JOURNAL OF BACTERIOLOGY, Dec. 1981, p. 926-932
0021-9193/81/120926-07$02.00/0

Vol. 148, No. 3

Regulation of Coenzyme A Biosynthesis

SUZANNE JACKOWSKI AND CHARLES O. ROCK*

Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee 38101

Received 29 May 1981/Accepted 1 August 1981

Coenzyme A (CoA) and acyl carrier protein are two cofactors in fatty acid metabolism, and both possess a 4'-phosphopantetheine moiety that is metabolically derived from the vitamin pantothenate. We studied the regulation of the metabolic pathway that gives rise to these two cofactors in an *Escherichia coli* β-alanine auxotroph, strain SJ16. Identification and quantitation of the intracellular and extracellular β-alanine-derived metabolites from cells grown on increasing β-alanine concentrations were performed. The intracellular content of acyl carrier protein was relatively insensitive to β-alanine input, whereas the CoA content increased as a function of external β-alanine concentration, reaching a maximum at 8 μM β-alanine. Further increase in the β-alanine concentration led to the excretion of pantothenate into the medium. Comparing the amount of pantothenate found outside the cell to the level of intracellular metabolites demonstrates that *E. coli* is capable of producing 15-fold more pantoic acid than is required to maintain the intracellular CoA content. Therefore, the supply of pantoic acid is not a limiting factor in CoA biosynthesis. Wild-type cells also excreted pantothenate into the medium, showing that the β-alanine supply is also not rate limiting in CoA biogenesis. Taken together, the results point to pantothenate kinase as the primary enzymatic step that regulates the CoA content of *E. coli*.

In *Escherichia coli*, thioesters of acyl carrier protein (ACP) function as substrates in the biosynthesis of fatty acids (32), and thioesters of coenzyme A (CoA) are substrates for fatty acid β-oxidation (4, 22). Both compounds possess a 4'-phosphopantetheine moiety that terminates in the biologically active sulfhydryl group. The biosynthesis of CoA in mammals and *E. coli* proceeds through five enzymatic steps, starting with the vitamin, pantothenic acid (6). The first step is the phosphorylation of pantothenate to 4'-phosphopantothenate by pantothenate kinase. Condensation of 4'-phosphopantothenate with cysteine is followed by a decarboxylation reaction to yield 4'-phosphopantetheine. Next, the 5'-AMP moiety of ATP is added to form dephospho-CoA, which is subsequently phosphorylated on the 3'-hydroxyl to form CoA. CoA serves as the 4'-phosphopantetheine donor in the biogenesis of ACP (7, 13, 23). *E. coli*, in contrast to mammals, is capable of synthesizing pantothenate (6) via the condensation of pantoic acid and β-alanine (18). Aspartate-1-decarboxylase is responsible for the formation of β-alanine (11, 35), and pantoate is formed from α-ketoisovaleric acid by a hydroxymethyltransferase (6, 24, 31) followed by an NADPH-dependent reductase (34).

Although the intracellular levels of CoA have been reported to vary by as much as sixfold, depending on the carbon source of the media (17), little is known about the regulation of CoA biosynthesis. Current concepts of regulation have focused on the control of β-alanine or pantoate formation since pantothenate synthetase activity appears to be present in large excess (18, 20). Powers and Snell (24) have suggested that ketopantoate hydroxymethyltransferase activity may be limiting on the basis of the in vitro inhibition of this enzyme by pantoate and CoA (24). On the other hand, Cronan (11) has suggested that β-alanine formation is the rate-limiting step in pantothenate biosynthesis and perhaps also rate limiting in CoA biosynthesis. Our interest in the regulation of lipid synthesis in *E. coli* led us to examine in more detail the mechanisms that control the intracellular content of CoA and ACP.

MATERIALS AND METHODS

Chemicals. Sources for supplies were P-L Biochemicals, Inc., CoA and [1-^{14}C]palmitoyl-CoA; Whatman, DEAE-cellulose (DE-52); Analabs, 250-μm Silica Gel H plates; Amersham Corp., ACS scintillation solution; New England Nuclear Corp., β-[3-^{3}H]alanine (specific activity, 38.1 Ci/mmol), D-[1-^{14}C]pantothenic acid (specific activity, 55.2 mCi/mmol), and Enhance spray; ICN Pharmaceuticals, [2-^{3}H]acetyl-CoA (spe-

cific activity, 15 Ci/mmol); Sigma Chemical Co., lysozyme, alkaline phosphatase, β-alanine, and D-pantothenate. All other chemicals and solvents were reagent grade or better.

Bacterial strains. All strains were derivations of *E. coli* K-12, and the media and transduction and transposition methods were those given previously (8, 9, 15). The experiments were performed with strain SJ16 (*panD*), which is defective in aspartate 1-decarboxylase. To construct a *panD* strain and facilitate moving the *panD* allele strain, we constructed SJ3 from strain YA139 (*panB*) by transduction with P1 phage grown on a random pool of tetracycline-resistant isolates of strain W1485::Tn*10*. Strain SJ3 was selected by its ability to grow on tetracycline without pantothenate, and the transposon insertion was found to be 98% contransducible with *panB*. Strain SJ10 was derived from the parent, strain AB354 (*panD*), by transduction with P1 phage grown on SJ3. Tetracycline-resistant recombinants were selected and scored for the *panD* allele. Strain SJ16 is a *panD zad*220::Tn*10* isolate from a transduction of strain UB1005 with P1 grown on strain SJ10. Strain UB1005 is a spontaneous nalidixic acid-resistant derivative of W1655F– (3).

Preparation of standards. ACP and CoA were resolved by DEAE-cellulose chromatography (DE-52) with a 0 to 0.55 M LiCl gradient. The elution position of CoA was 0.18 M LiCl, and that of ACP was 0.37 M LiCl, close to the values reported previously (23). The 4′-phospho[^{3}H]pantetheine standard was prepared by treating ACP (25) at pH 12, 37°C for 22 h in the presence of a 1,000-fold excess of dithiothreitol. Using this protocol, one radioactive species possessing an R_f of 0.43 in solvent I was obtained. However, harsher reaction conditions (higher pH, higher temperature, or omission of dithiothreitol) resulted in the additional formation of a more polar compound (R_f = 0.21; solvent I) which we believe to be the sulfonic acid of 4′-phosphopantetheine formed from the base-catalyzed cleavage of 4′-phosphopantetheine dimers. [^{3}H]pantetheine was prepared by treating 4′-phospho[^{3}H]pantetheine with 0.8 Sigma units per ml of bacterial alkaline phosphatase in 0.45 M Tris-hydrochloride, pH 8.0. [1-^{14}C]palmitoyl-ACP and [^{3}H]ACP dimers were prepared as described previously (26, 27). The 2′,4′-cyclophosphopantetheine was prepared by base-catalyzed hydrolysis of CoA as described by Michelson (19), and dephospho-CoA was prepared by alkaline phosphatase digestion of CoA, using the protocol described above.

Preparation of cell extracts. Extraction of β-alanine-derived metabolites was accomplished by a variation of the procedure of Clewell and Helinski (10). Washed cells were suspended in 0.5 ml of 25% sucrose–50 mM Tris-hydrochloride (pH 7.0) and allowed to stand for 5 min on ice. To this suspension 0.1 ml of lysozyme (5 mg/ml) in 0.25 M Tris-hydrochloride (pH 7.0) was added, and incubation was continued on ice for 5 min. Then 0.2 ml of 0.25 M EDTA (pH 7.0) was added, and after 5 min on ice 0.8 ml of 2-propanol was added. Next the solution was blended in a Vortex mixer and centrifuged at 10,000 × *g* for 10 min in a Sorvall SS-34 rotor to pellet precipitated cellular debris. The total extraction volume, but not the ratios or timing of the additions, was altered depending on the cell mass to be extracted. A minimum of 0.125 ml of 25% sucrose–50 mM Tris-hydrochloride (pH 7.0) was used for every 10 μl of cell pellet. In small-scale extractions, a 2-min spin in an Eppendorf microfuge sufficed for the last step. Extraction of radioactivity incorporated into SJ16 grown on 2 μM β-[3-^{3}H]alanine was >90%. The 2-propanol supernatant was spotted directly onto thin-layer plates or loaded onto DEAE-cellulose (see below).

Chromatography. When concentrated samples were desired or small quantities were to be processed, a batch elution procedure from DEAE-cellulose was used to separate CoA and ACP. The 2-propanol lysate was applied to a 0.5-ml column of Whatman DE-52 equilibrated with 10 mM bis-Tris, pH 6.5. The 2-propanol was washed from the column with 4 ml of 10 mM bis-Tris, pH 6.5. CoA was eluted with 3 ml of 0.25 M LiCl in 10 mM bis-Tris (pH 6.5), and ACP was eluted with 0.45 M LiCl in 10 mM bis-Tris, pH 6.5. Fraction size was 0.5 ml. Ion-exchange (23) or gel filtration (2) chromatography are the usual methods used to resolve CoA and ACP, but to process a large number of small samples for metabolic experiments, we routinely used thin-layer chromatography (Table

TABLE 1. *Mobilities of reference compounds on thin-layer plates*

Compound	R_f[a]	
	Solvent I	Solvent II
ACP	0.50	0.0
Palmitoyl-ACP	0.50	0.0
ACP dimer	0.50	0.0
CoA	0.28	0.0
Dephospho-CoA	0.40	0.14
Acetyl-CoA	0.28	0.0
Palmitoyl-CoA	0.46	0.0
Pantetheine	0.57	0.67
4′-Phosphopantetheine	0.43	0.0
2′,4′-Cyclophosphopantetheine	0.46	0.0
4′-Phosphopantetheine dimer	0.28	0.0
Pantothenate	0.57	0.61
4′-Phosphopantothenate	0.57	0.0
β-Alanine	0.36	0.28

[a] Thin-layer chromatography was performed on Silica Gel H layers developed in either solvent I (*n*-butanol-acetic acid-water, 5/2/4, vol/vol) or solvent II (ethanol-28% ammonium hydroxide, 4/1, vol/vol). Plates were developed to 14 cm from the origin, 0.5-cm sections were scraped into scintillation vials, and the Silica Gel was deactivated by the addition of 100 μl of water followed by 4 ml of ACS and counted to locate the position of the radioactive compounds. Reference radiolabeled compounds were prepared as described in the text. Recovery of radioactivity was at least 90% in all cases. Quenching was similar in all cases, as determined by the channels ratio method and a quench curve constructed with β-[3-^{3}H]alanine.

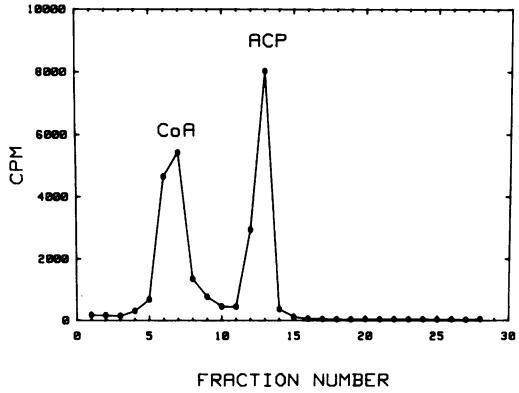

FIG. 1. *Separation of ACP and CoA by thin-layer chromatography. Shown is a thin-layer chromatogram of a Silica Gel H layer developed in solvent I as described in Table 1, footnote a. The sample was from a 2-propanol lysate of SJ16 grown on 0.5-μM β-[3-^{3}H]alanine. Fraction size was 0.5 cm. [^{3}H]ACP and [^{3}H]CoA prepared by DEAE-cellulose chromatography migrated identically to the peaks designated in the figure.*

1). Surprisingly, ACP had a faster mobility in solvent I than did CoA (Fig. 1) or other phosphorylated intermediates (Table 1). In all metabolic experiments, samples were run in both solvent I and II, which was sufficient to distinguish between all the compounds listed in Table 1. Two-dimensional chromatography was accomplished by development in solvent I followed by solvent II. Radioactive areas were located by spraying the plate with Enhance spray followed by fluorography at −80°C. Between these two chromatographic systems, positive identification of all metabolites listed in Table 1 was possible.

RESULTS

Utilization of β-alanine by strain SJ16. When strain SJ16 was grown overnight in the presence of 10 μM β-alanine and subcultured into media devoid of β-alanine, growth occurred equivalent to approximately four doublings before cessation. These results, which were observed repeatedly, suggested that strain SJ16 is capable of accumulating an intracellular reserve of β-alanine-derived metabolites. Thus, the total incorporation of β-alanine into strain SJ16 was determined as a function of β-alanine concentrations ranging from 0.5 to 32 μM (Fig. 2). Incorporation increased sevenfold as the β-alanine concentration was increased from 0.5 to 4 μM. Above that concentration, the response was less marked. Of the intracellular β-alanine derived metabolites, the CoA pool increased linearly from 3.5 to 27 pmol per 10^8 cells as a function of β-alanine concentration up to 4 μM β-alanine and reached a maximum level of 38 pmol per 10^8 cells at 8 μM (Fig. 3). ACP content, on the other hand, approximately doubled to an average of 3.4 pmol per 10^8 cells in the presence of increasing β-alanine. Pantothenate and 4′-phosphopantetheine were not detected at low β-alanine concentrations, but between 4 and 32 μM β-alanine, these metabolites increased steadily to levels of 8 and 7 pmol per 10^8 cells, respectively (Fig. 3). β-Alanine itself was only detected inside the cells at 32 μM external β-alanine (Fig. 3). Three β-alanine-derived metabolites, 4′-phosphopantothenoyl-1-cysteine, 4′-phosphopantothenate, and dephospho-CoA, were not found and were presumed to be below the level of detection. Taking into account the background radioactivity on our chromatograms, we set an upper limit of 3% on the concentration of these three metabolites with respect to the total pool.

The medium was also analyzed for β-alanine-derived metabolites in these same experiments (Fig. 4). At 0.5 μM β-alanine, 25% of the total label was found in the media. Greater than 88% of the extracellular label was found in 4′-phosphopantetheine with trace amounts of 4′-phosphopantothenate, β-alanine, pantothenate, and

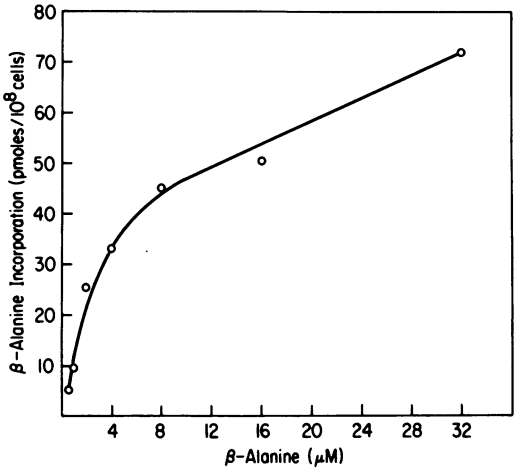

FIG. 2. *Incorporation of β-alanine into strain SJ16. Strain SJ16 was grown overnight at 37°C in minimal medium E (33) containing glucose (0.1%), methionine (0.01%), thiamine (0.001%), and β-alanine (10 μM). Cells were washed, suspended in the same media without β-alanine, and incubated for 7 h until growth had ceased. About 10^6 of the starved cells were used to inoculate 0.5 ml of the above media containing various concentrations of β-[3-^{3}H]alanine and grown overnight at 37°C in culture tubes. The specific activity of the 0.5 μM β-[3-^{3}H]alanine was 38.1 Ci/mmol and was decreased by half for each successively higher β-alanine concentration by the addition of an appropriate amount of cold β-alanine. With all β-alanine cultures, the final cell density was 3 × 10^9 colony-forming units per ml. Cells were harvested by centrifugation, washed twice with 1 ml of medium E, and assayed for incorporated radioactivity.*

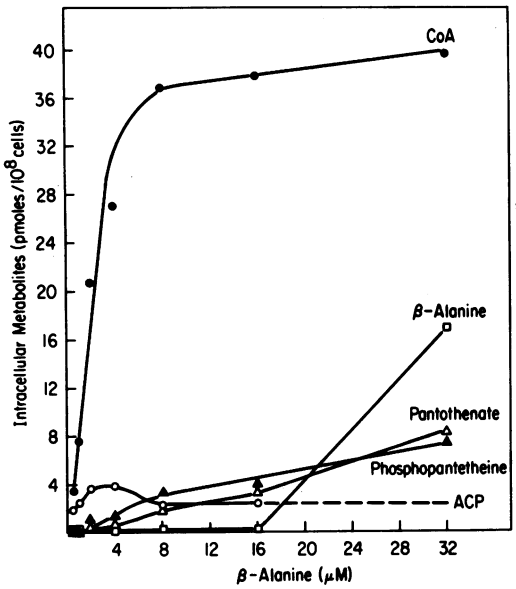

FIG. 3. *Utilization of β-alanine by strain SJ16: intracellular metabolites. The experimental protocol was the same as described in the legend to Fig. 2. Washed cells were extracted as described in the text, and the resulting 2-propanol lysates were chromatographed in both solvent I and II (Table 1) to identify and quantitate the intracellular β-alanine-derived metabolites. All samples were treated with 10 mM dithiothreitol before thin-layer chromatography. This treatment prevents the formation of dimeric species and converts mixed disulfides of CoA and glutathione, as well as thioesters of CoA (30), to free CoA, thus simplifying the analysis. Conversion of acyl-CoA to CoA was checked under our conditions and found to be complete, as judged by the quantitative formation of O-[2-³H]acetyldithiothreitol (R_f = 0.67 in solvent I) from [2-³H]acetyl-CoA (30), or O-[1-¹⁴C]palmitoyldithiothreitol (R_f = 0.75 in solvent I) from [1-¹⁴C]palmitoyl-CoA.*

pantetheine comprising the remainder. The phosphorylated compounds comprised an increasing proportion of the label in the medium between 0.5 and 4 μM β-alanine, where the level reached 22 pmol per 10^8 cells and remained at that level at higher β-alanine concentrations. The 4'-phosphopantothenate constituted 20% of the total pool of phosphorylated compounds at β-alanine concentrations greater than 4.0 μM. This species could be excreted from the cells, but our experiments cannot rule out the possibility of extracellular degradation of 4'-phosphopantetheine as the source of the 4'-phosphopantothenate. Pantothenate increased linearly between 4 and 16 μM β-alanine, becoming the major component of the extracellular label, and attained a maximal level of 600 pmol per 10^8 cells at 32 μM β-alanine (Fig. 4). After growth on 32 μM β-alanine, β-alanine comprised 26% of the media label (Fig. 4).

Production of pantothenate by strain UB1005. The possible role of β-alanine in the regulation of CoA biosynthesis could not be tested with strain SJ16. Therefore, the wild-type parent, strain UB1005, was tested for its ability to excrete pantothenate by microbiological assay with strain SJ16. Figure 5 shows the growth response of strain SJ16 to both β-alanine and pantothenate. The endpoints are similar in both cases, with maximum growth being achieved at 0.5 μM supplement, though in the lower concentration range pantothenate was consistently more efficient than β-alanine. These data (Fig. 5) were used as a standard curve to determine the concentration of growth-promoting substances excreted by strain UB1005. Dilutions of the media from strain UB1005 grown to 100 Klett units and stationary phase were found to possess growth-promoting activity equivalent to 9.0 and 12.5 μM pantothenate, respectively.

The media from UB1005 were chromatographed in solvent II, and the fractions containing growth-promoting activity were located (Fig. 6). All of the activity was found to co-chromatograph with the pantothenate standard (Fig. 6),

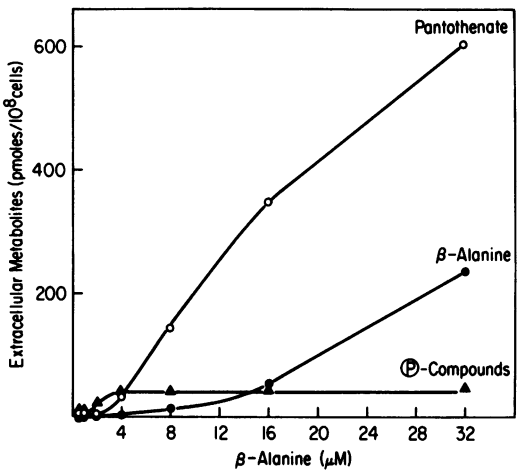

FIG. 4. *Utilization of β-alanine by strain SJ16: extracellular metabolites. The experimental protocol was the same as described in the legend to Fig. 2. At the end of the experiment, cells were removed from the media by centrifugation, and the supernatant (media) was chromatographed in both solvent I and II (see Table 1) to quantitate the level of extracellular β-alanine-derived metabolites. The phosphorylated compounds consisted primarily of 4'-phosphopantetheine. The identity of the phosphorylated compounds was confirmed by digestion with alkaline phosphatase followed by chromatography of the dephosphorylated species in both solvents I and II (see the text and Table 1).*

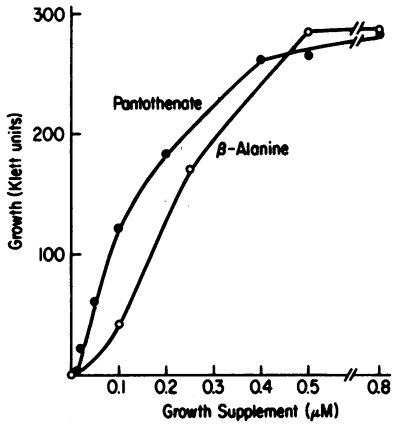

FIG. 5. *Growth response of SJ16 to β-alanine and pantothenate. Strain SJ16 was grown overnight in the presence of 10 μM β-alanine and depleted of its CoA reserves as described in the text. Approximately 10^6 cells were inoculated into 10 ml of medium E containing glucose (0.4%), methionine (0.01%), thiamine (0.001%), and the indicated amount of β-alanine or pantothenate. Cultures were incubated at 37°C for 15 h, and the turbidity of the culture was assessed with a Klett meter. These data were used as a standard curve to determine the pantothenate concentration in media from strain UB1005. In these experiments, strain UB1005 was grown in medium E containing glucose (0.4%), methionine (0.01%), and thiamine (0.001%).*

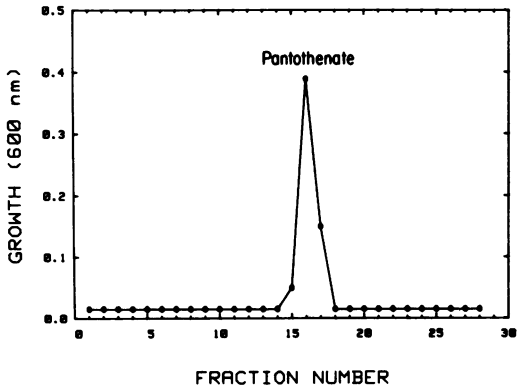

FIG. 6. *Excretion of pantothenate by strain UB1005. Strain UB1005 was grown on glucose (0.4%), methionine (0.01%), and thiamine (0.001%) in medium E overnight at 37°C. Cells were removed by filtration, and 20 μl of the media was applied to a Silica Gel H layer and developed in solvent II. Fractions of 0.5 cm of Silica Gel were placed in tubes and extracted with 1.0 ml of the above media. The Silica Gel was removed by centrifugation, and 0.8 ml of the supernatant was transferred to culture tubes and inoculated with 10^6 cells of strain SJ16 previously starved as described in the legend to Fig. 2. After incubation at 37°C for 15 h, the optical density of the culture was determined at 600 nm.*

demonstrating that strain UB1005 releases pantothenate into the media. Growth-promoting activity was not found at the origin, which is the migration position of phosphorylated compounds found in the medium. These experiments were performed on fresh media samples. Media samples that were several days old had an additional peak comigrating with β-alanine. The instability of pantothenate is well known, and the appearance of β-alanine in these samples due to pantothenate breakdown is the most plausible explanation for this finding.

DISCUSSION

The growth response of strain SJ16 to β-alanine or pantothenate was very similar (Fig. 5), although pantothenate was consistently observed to be a slightly better growth promoter at lower concentrations. The response of strain SJ16 to pantothenate was, in turn, quite comparable to that of strain YA139 (2). Our results concerning the intracellular content of β-alanine-derived metabolites are qualitatively similar to those reported for strain YA139 supplemented with pantothenate (2) in that the CoA content increases as a function of the supplement concentration in the media, whereas the ACP content remains relatively constant. However, quantitatively, Alberts and Vagelos (2) considerably underestimated the size of the CoA pool in strain YA139. Later studies with strain YA139 (23) are more in accord with our determinations of the relative CoA and ACP content of strain SJ16. Experiments in our laboratory in which both strain SJ16 and strain YA139 were labeled with D-[1-^{14}C]pantothenate also agree with the results in this report.

Phosphorylated pantothenate metabolites have been shown to be excreted by *E. coli* (23). On the basis of DEAE-cellulose chromatography, Powell et al. (23) concluded that the species was probably 4′-phosphopantetheine since Brown (5) reported that 4′-phosphopantothenate was not detectable in *E. coli*. Our chromatographic results show that the majority of the excreted phosphorylated material was 4′-phosphopantetheine, but 4′-phosphopantothenate was also detected, particularly at the higher β-alanine concentrations. Whether this is due to the excretion of 4′-phosphopantothenate or to the breakdown of 4′-phosphopantetheine is not known. Pantetheine was also detected in the media. This species usually accounted for less than 5% of the 4′-phosphopantetheine peak, and probably its presence was due to the dephosphorylation of 4′-phosphopantetheine. The major intracellular intermediate in the CoA biosynthetic pathway was also 4′-phosphopantetheine.

This intermediate was detected previously in *E. coli* by Brown (5) and Powell et al. (23), and also Kuwagata (16) and Nakamura et al. (21) reported that 4'-phosphopantetheine was the only intermediate in CoA biosynthesis with a significant pool in rat liver. Thus, the conversion of 4'-phosphopantetheine to dephospho-CoA may be the slowest step between 4'-phosphopantothenate and CoA in *E. coli*, as well as other organisms.

The maximal intracellular CoA level was attained in the presence of 8 μM β-alanine. It is evident from the amount of pantothenate found in the medium at this point and at higher β-alanine concentrations that strain SJ16 is capable of synthesizing a large excess of pantothenate over that required to maintain the CoA content. Therefore, the supply of pantoate, a direct precursor of pantothenate, is not limiting to CoA biosynthesis. Pantothenate excretion was also shown to be a normal process in wild-type cells. Metabolite(s) capable of supporting the growth of pantothenate auxotrophs have previously been shown to be excretion products of wild-type *E. coli* (12). We identified this material as pantothenate by thin-layer chromatography (Fig. 6) and showed that the medium from stationary-phase strain UB1005 contains 450 pmol per 10^8 cells (12.5 μM) of pantothenate. This value is similar to that excreted by strain SJ16 in the presence of 16 to 32 μM β-alanine (Fig. 4); therefore, we conclude that neither β-alanine nor pantoate production restricts CoA formation. Only at 32 μM β-alanine does the amount of pantothenate in the medium deviate from the linear relationship established at lesser β-alanine concentrations. In addition, appreciable quantities of unmetabolized β-alanine remained in the medium, and β-alanine was a major constituent of the intracellular pool. These data strongly suggest that between 16 and 32 μM β-alanine, pantoate production becomes limiting to pantothenate synthesis.

Taken together, the data demonstrate that pantothenate kinase is the primary regulatory site in CoA biosynthesis. Comparison between the buildup of pantothenate in the medium and the levels of all other intracellular intermediates in CoA synthesis clearly shows that regulation at the pantothenate kinase step is approximately 100-fold more efficient than at other possible sites in the pathway. The biochemical mechanism by which the CoA pool can influence the activity of pantothenate kinase is not known, but allosteric control seems likely. Pantothenate kinase activity has been detected in *E. coli* (6), but mechanistic details are lacking. However, pantothenate kinase from rat kidney (14), rat liver (1), and a variety of microorganisms (28, 29) has been reported to be strongly inhibited by CoA. In the case of the rat kidney enzyme (14), it has been shown that the CoA inhibition is noncompetitive with respect to pantothenate.

Our observations are also intriguing when viewed from a nutritional standpoint. Since pantothenate is one of the B-complex of vitamins and is a nutritional requirement of the host mammal, the finding that wild-type *E. coli* excretes pantothenate points to a role for intestinal flora in providing this vitamin to the host.

ACKNOWLEDGMENTS

We thank John Cronan for his comments and for providing the facilities for construction of strain SJ3 and Kim Lee for his excellent technical assistance.

This work was supported by National Institutes of Health grant GM 28035, Cancer Center Support (CORE) grant CA 21765 from the National Cancer Institute, American Cancer Society grant IN 99-G, and ALSAC.

LITERATURE CITED

1. **Abiko, Y., S.-I. Ashida, and M. Shimizu.** 1972. Purification and properties of D-pantothenate kinase from rat liver. Biochim. Biophys. Acta **268**:364–372.
2. **Alberts, A. W., and P. R. Vagelos.** 1966. Acyl carrier protein. VIII. Studies of acyl carrier protein and coenzyme A in *Escherichia coli* pantothenate or β-alanine auxotrophs. J. Biol. Chem. **241**:5201–5204.
3. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
4. **Binstock, J. F., A. Pramanik, and H. Schulz.** 1977. Isolation of a multi-enzyme complex of fatty acid oxidation from *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74**:492–495.
5. **Brown, G. M.** 1959. Assay and distribution of bound forms of pantothenic acid. J. Biol. Chem. **234**:379–382.
6. **Brown, G. M.** 1959. The metabolism of pantothenic acid. J. Biol. Chem. **234**:370–378.
7. **Burton, D. N., C. Sobhy, R. A. Jenik, and J. W. Porter.** 1979. Interconversion of apo- and holofatty acid synthetases of rat and pigeon liver. Methods Enzymol. **62**:249–262.
8. **Clark, D., and J. E. Cronan, Jr.** 1980. *Escherichia coli* mutants with altered control of alcohol dehydrogenase and nitrate reductase. J. Bacteriol. **141**:177–183.
9. **Clark, D., and J. E. Cronan, Jr.** 1980. Acetaldehyde coenzyme A dehydrogenase of *Escherica coli*. J. Bacteriol. **144**:179–184.
10. **Clewell, D. B., and D. R. Helinski.** 1969. Supercoiled circular DNA-protein complex in *Escherichia coli*: purification and induced conversion to an open circular DNA form. Proc. Natl. Acad. Sci. U.S.A. **69**:2110–2114.
11. **Cronan, J. E., Jr.** 1980. β-Alanine synthesis in *Escherichia coli*. J. Bacteriol. **141**:1291–1297.
12. **Davis, B. D.** 1950. Studies on nutritionally deficient bacterial mutants isolated by means of penicillin. Experientia **6**:41–80.
13. **Elovson, J., and P. R. Vagelos.** 1968. Acyl carrier protein. X. Acyl carrier protein synthetase. J. Biol. Chem. **243**:3603–3611.
14. **Karasawa, T., K. Yoshida, K. Furukawa, and K. Hosoki.** 1972. Feedback inhibition of pantothenate kinase by coenzyme A and possible role of the enzyme for the regulation of cellular coenzyme A level. J. Biochem. **71**:1065–1067.
15. **Kleckner, N., J. Roth, and D. Botstein.** 1977. Genetic

engineering *in vivo* using translocatable drug resistance elements. J. Mol. Biol. **116**:125–154.
16. **Kuwagata, M.** 1971. Incorporation of ^{14}C-pantothenic acid into an intermediate of CoA biosynthesis in rat liver. Vitamins **43**:78–86.
17. **Loewen, P. C.** 1978. Levels of coenzyme A-glutathione mixed disulfide in *Escherichia coli*. Can. J. Biochem. **56**:753–759.
18. **Maas, W. K.** 1952. Pantothenate studies. III. Description of the extracted pantothenate-synthesizing enzyme of *Escherichia coli*. J. Biol. Chem. **198**:23–32.
19. **Michelson, A. M.** 1964. Synthesis of coenzyme A. Biochim. Biophys. Acta **93**:71–77.
20. **Miyatake, K., Y. Nakano, and S. Kitaoka.** 1979. Pantothenate synthetase from *Escherichia coli* (D-pantoate: β-alanine ligase (AMP-forming), EC 6.3.2.1). Methods Enzymol. **62**:215–219.
21. **Nakamura, T., T. Kusunoki, K. Soyama, and M. Kuwagata.** 1969. Studies on the distribution of pantothenic acid, coenzyme A and their intermediates in rat liver. III. The isolation of pantothenic acid, 4′-phosphopantetheine and coenzyme A by using column chromatography. Vitamins **40**:412–415.
22. **O'Brien, W. J., and F. E. Frerman.** 1977. Evidence for a complex of three beta-oxidation enzymes in *Escherichia coli*: induction and localization. J. Bacteriol. **132**: 532–540.
23. **Powell, G. L., J. Elovson, and P. R. Vagelos.** 1969. Acyl carrier protein. XII. Synthesis and turnover of the prosthetic group of acyl carrier protein *in vivo*. J. Biol. Chem. **244**:5616–5624.
24. **Powers, S. G., and E. E. Snell.** 1976. Ketopantoate hydroxymethyltransferase. II. Physical, catalytic and regulatory properties. J. Biol. Chem. **251**:3786–3793.
25. **Pugh, E. L., and S. J. Wakil.** 1965. Studies on the mechanism of fatty acid synthesis. XIV. The prosthetic group of acyl carrier protein and the mode of its attachment to the protein. J. Biol. Chem. **240**:4727–4733.
26. **Rock, C. O., J. E. Cronan, Jr., and I. M. Armitage.** 1981. Molecular properties of acyl carrier protein derivatives. J. Biol. Chem. **256**:2669–2674.
27. **Rock, C. O., and J. L. Garwin.** 1979. Preparative enzymatic synthesis and hydrophobic chromatography of acyl-acyl carrier protein. J. Biol. Chem. **254**:7123–7128.
28. **Shimizu, S., K. Kubo, Y. Tani, and K. Ogata.** 1973. Purification and properties of pantothenate kinase from *Brevibacterium ammoniagenes* IFO 12071. Agric. Biol. Chem. **37**:2863–2870.
29. **Shimizu, S., K. Kubo, H. Morioka, Y. Tani, and K. Ogata.** 1974. Some aspects of the enzyme activities involved in coenzyme A biosynthesis in various microorganisms. Agric. Biol. Chem. **38**:1015–1021.
30. **Stokes, G. B., and P. K. Stumpf.** 1974. Fat metabolism in higher plants. The nonenzymatic acylation of dithiothreitol by acyl coenzyme A. Arch. Biochem. Biophys. **162**:638–648.
31. **Teller, J. H., S. G. Powers, and E. E. Snell.** 1976. Ketopantoate hydroxymethyltransferase. I. Purification and role in pantothenate biosynthesis. J. Biol. Chem. **251**:3780–3785.
32. **Vagelos, P. R.** 1973. Acyl group transfer, *In* P. D. Boyer (ed.), The Enzymes, vol. 8, 3rd ed. Academic Press, Inc. New York.
33. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **250**:2311–2314.
34. **Wilkin, D. R., H. L. King, Jr., and D. E. Dyar.** 1975. Ketopantoic acid and ketopantoyl lactone reductases. Stereospecificity of transfer of hydrogen from reduced nicotinamide adenine dinucleotide phosphate. J. Biol. Chem. **250**:2311–2314.
35. **Williamson, J. M., and G. M. Brown.** 1979. Purification and properties of L-aspartate-α-decarboxylase, an enzyme that catalyzes the formation of β-alanine in *Escherichia coli*. J. Biol. Chem. **254**:8074–8082.