Acyl Carrier Protein Metabolism and Regulation of Fatty Acid Biosynthesis by *Lactobacillus plantarum*

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Endogenous fatty acid biosynthesis in the bacterium *Lactobacillus plantarum* is greatly decreased upon addition of exogenous fatty acids (Henderson, T. A., and McNeil, J. J. (1966) Biochem. Biophys. Res. Commun. 25, 662-669). We have demonstrated the presence of five pantothenate-containing compounds in *L. plantarum* which have been identified by co-chromatography with authentic samples: pantothenate, 4'-phosphopantetheine, 3'-dephospho-Coenzyme A, coenzyme A, and acyl carrier protein (ACP). The concentrations of the above pantothenate-containing compounds were found to be: 0.009, 0.13, 0.067, 0.69, and 0.22 nmol/mg of protein, respectively. *L. plantarum* ACP was shown to have a molecular weight near that of *Escherichia coli* ACP but to have a lower isoelectric point (pI = 3.75). Oleate in the presence of Triton X-100 was found to reduce the concentration of ACP by 80% with little effect on the concentrations of the other pantothenate-containing compounds. The synthesis of ACP apparently ceased soon after the addition of oleate and the rate of decrease in concentration of ACP was quantitatively consistent with the previously observed rate of decrease in the initial rate of fatty acid biosynthesis in this organism (Weeks, G., and Wakil, S. J. (1970) J. Biol. Chem. 245, 1913-1921). Thus, the change in rate of fatty acid biosynthesis in *L. plantarum* upon addition of oleate to the medium can be quantitatively related to the concentration of ACP (and probably to the concentrations of co-repressible enzymes of fatty acid biosynthesis).

Coenzyme A (CoA), a pantothenate-containing cofactor, participates in a great variety of biochemical reactions in which acyl groups are transferred or metabolized. In bacteria and in plants, acyl carrier protein, also a pantothenate-containing cofactor, is a specific and soluble cofactor for fatty acid biosynthesis (1). Acyl carrier peptides are apparently also components of the high molecular weight complexes found in mammals (2), birds (3), and in yeast (4). In all these cases acyl groups are covalently bound as thioesters to the terminal thiol group of the 4'-phosphopantetheine moiety. The pantetheine-P prosthetic group is common to both CoA and ACP, and CoA has been shown to be the precursor for the biosynthesis of the prosthetic group of ACP in *Escherichia coli* (5, 6). Powell et al. (6) have developed a model in which the pantothenate-containing metabolites in *E. coli* are biosynthesized in the following order:

\[ \text{Pantothenate} \rightarrow \text{pantetheine-P} \rightarrow \text{dephospho-CoA} \rightarrow \text{CoA} \rightarrow \text{ACP} \]

An exchange between intracellular pantetheine-P and the exogenous pantothenate and pantetheine-P of the medium and the rapid turnover (loss and concomitant replacement) of the pantetheine-P prosthetic group of ACP were obligatory features of the model.

We have chosen to extend previous studies on the metabolism of pantothenate and the prosthetic group of ACP by investigating pantothenate metabolism in the gram-positive bacterium *Lactobacillus plantarum*. This organism requires pantothenate for growth and has been used extensively for the bioassay of pantothenate (7). The mechanism of this effect was attributed by Henderson and McNeil (8) to feedback inhibition of acetyl-CoA carboxylase. Weeks and Wakil (9) have presented data which support repression of both ACP and enzymes of fatty acid biosynthesis in *L. plantarum* as the significant regulatory event to account for the decrease in

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*This work was undertaken by J. E. S. in partial fulfillment of the requirements of the M.S. degree in chemistry. The work was presented on October 24, 1974, at the Southeastern Regional American Chemical Society Meeting in Richmond, Va.

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The abbreviations used are: ACP, acyl carrier protein; dephospho-CoA, 3'-dephospho-Coenzyme A; pantetheine, 4'-phosphopantetheine; apoACP, acyl carrier protein lacking the 4'-phosphopantetheine prosthetic group.

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4706
endogenous fatty acid biosynthesis in this organism. The existence of these regulatory features in *L. plantarum* together with the opportunity for characterization of common features of pantothenate metabolism led us to the following studies.

**EXPERIMENTAL PROCEDURES**

[1-14C]Pantothenate (4.3 mCi/mmol), [9,10-3H]oleic acid (800 mCi/mmol), and [U-14C]isoleucine (1,700 mCi/mmol) were purchased from New England Nuclear Corp. [1-14C]Pantothenate was bioassayed (7) giving a specific activity of 9.5 mCi/mmol. 4'-Phosphopantetheine, 4'-phosphopantothenic acid, and 4'-phosphopantelythycysteine were the kind gifts of Dr. O. Nagase (Central Research Laboratory, Daiichi Seiyaku Co., Ltd., Tokyo). [1-14C]Pantothenate-labeled acyl carrier protein prepared from *Escherichia coli* was a gift from Dr. P. R. Vagelos (Merck, Sharp & Dohme Research Laboratories, Rahway, N.J.). Coenzyme A was purchased from P-L Biochemicals and dephospho-coenzyme A was prepared according the method of Wang et al. (10). Acrylamide and N,N'-methylenebisacrylamide were purchased from Eastman Kodak. Acrylamide was recrystallized from chloroform (11) and methylenebisacrylamide was recrystallized from acetic (11). N,N'-Diallyl tartardiamide (Serva) was used without purification. Ammonium hydroxide pH 3 to 5 were purchased from E. Merck. Acrylamide was recrystallized from chloroform (11).

**Concentration of Pantothenate-containing compounds** were the only parameters of interest, a partially defined commercial medium was employed (Bacto-Pantothenate A.O.A.C. Medium, Difco Laboratories Inc.). Filter-sterilized sodium [1-14C]pantothenate or unlabeled, filter-sterilized calcium pantothenate was added to a final concentration of 0.5 μM. Triton X-100, oleate, and water were autoclaved and added to the medium to achieve a final concentration of 100 μg of Triton X-100 and 20 μg of oleate per ml of medium. When oleate or palmitate were employed alone, they were dissolved in the hot medium after autoclaving, with the aid of a magnetic stirring bar, at a concentration of 10 mg/200 ml of medium.

When the stability of the protein of ACP was to be assayed, a defined medium was prepared in which the casamino acids present in the Bacto-pantothenate medium (Difco) was replaced with 10 mM M dithiothreitol and further fractionated by disc gel electrophoresis. The gels were sliced and assayed for radioactivity as previously described (14, 15). When the turnover of ACP in exponentially growing cells in the absence of oleate was determined the pooled ACP from *DEAE-cellulose fractionation* was further purified by chromatography on A-25 DEAE-Sephadex (Pharmacia) as previously described (15).

**Identification of Pantothenate-containing Metabolites from Lactobacillus plantarum** was verified by comparison with the elution conductivity of authentic standards under identical fractionation conditions. The standards employed included 4'-phosphopantetheine and 4'-phosphopantelythycysteine. Cell-free radioactive medium could be diluted with deionized water at 4°C until the conductivity could be diluted with deionized water at 4°C until the conductivity was below 400 μS/cm and was dialyzed against an anion exchange resin, AG 1-X8 (Bio-Rad) in the formate form. The radioactive compound (pantothenate) was eluted from the column by a convex gradient of ammonium formate (0 to 1.5 M) in 0.6 M formic acid.

**Characterization of L. plantarum ACP**—The isoelectric point was determined by the method of Wrigley (16) and the molecular weight estimated by gel filtration on a small column (0.5 x 10 cm) of Sephadex G-50 (Pharmacia) equilibrated with 20 mM KPi (pH 6.2), 0.3 mM LiCl, and 10 μM in 1.8 M in β-mercaptoethanol using blue dextran (Pharmacia), cytochrome c (Sigma Chemical Co.), [14C]isoleucine, and authentic E. coli [14C]ACP as molecular weight standards. The radioactive compound was pooled and dialyzed against 2 liter of 10 mM M dithiothreitol and further fractionated by disc gel electrophoresis.

**Radioassay**—Samples were assayed in a toluene/Triton X-100 scintillation mixture (17) adjusted to a final concentration of 10% water and assayed in the Packard model 3320 liquid scintillation spectrometer. Dual-labeled samples were analyzed using programmable calculators or a PL/I program for the IBM 370/158 computer.

**RESULTS**

Since incomplete information was available (18) on the kinds and amounts of the pantothenate-containing metabolites present in *Lactobacillus plantarum* (ATCC 8014) we made these measurements first. The organism was grown on medium supplemented with [1-14C]pantothenate for 10 generations and the extracts fractionated using DEAE-cellulose. A typical separation is shown in Fig. 1. The compounds were identified by comparison of the observed elution conductivities of the radioactive with the elution volumes of samples of authentic standards. We found five [14C]pantothenate-containing compounds to be present in appreciable quantities in *L. plantarum*: pantothenate, ACP, CoA, diphospho-CoA, and pantetheine-P. The component requiring the highest salt concentration for elution upon DEAE-cellulose chromatography was tentatively identified as ACP. Upon disc gel electrophoresis we observed a noticeably faster mobility for this fraction as compared with the ACP of *Escherichia coli*. Since we wished to be certain we were working with the ACP and not some smaller pantothenate derivative, we proceeded to characterize this compound more fully. We compared the molecular weight of the *L. plantarum* compound with that of *E. coli* ACP using gel filtration; the similar elution volumes (Fig. 2) suggest that the molecular weights of these compounds are similar. The isoelectric point of this fraction from *L. plantarum* was found to be 3.75 (Fig. 3) somewhat lower than the value of 4.2 found for *E. coli* ACP (1). *L. plantarum* extracts show

**ACP in Lactobacillus plantarum** 4707
biological ACP activity (9). Thus, we concluded that this fraction was indeed ACP, the higher mobility of L. plantarum ACP upon disc gel electrophoresis is presumed to be a consequence of the more acidic nature of the protein; not a molecular weight difference as compared with the ACP of E. coli.

The concentrations of these pantothenate-containing compounds were determined by pooling the fractions containing one component and determining the total radioactivity. The specific activity of all these pantothenate-containing compounds must be the same as the pantothenate of the medium after 10 generations since the organism depends entirely on the exogenous supply for growth. Thus, the concentrations (nanomoles per mg of protein) of each of these compounds in the extracts was calculated from the radioactivity of the isolated compounds and the specific activity of the pantothenate added to the medium. The average concentrations obtained for exponentially growing cells are given in Table I. Cells harvested from medium containing growth-limiting concentrations of [14C]pantothenate (0.05 μM) have greatly reduced concentrations of all the pantothenate-containing compounds including ACP (not shown). This observation contrasts with the E. coli system where the concentration of ACP is maintained at the apparent expense of the other pantothenate-containing compounds (5). Since later experiments deal with the effects of Triton X-100 on the concentrations of these compounds, we determined these concentrations in the presence of the detergent in exponentially growing cells. Table I shows that with Triton X-100 alone concentrations of ACP and pantetheine-P (at three different cell densities) are apparently decreased to 64% and 69%, respectively. However, when oleate is present together with Triton X-100 the average concentration of ACP is decreased to 19% of the control level. Oleate alone does not decrease the ACP concentration, nor does palmitate, a saturated fatty acid, either in the presence or absence of Triton X-100 (Table I).

To learn how rapidly L. plantarum adjusts its ACP concentration in response to exogenous oleate we grew cells on medium containing [14C]pantothenate (and supplemented with [3H]isoleucine). At a suitable cell density we harvested
The values given are triplicate determinations except for the experiments in which palmitate was one of the supplements. Those values were from duplicate experiments but in the two cases where no standard deviations are indicated, were single determinations.

<table>
<thead>
<tr>
<th>Addition</th>
<th>ACP</th>
<th>CoA</th>
<th>Panthenate-P</th>
<th>DeoxycoA-CoA</th>
<th>Panthothenate</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.22 ± 0.03</td>
<td>0.69 ± 0.02</td>
<td>0.13 ± 0.01</td>
<td>0.067 ± 0.004</td>
<td>0.009 ± 0.009</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>0.14 ± 0.01</td>
<td>0.52 ± 0.06</td>
<td>0.09 ± 0.03</td>
<td>0.063 ± 0.007</td>
<td>0.014 ± 0.004</td>
</tr>
<tr>
<td>Palmitate</td>
<td>0.22 ± 0.01</td>
<td>0.24 ± 0.16</td>
<td>0.03 ± 0.03</td>
<td>0.14 ± 0.08</td>
<td>0.002 ± 0.002</td>
</tr>
<tr>
<td>Palmitate &amp; Triton X-100</td>
<td>0.39 ± 0.06</td>
<td>1.3 ± 0.03</td>
<td>0.4</td>
<td>0.2</td>
<td>0.009 ± 0.0004</td>
</tr>
<tr>
<td>Oleate</td>
<td>0.3 ± 0.1</td>
<td>0.8 ± 0.4</td>
<td>0.19 ± 0.3</td>
<td>0.16 ± 0.16</td>
<td>0.009 ± 0.009</td>
</tr>
<tr>
<td>Oleate &amp; Triton X-100</td>
<td>0.042 ± 0.005</td>
<td>0.5 ± 0.06</td>
<td>0.075 ± 0.007</td>
<td>0.07 ± 0.01</td>
<td>0.06 ± 0.03</td>
</tr>
</tbody>
</table>

Table 1
Concentrations of pantothenate-containing compounds in Lactobacillus plantarum

Fig. 4. The effect of oleate on ACP mass. The solid points are experimental data for 14C in ACP (9.5 mCi/nmol). The dotted lines are generated assuming growth of the initial mass of ACP at some fraction (0.1x, 0.5x and full growth) of the growth rate, μ, of the cells. Triton X-100 was present.

and washed these cells and at time zero we resuspended them in medium containing Triton X-100, oleate, and [14C]pantothenate (supplemented with unlabeled isoleucine). Samples were taken at suitable intervals, extracted, and fractionated on DEAE-cellulose. From the 14C/pantothenate specific activity the mass of each pantothenate-containing compound was calculated. Our experimental results (points) are shown in Fig. 4 along with theoretical curves assuming that ACP is no longer synthesized (no growth) after addition of oleate or that it is synthesized at fractions of the normal exponential growth rate required to keep the concentration of ACP a constant function of cell mass (μ = 1.0). We found that the other pantothenate-containing compounds continued to increase exponentially (constant intracellular concentration) after the addition of Triton X-100 and oleate with the exception of pantothenate, which increased more rapidly than predicted.

The decreasing ACP concentration could be the result of several processes including breakdown and resynthesis of the protein portion of the ACP molecule. Accordingly we had designed our experimental procedure on the effects of oleate (above) to test the possibility of turnover of the protein moiety as well. The cells in the previous experiment, which were grown with [3H]isoleucine, washed, and resuspended in medium containing Triton X-100 and oleate in the presence of unlabeled isoleucine, were extracted and fractionated on DEAE-cellulose. The ACP fraction was further purified by disc gel electrophoresis and assayed for radioactivity. A typical disc gel separation is shown in Fig. 5. The ratio of 3H to 14C is a measure of the isoleucine content (nmol) per nmol of pantothenate-containing prosthetic group. If the protein were being broken down and resynthesized, isoleucine would be lost from the ACP. Since the ACP mass is constant after addition of oleate (Fig. 5) a constant ratio of SH to 14C (Fig. 6) suggests that no [3H]isoleucine is lost from the protein. Thus, no turnover of the ACP protein is occurring. We have also looked for turnover of the protein of ACP in the absence of oleate in exponentially growing cells. The experimental design was similar but further fractionation of the ACP was carried out on DEAE-Sephadex. The experimental values for 3H/14C decreased about as anticipated (15) (data not shown). Thus, the protein of ACP does not turnover and the changes observed are a consequence of repression of synthesis of ACP not breakdown.

DISCUSSION
As first recognized by Henderson and McNeil (8), L. plantarum is one of a few bacteria that respond to the availability of fatty acids in the medium by markedly decreases.
ACP in Lactobacillus plantarum

The concentrations of these metabolites in L. plantarum were all found to decrease to a low level upon depletion of exogenous pantothenate in contrast to E. coli cells which maintain the ACP concentrations while the concentration of the remaining pantotheinate-containing compounds is decreased (5, 6). We have not further studied the influence of exogenous pantotheinate concentrations on the endogenous concentration of these metabolites.

We have also collected in vivo kinetic data for the metabolism of the pantothenate-containing compounds in L. plantarum. Inclusion of an endogenous pool of pantothenate in addition to the exogenous pool:

\[
\text{Pantothenate} \rightarrow \text{exogenous} \rightarrow \text{endogenous}
\]

introduce one more independent variable into the differential equations describing the most suitable (and simplest) model. Thus this model is not determinate as was the most suitable model in the case of E. coli (6) and an extraneous parameter had to be arbitrarily specified. With this reservation we found that a successful model for L. plantarum included all the features of the model describing pantothenate metabolism in E. coli including rapid turnover of the prosthetic group of ACP (1/%) of the total L. plantarum ACP pool per min) and exchange of endogenous pantothenate with the medium. Two other kinds of enzymes present in microorganisms are now known to possess pantetheine-P prosthetic groups, the antibiotic polyolipid-synthesizing complexes such as the tyrosine-synthesizing polypeptide of Bacillus brevis (24, 25) and the citrate ligase of Klebsiella aerogenes (26, 27). The existence of two other kinds of enzymes possessing the same prosthetic group might permit one to establish whether turnover of the pantetheine-P prosthetic group is a common characteristic of such enzymes or is specific for those enzymes involved in lipid biosynthesis.

We found that oleate, in the presence of Triton X-100, exerted a relatively specific effect (within the precision of the measurements) in decreasing the ACP concentration in L. plantarum. The effect of the unsaturated fatty acid, oleate, was anticipated from previous results on the effects of unsaturated and cyclopropane fatty acids, but not saturated fatty acids, in decreasing the rates of fatty acid biosynthesis in L. plantarum (9). Triton X-100 alone seems to decrease the ACP concentration somewhat but apparently not in combination with palmitate. Thus, the Triton X-100 plus oleate effect is not simply a surfactant effect. Detergent may be required to provide the fatty acid in a suitably dispersed form at the cell surface or may alter the permeability of the cell membrane. The latter mechanism might explain the large increase in endogenous pantotheinate observed when Triton X-100 and oleate were added to cells in the presence of exogenous [14C]pantothenate.

The mechanism of control of endogenous fatty acid biosynthesis in L. plantarum is controversial. Henderson and McNeil (8) proposed that the major controlling effect is feedback inhibition of acetyl-CoA carboxylase by fatty acyl-CoA compounds. Early kinetic studies of the rat liver acetyl-CoA carboxylase support this idea (28) and recent in vivo studies suggest that such control may be of major importance in mammalian systems (29, 30). Weeks and Wakil (9), on the other hand, have shown that the enzymatic activities of malonyl-CoA-ACP transacylase, \( \beta \)-ketoacyl-ACP synthetase, \( \beta \)-ketoacyl-ACP reductase, \( \beta \)-hydroxoyacyl-ACP dehydrase, \( \beta \)-hydroxyoctanoyl-ACP dehydrase, and enoyl-ACP reductase are reduced about 80%. The activities of acetyl-CoA carboxylase and the overall conversion of [14C]acetate into fatty acids are reduced even more. They observed a little more than 2-fold decrease in ACP concentration when cells grown in the presence of Triton X-100 were compared with cells grown in the presence of oleate and Triton X-100 as measured by an
ACP in Lactobacillus plantarum

We have shown that ACP synthesis in cultures of L. plantarum is repressed essentially instantaneously upon the addition of exogenous oleate and is maintained (without turnover of ACP protein) for at least two generations at a constant mass equal to that present at the time of addition of oleate. Since the cells are growing exponentially, the concentration of ACP (mass of ACP per unit of cell mass) will decrease exponentially with time. If fatty acids are synthesized in these cells at a rate directly proportional to ACP concentrations (or at a rate directly proportional to the concentration of any of the enzymes of fatty acid biosynthesis coordinately repressed with ACP) one can predict that the instantaneous rate of fatty acid biosynthesis is exactly one-half as rapid after a doubling of cell mass after addition of oleate. This predicted decrease in rate is essentially what has been observed (Weeks and Wakil, Fig. 3B, in Ref. 9). Thus, the kinetics of repression of ACP (or co-repressible enzymes of fatty acid biosynthesis or both) can quantitatively account for the degree of control of fatty acid biosynthesis in L. plantarum following the addition of exogenous fatty acids.

Acknowledgments—We would like to acknowledge the work of Miss Nancy Jo Law carried out as part of the National Science Foundation Student Training Project in biology for talented high school students at Clemson in the summer of 1973. We also want to express our appreciation to Dr. Henry R. Bungay III, now of Worthington Biochemical Corp., for introducing us to the use of the CSMP computing package at Clemson University. We also gratefully acknowledge the help of Dr. J. J. McNeil, North Carolina State University, for timely suggestions during the initial stages of this work. G. L. Powell acknowledges Clemson University and the National Science Foundation for support while on sabbatical leave and for secretarial help with the manuscript in the laboratories of O. H. Griffith and P. C. Jost, Molecular Biology Institute, University of Oregon.

REFERENCES

17. Patterson, M. A., and Green, R. C. (1965) Anal. Chem. 37,
ACP in Lactobacillus plantarum

854–861
Acyl carrier protein metabolism and regulation of fatty acid biosynthesis by Lactobacillus plantarum.
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