Snoswell (10). All enzyme assays were performed in duplicate.

RESULTS AND DISCUSSION

Figure 1 shows the variations in enzyme activities in sample extracts as a function of bacterial culture age. The first sample was taken when the bacteria were still in exponential growth; the second, third, and fourth samples were taken during post-exponential growth; the fifth sample was taken after the cells had reached the stationary phase; the sixth sample was

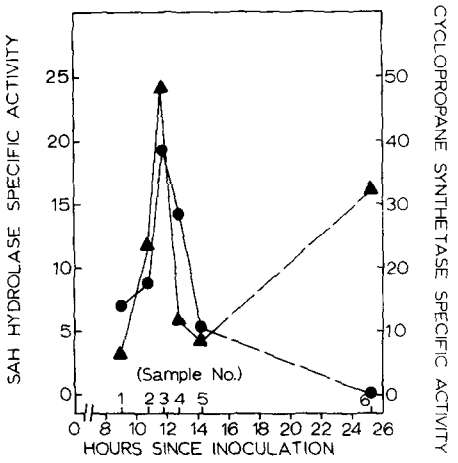

Figure 1: Variations in the activities of cyclopropane synthetase and SAH hydrolase in L. plantarum passing from exponential into stationary growth. ▲, specific activity of cyclopropane synthetase, expressed as (cpm mg^{-1} 30 min^{-1}) X 10^{-3}; ●, specific activity of SAH hydrolase, expressed as (μmole ribosylhomocysteine mg^{-1} 30 min^{-1}) X 10.

The cyclopropane synthetase incubation mixture contained 0.28 mg lipid, 10 nmoles [^{3}H]methyl-S-adenosylmethionine (0.9 μCi), and 0.05 ml cell-free extract. Total volume was 0.1 ml. Incubation was carried out at 30°C for 30 minutes.

SAH hydrolase incubation mixture contained 0.5 μmoles SAH, 10 μmoles potassium phosphate at pH 6.5, and 0.05 ml cell-free extract. Total volume was 0.2 ml. Incubation was carried out at 30°C for 30 minutes.

---

taken after the cells had been in the stationary phase for several hours. Each datum point represents the average specific activity of two samples harvested at the same time, but independently disrupted and assayed. The data shown are for the dialyzed extracts; activities of the undialyzed extracts followed the same curve shape and are omitted for simplicity. As the bacteria grow into the late exponential phase, the activities of cyclopropane synthetase and SAH hydrolase remain relatively low. There is an abrupt increase in the activities of both enzymes during post-exponential growth, and a subsequent rapid decrease as the bacteria enter the stationary phase. After the cells have remained in the stationary phase for several hours,

the activity of cyclopropane synthetase is seen to have increased, whereas that of SAH hydrolase has decreased to an extremely low level.

Low *in vitro* cyclopropane synthetase activities in certain samples could be due to the presence of high activities of enzymes which degrade SAM (e.g., SAM decarboxylase). Therefore, an experiment was performed in which sample 3 extract was added to a reaction mixture previously incubated with sample 5 extract. It was found, using the appropriate controls, that the activities of cyclopropane synthetase from samples 3 and 5 were additive. Therefore, the low synthetase activity of sample 5 could not be due to substrate depletion during the incubation period.

We determined that the observed changes in enzyme activities were likely not caused by inadvertant variations in sample preparation by examining the activity of the NAD-independent D- and L-lactate dehydrogenases in each sample. The specific activities of the dehydrogenases were found to be constant in all of the growth samples, thus indicating that the observed changes in cyclopropane synthetase and SAH hydrolase activities were characteristic for those enzymes.

We propose that when both the synthetase and hydrolase are at maximal activities, the organism will produce cyclopropane fatty acid at the greatest rate, since product inhibition of cyclopropane synthetase will be relieved by degradation of SAH by the hydrolase. To support this proposal, we determined that a high activity of SAH hydrolase does prevent *in vitro* inhibition of cyclopropane synthetase. Cyclopropane synthetase was assayed in the presence of 0.1 mM SAH. The synthetase of sample 3, which is high in SAH hydrolase activity, was inhibited only 15%,

TABLE I

Content and Rate of Production of Cyclopropane Fatty Acid in L. plantarum Passing from Exponential into Stationary Growth

Sample	Cyclopropane Fatty Acid* (% of Total Fatty Acids)	Rate of Production (Increase in %/Hour)
#1	1.7	-
#2	2.8	0.6
#3	21.7	18.9
#4	24.2	2.5
#5	37.5	8.9
#6	53.3	1.4

*Methyl esters were prepared as in Methods and Materials. Esters were analyzed by gas chromatography using a 1/8" column of 15% diethyleneglycol succinate on Chromosorb W at 170°C.

whereas the synthetase of sample 6, which has a low activity of hydrolase, was inhibited 80%. Changes in the relative activities of cyclopropane synthetase and SAH hydrolase could, therefore, determine the rate of cyclopropane fatty acid synthesis.

In accord with this proposal, Table 1 shows the percent content and rate of production of cyclopropane fatty acids in the various samples. It is significant that the greatest rate of synthesis occurs during the period when both enzymes are reaching their maximal activities. Although the late stationary cells contain considerable synthetase activity, the rate of cyclopropane fatty acid production is quite low. Apparently, product inhibition by SAH dominates during this growth phase due to low SAH hydrolase activity. Thus, high synthetase activity is not sufficient by itself to cause rapid production of cyclopropane fatty acids. The relative activities of both enzymes is the regulating factor.

CONCLUSION

These studies indicate that the production of cyclopropane fatty acids in L. plantarum is, at least in part, regulated by the enzymes cyclopropane synthetase and SAH hydrolase. High activities of SAH hydrolase prevent product inhibition of the synthetase by SAH. When both enzymes are at maximal activities, the organism produces cyclopropane fatty acid at the greatest rate. Studies now in progress are designed to determine whether induction and degradation phenomena are involved in this proposed mechanism of regulation.

Acknowledgement: This work was supported in part by a grant from The Robert A. Welch Foundation of Texas, Grant No. B-133.

REFERENCES

1. Christie, W. W. in Topics in Lipid Chemistry (Gunstone, F. D., ed.) John Wiley & Sons, New York (1970) I, 6-7.
2. Law, J. H., Zalkin, H., and Kaneshiro, T. (1971) Biochim. Biophys. Acta, 70, 143-151.
3. Croom, J. A., and McNeill, J. J. (1961) Bact. Proc., p. 170.
4. Kates, M., Adams, G. A., and Martin, S. M. (1964) Can. J. Biochem. 42, 461-479.
5. Chung, A. E., and Law, J. H. (1964) Biochemistry 3, 1989-1993.
6. Duerre, J. A. (1962) J. Biol. Chem. 237, 3737-3741.
7. Brian, B. L., and Gardner, E. W. (1967) Appl. Microbiol. 15, 1499-1500.
8. Goldfine, H. (1966) J. Lipid Res. 7, 146-149.
9. Mattson, A. N., and Tensen, C. O. (1950) Anal. Chem. 22, 182-184.
10. Snoswell, A. M. in Methods in Enzymology (Wood, W. A., ed.) Academic Press, New York (1966) IX, 321-322.