JOURNAL OF BACTERIOLOGY, May 1996, p. 2662–2667 Vol. 178, No. 9
0021-9193/96/$04.00+0

The Apparent Coupling between Synthesis and Posttranslational Modification of *Escherichia coli* Acyl Carrier Protein Is Due to Inhibition of Amino Acid Biosynthesis

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Received 8 September 1995/Accepted 23 February 1996

Acyl carrier protein (ACP) is modified on serine 36 by the covalent posttranslational attachment of 4′-phosphopantetheine from coenzyme A (CoA), and this modification is required for lipid biosynthesis. Jackowski and Rock (J. Biol. Chem 258:15186–15191, 1983) reported that upon depletion of the CoA pool by starvation for a CoA precursor, no accumulation of the unmodified form of ACP (apo-ACP) was detected. We report that this lack of apo-ACP accumulation results from decreased translation of the *acpP* mRNAs because of the limitation of the synthesis of glutamate and other amino acids made directly from tricarboxylic acid cycle intermediates.

The fatty acids that constitute the membrane lipids of *Escherichia coli* are assembled through a series of reactions that are highly conserved in nature and that result in progressive two-carbon additions to the growing fatty acid chain. Acyl carrier protein (ACP) functions as the carrier of fatty acids throughout these reactions as well as in the transfer of the completed fatty acids to glycerol 3-phosphate, the first committed step of phospholipid biosynthesis. Fatty acids are attached to ACP through a thioester linkage to the sulfhydryl group of a phosphopantetheine moiety covalently bound to ACP (21). The 4′-phosphopantetheine moiety is transferred from coenzyme A (CoA) to a specific serine of ACP (serine 36) by the enzyme holo-ACP synthase (7, 20). The prosthetic group can be enzymatically removed by the action of ACP phosphodiesterase (9, 29), which cleaves the phosphodiester bond between Ser-36 and the phosphate of the 4′-phosphopantetheine, resulting in formation of apo-ACP and free 4′-phosphopantetheine. The actions of holo-ACP synthase and ACP phosphodiesterase constitute a putative futile cycle, and this cycle has been hypothesized to play a role in the regulation of fatty acid biosynthesis (8, 28), but few data are available.

Several groups have reported that in vivo pools of ACP consist almost entirely of holo-ACP (ACP modified by 4′-phosphopantetheine attachment); little or no apo-ACP is present (8, 13, 18). However, differing results have been reported for cells in which the cellular CoA pool was depleted by starvation of strains auxotrophic for pantothenate (a CoA precursor) or β-alanine, a pantothenate (hence CoA) precursor. In the first report, a small accumulation (<25%) of the unmodified (apo) form of ACP was found. However, interpretation of the results of these experiments was complicated by the indirect assay used to assay apo-ACP. Later, direct measurements of ACP pool compositions by Jackowski and Rock (13) gave an opposing result. By use of a direct gel electrophoretic assay, these workers were unable to detect accumulation of apo-ACP in cells with depleted CoA pools. The trivial explanation, that apo-ACP is unstable in vivo, was ruled out by the large accumulation of apo-ACP observed in a mutant strain (*acpS*) deficient in holo-ACP synthase (13). Since the *acpS* mutant has a normal CoA pool, it seemed possible that the lack of apo-ACP synthesis in CoA-depleted cells could be explained if CoA is a positive regulator of ACP transcription or translation. This putative regulatory system would prevent accumulation of apo-ACP, a species recently shown to be a potent inhibitor of cell growth (18). We now report a reexamination of the question of apo-ACP accumulation during pantothenate starvation and show that the results of Jackowski and Rock (13) are due to an unexpected limitation of the amino acid supply engendered by depletion of the CoA pools.

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MATERIALS AND METHODS

Bacterial strains and media. The culture medium used was either rich broth (6) or minimal salts medium E (5) supplemented with 0.4% glucose and 0.1% vitamin-free casein hydrolysate (Difco). Spectinomycin (30 μg/ml), ampicillin (100 μg/ml), kanamycin (40 μg/ml), tetracycline (10 μg/ml), pantothenate (120 nM), and β-alanine (5 μM) were added as required. Solid media contained 1.5% agar. All cultures were grown at 37°C. The growth of liquid cultures was monitored with a Klett-Summerson colorimeter equipped with a green filter. All strains used in this work are derivatives of *Escherichia coli* K-12. Strain SJ16 (*metB1 relA1 spoT1* λ⁻ λʳ *gyrA216 panD2 zad-220*::Tn*10*) (12) was used in all CoA depletion experiments. Strain YZ3 (*polA1 metB1 relA1 spoT1* λ⁻ λʳ *gyrA216 panD2 zig-621*::Tn*10*) was used as the host strain for all of the CoA depletion experiments involving the *acpP*::*lac* and *acpP*::*cat* fusion constructs and control plasmids.

Plasmid pDK688 was constructed by ligating the *lacZ*-containing *Pst*I fragment of pMC1871 (2) into pKK223-3 (Pharmacia) digested with *Pst*I, placing *lacZ* under control of the *tac* promoter. Plasmid pYZ2 was constructed by ligating the *Sma*I-*Sal*I fragment of pMC1871 containing the *lacZ* into the *Pvu*II site of the *acpP* gene of plasmid pMR24 (26), which results in an in-frame translational fusion of *lacZ* to *acpP*. Plasmid pMR29 was constructed by ligating the promoterless *cat* gene containing a *Hin*dIII fragment of pCM1 (Pharmacia) into pMR24 (26), which had been digested with *Pvu*II. The result is a transcriptional fusion of the *cat* gene with *acpP*.

The copy number of the fusion plasmids used in this study was reduced to 1 to 2 copies per cell by the technique of Henry and Cronan (11). This procedure employs a mutant derivative of Tn*1000* present on the F plasmid that is unable to resolve the cointegrates formed as the initial reaction of transposition. When introduced by conjugation into a *polA* strain (which prevents normal plasmid replication), the replication of these cointegrates becomes dependent on the F plasmid and the cointegrates have a copy number of 1 to 2 copies per cell (11).

Depletion of intracellular CoA pools. Intracellular CoA pools were reduced by culturing a strain auxotrophic for β-alanine (a precursor of CoA) in the absence of supplement. Briefly, SJ16 (a β-alanine auxotroph) was cultured in minimal medium E supplemented with 0.4% glucose and 0.01% methionine until the cells reached early log phase (ca. 3×10^8 cells per ml). The cells were then washed free of the supplements by trapping the cells on a sterile 0.45-μm filter (Nalgene) and washing with an excess of medium lacking any supplements except glucose.

The cells were then resuspended in the same medium and cultured in the presence or absence of β-alanine.

Analysis of ACP pools. Extracts were also prepared by a nondenaturing method as described previously (13). Briefly, the cells were cultured as described above, and the cells were collected by centrifugation at 16,000 × *g* and resuspended in lysis buffer (50 mM Tris-HCl [pH 7], 6 mM EDTA, 10% Triton X-100, 0.6 mg of lysozyme per ml). The suspension was then quickly frozen and kept at −80°C until ready for fractionation. Upon thawing, the suspension was centrifuged at 16,000 × *g* to remove particulate matter and fractionated on a 20% polyacrylamide gel as described previously (13). Upon completion of electrophoresis, the gel extracts were visualized by staining with Coomassie blue.

ACP pool compositions were also analyzed by urea-polyacrylamide gel electrophoresis (urea-PAGE) as described previously (25). Briefly, extracts were prepared as described above, urea was added to 2.5 M, and then sample buffer was added to a total volume of 50 μl. Samples were then applied to a 13% polyacrylamide gel containing 2.5 M urea for a run of approximately 2.5 h at 15°C. Upon completion of electrophoresis, the gel extracts were visualized by staining with Coomassie blue.

Transcriptional analysis of *acpP*. Analysis of the transcription of *acpP* was performed by Northern (RNA) analysis as described previously (26). Briefly, cultures of SJ16 were grown to log phase and then washed free of β-alanine as described above. At various time intervals, samples were taken for RNA extraction (19), and the RNA samples were fractionated on a formaldehyde gel (22). After electrophoresis, the RNA was then transferred to a nylon membrane and immobilized by UV irradiation with a Stratalinker (Stratagene). The membrane-bound RNA was then hybridized to a ^{32}P-labeled probe generated by random priming of an *acpP*-containing *Taq*I fragment. The membrane was then washed with 0.5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 57°C for 30 min to remove nonspecific binding of labeled probe and exposed to a Molecular Dynamics PhosphorImager screen for quantitation.

Assay of β-galactosidase. Derivatives of strain YZ3 which contain the *acpP*::lac and pKK223-3::lac protein fusions in single copy were cultured in the presence of β-alanine until the cells reached log phase. The cells were then washed free of the supplements as described above and cultured in the presence or absence of β-alanine, and 1-ml samples were removed at various time points. β-Galactosidase activity was assayed as described previously (23).

Assay of chloramphenicol acetyltransferase activity. Transcriptional fusions of chloramphenicol acetyltransferase to *acpP* were depleted of intracellular CoA reserves as described above. The cells then were collected by centrifugation at 16,000 × *g* and resuspended in 10 mM Tris-HCl (pH 7; 100 μl), and extracts were prepared by sonication. The cell debris was removed from the extracts by centrifugation at 16,000 × *g*. The extracts were then assayed as described previously (27). The reaction mixture included 100 mM Tris-HCl (pH 7.8), 0.1 mM acetyl-CoA, 0.4 mg of 5,5′-dithiobis-2-nitrobenzoic acid per ml, and 10 to 20 μg of cell extract protein. Chloramphenicol (0.1 mM) was then added, and the increase in adsorption at 412 nm was measured with the aid of a spectrophotometer. One unit of activity is defined as 1 nmol of chloramphenicol-dependent thionitrobenzoic acid formed per minute.

RESULTS

An unusual form of ACP is present in CoA-depleted cells. In agreement with previous workers (8, 13), we were unable to detect apo-ACP in wild-type cells grown under normal culture conditions (Fig. 1). However, by employing the conformationally sensitive PAGE assay used by Jackowski and Rock (13), we detected a considerable accumulation of an unusual form of ACP in cells with depleted CoA pools resulting from starvation for β-alanine (Fig. 1). The electrophoretic mobility of this form of ACP on a conformationally sensitive polyacrylamide gel suggested that it was apo-ACP. To confirm the presence of apo-ACP, we purified the ACP fraction from β-alanine-starved cultures and analyzed it by electrospray mass spectrometry (17). The ACP fraction from β-alanine-starved cultures included a significant fraction of ACP which had the molecular weight (i.e., 8,548) expected for apo-ACP plus the N-terminal methionine (17). The presence of the methionine residue was confirmed by N-terminal amino acid sequencing. The β-alanine-starved cultures contained a small pool of holo-ACP, and the mass (8,995 Da by electrospray mass spectrometry) indicated that this species also retained the N-terminal methionine. These results suggest that the methionine aminopeptidase action was less efficient during CoA limitation. The amino-terminal methionine of ACP is normally removed efficiently (18), and our mass spectral analyses of purified ACP from cells with normal CoA pools has confirmed both the efficiency of N-terminal processing and the lack of apo-ACP (17). It should be noted that we utilized the same *E. coli* strain studied by Jackowski and Rock (13).

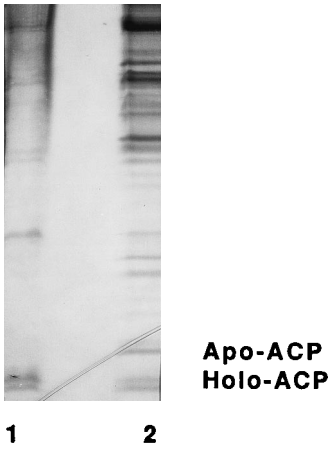

FIG. 1. Accumulation of apo-ACP in extracts of CoA-depleted cells. Strain SJ16 was grown to log phase in 10 ml of minimal medium E in the presence of 0.2% glucose, 1 μM β-alanine, and 0.1% casein hydrolysate. The cells were then retained on a filter, washed free of the supplements, and resuspended and cultured for 12 h in 100 ml of the same medium in the presence or absence of β-alanine. The culture grown in the presence of β-alanine (which grew to a higher final optical density) was diluted such that it contained a cell concentration that matched that of the culture grown without β-alanine. The cells were harvested and extracts were prepared as described in Materials and Methods. The extract proteins were then fractionated by conformationally sensitive PAGE as described in Materials and Methods and visualized by staining with Coomassie blue. Lanes: 1, strain SJ16 supplemented with β-alanine; 2, strain SJ16 lacking β-alanine. The doublet for holo-ACP is due to the presence of short-chain acyl-ACP species.

Detection of apo-ACP in CoA-limited cells cultured in the presence of exogenous amino acids. Comparison of our experimental conditions with those of Jackowski and Rock (13) suggested that a difference in growth media might account for the differing observations concerning apo-ACP accumulation. We cultured a β-alanine (*panD*) auxotrophic strain in minimal medium supplemented with vitamin-free casein hydrolysate as a source of amino acids, whereas the studies of Jackowski and Rock (13) were conducted with a minimal medium supplemented only with methionine (the strain used is a methionine auxotroph). To determine if the addition of amino acids allowed detection of apo-ACP, we depleted the CoA pools by starvation for β-alanine and examined the ACP species that accumulated in the presence or absence of the casein hydrolysate supplement. In the absence of casein hydrolysate, we could not detect the accumulation of apo-ACP (17), a result in agreement with that of Jackowski and Rock (13). However, in cultures supplemented with casein hydrolysate, a pool of apo-ACP was readily apparent (Fig. 1). There also appeared to be some conversion of apo-ACP to the holo form consistent with the presence of some nonesterified CoA in the starved cells (ca. 5 μM) (15) and the Michaelis constant of ACP synthase for CoA (50 μM) (20).

A straightforward explanation for the casein hydrolysate stimulation of ACP accumulation seen under CoA-limiting conditions is that a reduction in intracellular CoA concentration could have resulted in a decreased rate of amino acid synthesis and thus hindered translation of the *acpP* mRNAs. Glutamate is the major amino acid present in casein hydrolysate, constituting ca. 21% of the amino acids (1), and ACP is an unusually acidic protein and thus very rich in the amino acids

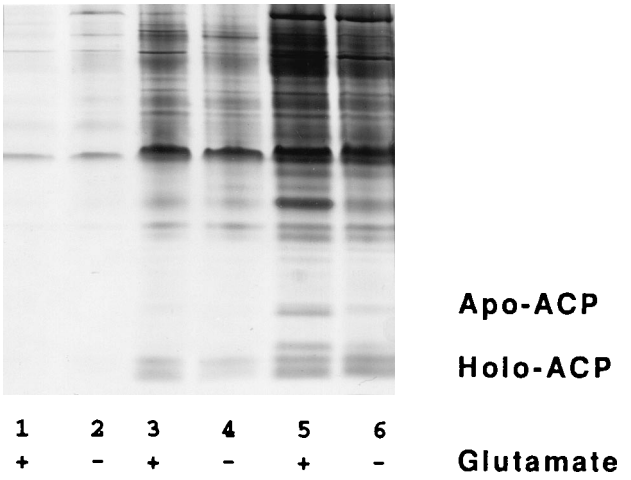

FIG. 2. Accumulation of apo-ACP in extracts of CoA-depleted cells supplemented with glutamate. Strain SJ16 was grown to log phase in minimal medium E supplemented with glucose and 0.01% methionine in the presence of 1 μM β-alanine. The cells were then retained on a filter, washed free of the supplements, and cultured in 60 ml of the same methionine-supplemented medium lacking β-alanine in the presence or absence of 0.01% sodium glutamate. Samples containing equal numbers of cells (10 to 20 ml) were removed, extracts were prepared by sonication, and the ACP species were partially purified by isopropanol fractionation. The 50% isopropanol supernatants were dialyzed against 20 mM Tris-HCl (pH 7), and the ACP species were recovered by acid precipitation. The partially purified ACP was then analyzed by urea-PAGE and stained with Coomassie blue as described in Materials and Methods. Urea-PAGE allows separation of ACP species on the basis of the posttranslational modification and acylation states (25). Lanes: 1, 3, and 5, preparations from cells grown in the presence of glutamate and harvested at 0, 2, and 4 h, respectively, after β-alanine removal; 2, 4, and 6, preparations from cells grown in the absence of glutamate and harvested at 0, 2, and 4 h, respectively. The doublet for holo-ACP is due to the short-chain acyl-ACP species. The amounts of total protein loaded per lane for the glutamate experiment were as follows: lane 1, 40 μg; lane 2, 66.4 μg; lane 3, 59.7 μg; lane 4, 64 μg; lane 5, 136 μg; and lane 6, 132 μg.

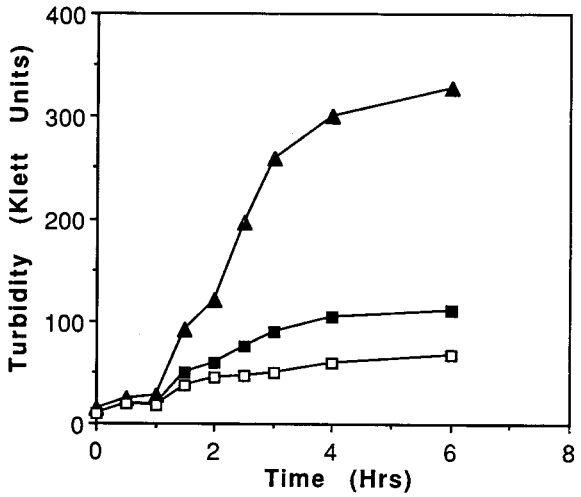

FIG. 3. Stimulation of growth in CoA-depleted cells upon addition of glutamate. Strain SJ16 was cultured in minimal medium E in the presence of methionine (0.01%) and β-alanine (1 μM) to log phase and then washed free of supplements. The cells were then resuspended in fresh medium and cultured in the presence or absence of β-alanine and glutamate. Turbidity was monitored with the aid of a Klett-Summerson colorimeter equipped with a green filter. Symbols: ▲, supplemented with methionine plus β-alanine; □, supplemented with methionine; ■, strain SJ16 supplemented with methionine and glutamate (0.1 mg/ml).

glutamate and aspartate derived by addition of ammonium directly to tricarboxylic acid (TCA) cycle intermediates. Acetyl-CoA is required for entry of carbon into the TCA cycle, and succinyl-CoA is a key intermediate of the cycle. Given that glutamate and aspartate plus the amidated forms of these acids, i.e., glutamine and asparagine, constitute 34% (26 of the 77 residues) of ACP and given the abundance of ACP in *E. coli*, a decreased flux through the TCA cycle could provide the preferential decrease in ACP synthesis relative to bulk protein synthesis reported by Jackowski and Rock (13). To test the role of the TCA cycle limitation in ACP synthesis, we replaced casein hydrolysate with methionine (the strain used is a methionine auxotroph) plus either glutamate or aspartate. The addition of glutamate (Fig. 2) restored apo-ACP synthesis and was nearly as effective as casein hydrolysate, whereas aspartate supplementation was relatively ineffectual. Thus, decreased glutamate synthesis seemed to be the major reason for the lack of apo-ACP accumulation in the cells having depleted CoA pools. In addition, supplementation with glutamate increased the growth rate and growth yield of the strain under conditions of CoA depletion (Fig. 3), indicating that these cells were deficient in this amino acid.

Transcription of *acpP* in CoA-depleted cells. The ability of amino acids to stimulate production of apo-ACP suggested that the primary problem was translational in nature. However, the possibility remained that part of the decrease in intracellular ACP seen during CoA depletion was the result of transcriptional regulation of *acpP*. The gene encoding ACP lies within a cluster of genes and is transcribed from three different promoters (31). We therefore analyzed transcription of *acpP* by Northern analysis and found no decrease in the level of the *acpP* mRNA species during CoA depletion (Fig. 4).

This result was confirmed by measuring the level of expression of a transcriptional fusion in which the *acpP* promoters drove chloramphenicol acetyltransferase expression. Depletion of CoA reserves did result in a decrease in *acpP*-dependent chloramphenicol acetyltransferase activity (Table 1).

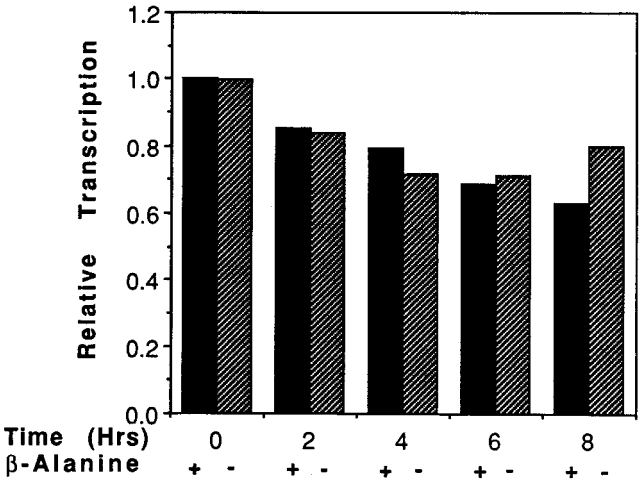

FIG. 4. Transcription of *acpP* during CoA limitation. Strain SJ16 was grown to log phase in minimal medium supplemented with 0.2% glucose, 0.1% casein hydrolysate, and 1 μM β-alanine. The cells were then washed free of the supplements and cultured in the same medium in the presence or absence of β-alanine as described in Materials and Methods. RNA was then extracted, fractionated by gel electrophoresis, blotted onto a membrane, and probed with a labeled *acpP*-containing fragment. The amount of radioactivity that remained bound to the membrane was then quantitated by use of a PhosphorImager. The numbers 0 to 8 refer to the times (in hours) at which samples were taken for the RNA preparation from the cultures.

TABLE 1. Chloramphenicol acetyltransferase activities of *acpP*::Cat transcriptional fusions[a]

Strain	β-Alanine	CAT activity at[b]:			
		0 h	2 h	4 h	6 h
acpP::Cat	+	456	392	579	810
acpP::Cat	−	569	387	408	612
pACYC184	+	665	1,008	1,305	931
pACYC184	−	585	898	1,045	804

[a] Derivatives of strain SJ16 that contain single-copy plasmids placing the chloramphenicol acetyltransferase (*cat*) gene under control of the *acpP* promoter were cultured in minimal medium to log phase, washed free of β-alanine as described in Materials and Methods, and cultured in the presence and absence of β-alanine. Samples were then removed at 0, 2, 4, and 6 h (time of starvation) and assayed for chloramphenicol acetyltransferase activity.

[b] Chloramphenicol acetyltransferase (CAT) activity was measured in cultures at 0, 2, 4, and 6 h after removal of the β-alanine supplement. Plasmid pACYC184 was used as the *acpP*-independent *cat* promoter. Activities are expressed in nanomoles of thionitrobenzoic acid formed per minute. The values given are the means of three separate trials.

However, the activity of an *acpP*-independent chloramphenicol acetyltransferase promoter (the *cat* gene present on plasmid pACYC184) also decreased during CoA depletion (Table 1). Comparison of the relative decreases in activity between the two promoters indicated no regulation of *acpP* transcription by intracellular CoA level.

Intracellular CoA concentration does not regulate translational initiation on the *acpP* mRNAs. The results presented above indicate that CoA limitation does not decrease *acpP* mRNA levels. However, the possibility remained that *acpP* expression was regulated at the level of translational initiation. We tested this possibility by use of a translational fusion which joined the *lacZ* gene to *acpP* in such a way that a fused protein was produced. The fusion retained the upstream noncoding sequence and the DNA sequence encoding the first 14 N-terminal amino acid residues of *acpP* plus all of the *lacZ* coding sequence except the first 8 residues. If initiation of *acpP* mRNA translation of *acpP* was regulated, then expression of β-galactosidase encoded by the *acpP-lacZ* fusion would be expected to decrease during CoA limitation as do ACP levels. The β-galactosidase activity of the *acpP-lacZ* fusion decreased upon CoA depletion (Table 2), but the rate of decrease was no greater than that seen for a control plasmid in which β-galactosidase was expressed from the *tac* promoter of plasmid pDK688. The comparable decreases in activity indicated that the decreased ACP level of CoA-depleted cells cannot be attributed to a specific defect in translational initiation of the *acpP* mRNAs.

TABLE 2. β-Galactosidase activities of *acpP*::*lacZ* translation fusions in CoA-depleted cultures[a]

Strain	β-Alanine	β-Galactosidase activity at[b]:			
		0 h	2 h	4 h	6 h
acpP::*lacZ*	+	480	903	1,040	1,032
acpP::*lacZ*	−	452	807	694	815
p*tac-lacZ*[c]	+	186	160	169	137
p*tac-lacZ*	−	74	38	44	54

[a] Strain SJ16 derivatives harboring a single-copy in-frame fusion of β-galactosidase to *acpP* were prepared as described in Materials and Methods and grown in minimal medium in the presence of methionine and β-alanine to log phase. The cells were then washed free of the supplements, resuspended in the same medium in the presence or absence of β-alanine, and cultured. Samples were removed at 0, 2, 4, and 6 h (time of starvation), and β-galactosidase activity was measured.

[b] Activities are expressed in Miller units (23). Values are the averages of three separate trials.

[c] The *tac* promoter of plasmid pDK688 (pKK223-3::*lacZ*) is insensitive to catabolite repression, thus allowing for measurement of β-galactosidase activity in cells grown in the presence of glucose.

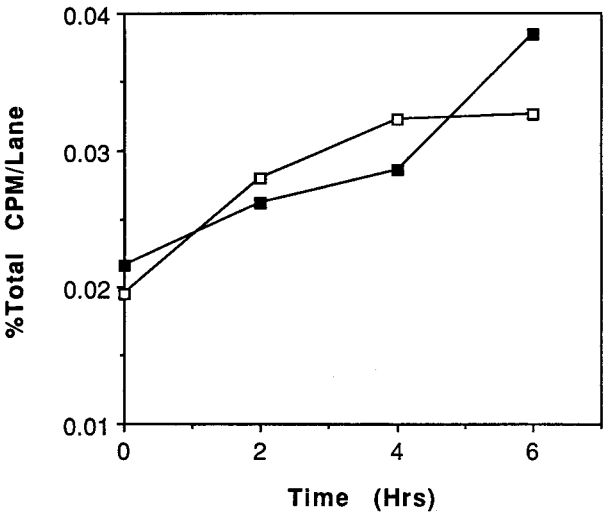

FIG. 5. Conversion of holo-ACP to apo-ACP during CoA depletion. Strain SJ16 was cultured in minimal medium containing 0.2% glucose, 0.1% casein hydrolysate, 1 μM β-alanine, and ^{35}S-Trans label (50 μCi/ml) to the log phase to label the intracellular ACP. The cells were washed free of the supplements and then cultured without β-alanine in medium plus cold methionine, in the presence or absence of 0.1% casein hydrolysate. Samples were removed at the time points listed at the bottom, and extracts were prepared. The samples were then fractionated by urea-PAGE, and the amount of apo-ACP was quantitated with a PhosphorImager. The amounts of apo-ACP are expressed as the percentages of the total amount of radioactivity present in the entire lane. Symbols: ■, apo-ACP levels in CoA-depleted cells cultured in the presence of casein hydrolysate; □, apo-ACP levels in CoA-depleted cells cultured in the presence of methionine.

Depletion of intracellular CoA does not stimulate conversion of holo-ACP to apo-ACP. An alternative explanation for the increase in apo-ACP seen under these conditions was that the addition of casein hydrolysate or glutamate stimulated ACP phosphodiesterase activity, thus yielding a pool of apo-ACP. However, depletion of CoA pools has been reported to give only a minor stimulation of the rate of ACP prosthetic group turnover (14, 17). Moreover, supplementation with casein hydrolysate had only a negligible effect on the conversion of holo-ACP (labeled before β-alanine starvation) to apo-ACP (Fig. 5). At later times (6 h after the onset of starvation), a small (ca. 1.5-fold) stimulation of this conversion was seen, but this was insufficient to explain the marked accumulation of apo-ACP seen earlier during starvation.

DISCUSSION

The prosthetic group of ACP provides the thiol group responsible for tethering fatty acid chains to ACP, and thus apo-ACP, which lacks the prosthetic group, cannot act as a fatty acid carrier. In addition, apo-ACP has recently been found to be toxic to *E. coli* through inhibition of lipid metabolism (18). These results raised the possibility that the synthesis of ACP might be coupled to the availability of the donor of the 4′-phosphopantetheine prosthetic group, i.e., CoA. However, our data give no evidence for coupling of ACP and CoA synthesis. The apparent coupling previously observed is an artifact of decreased amino acid (chiefly glutamate) synthesis that results from depletion of the intracellular pools of acetyl-

CoA. The lack of glutamate results in a decreased synthesis of apo-ACP, and much of the remaining apo-ACP synthesized is probably converted to holo-ACP by utilization of the low (but significant) residual supply of CoA.

Previous work showed that starvation for pantothenate precursors resulted in a drastic decrease in succinyl-CoA levels, with lesser effects on the levels of nonesterified CoA and acetyl-CoA (15). Jackowski and Rock (15) suggested that this loss of succinyl-CoA resulted in decreased amino acid and (hence) protein synthesis, which gave growth stasis. However, we found that supplementation of minimal medium with succinate or a mixture of methionine, diaminopimelic acid, and lysine (the amino acids synthesized from succinyl-CoA) failed to increase growth of CoA-depleted cultures or result in accumulation of apo-ACP in these cultures (17). It therefore seems that the decreased succinyl-CoA does not have a direct effect on protein synthesis but is another symptom of the low glutamate (hence 2-oxoglutarate) levels resulting from decreased TCA cycle function. Although glutamate is known to be the major TCA cycle-derived amino acid (4), it should be noted that 2-oxoglutarate derived by deamination of glutamate could enter the TCA cycle and thus become a source of aspartate carbon. In contrast, aspartate cannot become a source of glutamate carbon without input of acetyl-CoA. Consistent with this reasoning, the addition of aspartate failed to give accumulation of apo-ACP under CoA-limited conditions, indicating that glutamate plays the major role. Moreover, glutamate addition also resulted in increased total protein synthesis early in pantothenate starvation as well as increased growth.

A straightforward explanation for the glutamate stimulation of apo-ACP accumulation seen under CoA-limited conditions is that decreased levels of the acetyl-CoA required for entry of carbon into the TCA cycle result in a decreased rate of glutamate (and glutamate-derived amino acid) synthesis, resulting in hindered translation of the *acpP* mRNAs. ACP is an unusually acidic protein and is thus very rich (26 of the 77 residues) in the amino acids glutamate, glutamine, aspartate, and asparagine, which are derived by NH_2 addition to TCA cycle intermediates.

We envision the following scenario for the selective decrease in ACP synthesis seen upon glutamate starvation (it is important to note that we are unable to differentiate glutamate and glutamine). Glutamate is normally the most abundant intracellular amino acid of *E. coli* (1), and thus ACP synthesis is not normally limited by the supply of this amino acid. However, during CoA limitation, the synthesis of glutamate slows and becomes limiting to overall protein synthesis. In the absence of the cognate aminoacyl-tRNA, a ribosome has some probability of simply falling off the mRNA, resulting in premature translation termination (10, 16, 24). Under glutamate-limiting conditions, the synthesis of proteins with few glutamate residues would be favored because there will be few chances for ribosome stalling and dissociation. In contrast, translation of the *acpP* mRNAs should involve many more stalling events, one at each of the many glutamate codons. Any ribosome that remains on the mRNA after stalling at a glutamate codon will be again in jeopardy when the next glutamate codon is encountered. Thus, the sheer number of glutamate codons in *acpP* will result in few ribosomes completing translation of the message and the observed preferential inhibition of ACP synthesis. Moreover, ACP has five instances of 2 to 3 consecutive glutamate (or glutamine) residues.

An alternative explanation for the increase in apo-ACP seen under these conditions was that the addition of casein hydrolysate or glutamate stimulated the turnover of the holo-ACP prosthetic group, thus yielding a pool of apo-ACP. However, depletion of CoA pools results in only a minor stimulation of the rate of ACP prosthetic group turnover (14, 17). Moreover, supplementation with casein hydrolysate had only a very small effect on the turnover of the prosthetic group, although at later time points (6 h of starvation), a small (ca. 1.5-fold) stimulation of turnover was seen (17).

In conclusion, the apparent downregulation of ACP synthesis found under conditions of CoA limitation is an artifact of decreased translational efficiency arising from a decreased ability to synthesize glutamate and other TCA cycle-derived amino acids. Despite the indirect nature of the method utilized to detect the presence of apo-ACP during CoA depletion, the results of Elovson and Vagelos (8) are consistent with the results presented here. Interestingly, the extracts in which they detected apo-ACP were prepared from cells cultured in minimal media supplemented with glycerol as a carbon source. Growth on glycerol as a carbon source results in a ca. twofold decrease in the total concentration of CoA but results in a considerable change in the relative pool sizes of CoA esters (30). We have also found that depleting cells of CoA in the presence of glycerol resulted in detectable pools of apo-ACP (17). This suggests that the conflicting results seen previously in the literature were most likely caused by the media used.

The inhibitory effects of apo-ACP on acylation of glycerol 3-phosphate in vivo and in vitro raise the possibility that it could play a role in regulation of fatty acid synthesis in certain situations. However, the only currently known physiological situation under which a pool of apo-ACP can be detected is extended depletion of the CoA pools in the presence of amino acids. CoA is synthesized from amino acids and amino acid biosynthetic intermediates as precursors, so it is difficult to imagine that a physiological condition exists where the cells would produce amino acids but not CoA. However, so little is currently known about the enzymes that interconvert holo-ACP and apo-ACP that this turnover could play an important regulatory role under conditions that have not yet been tested.

ACKNOWLEDGMENTS

The work was supported by National Institutes of Health grant AI15650.

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