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Biosynthesis and Degradation Both Contribute to the Regulation of Coenzyme A Content in *Escherichia coli*

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Escherichia coli **mutants [*coaA16*(Fr); Fr indicates feedback resistance] were isolated which possessed a pantothenate kinase activity that was refractory to feedback inhibition by coenzyme A (CoA). Strains harboring this mutation had CoA levels that were significantly elevated compared with strains containing the wild-type kinase and also overproduced both intra- and extracellular 4′-phosphopantetheine. The origin of 4′-phosphopantetheine was investigated by using strain SJ135 [*panD* Δ(*aroP-aceEF*)], in which synthesis of acetyl-CoA was dependent on the addition of an acetate growth supplement. Rapid degradation of CoA to 4′-phosphopantetheine was triggered by the conversion of acetyl-CoA to CoA following the removal of acetate from the media. CoA hydrolysis under these conditions appeared not to involve acyl carrier protein prosthetic group turnover since [acyl carrier protein] phosphodiesterase was inhibited equally well by acetyl-CoA or CoA. These data support the view that the total cellular CoA content is controlled by modulation of biosynthesis at the pantothenate kinase step and by degradation of CoA to 4′-phosphopantetheine.**

Coenzyme A (CoA) and CoA thioesters are essential cofactors in numerous biosynthetic and energy-yielding metabolic pathways and regulate several key reactions of intermediary metabolism such as pyruvate dehydrogenase and phosphoenolpyruvate carboxylase (for reviews, see references 1 and 4). Metabolic labeling experiments illustrate that the cellular CoA concentration fluctuates depending on the carbon source and growth state of *Escherichia coli* (8, 10, 18). The regulation of pantothenate kinase appears to be the most important determinant of the CoA biosynthetic rate since 15 times more pantothenate is produced than is phosphorylated (6), suggesting that a maximum of 25% of the catalytic capacity of the enzyme is expressed in normally growing cells (18). Pantothenate kinase activity in vitro is inhibited by CoA and less effectively by CoA thioesters, and CoA biosynthesis in vivo is curtailed by the conversion of acetyl-CoA to CoA (18). The competitive inhibition of pantothenate kinase by CoA with respect to ATP coordinates CoA synthesis with the energy state of the cell (18). Less is known about the contribution of CoA degradation to determination of the intracellular CoA level. Excretion of 4′-phosphopantetheine accompanies decreased CoA production, and during logarithmic growth, the majority of the extracellular 4′-phosphopantetheine is derived from CoA via acyl carrier protein (ACP) prosthetic group turnover rather than new synthesis (8). The purposes of this study were to assess the contribution of feedback inhibition of pantothenate kinase to the overall regulation of cellular CoA content and to determine whether the 4′-phosphopantetheine cycle is the only mechanism for CoA degradation.

MATERIALS AND METHODS

Materials. Sources of supplies were as follows: Amersham Corp., ACS scintillation solution; Amicon Corp., YMT ultrafiltration membranes; Analabs Inc., 250-μm Silica Gel H plates; Fisher Chemical Co., high-pressure liquid chromatography (HPLC)-grade monobasic potassium phosphate; Dupont, NEN Research Products, β-[3-^{3}H]alanine (specific activity, 120 Ci/mmol) and D-[1-^{14}C]pantothenic acid (specific activity, 57.0 Ci/mol); Pharmacia P-L Biochemicals, Inc., ATP, 3′,5′-ADP, acetyl-CoA, and CoA; Research Organics, Inc., dithiothreitol; Sigma Chemical Co., β-alanine, D-pantothenate, β-mercaptoethanol, tetracycline hydrochloride, lysozyme, and bovine serum albumin; The Separations Group, Vydac C-18 HPLC column; and Whatman, Inc., DE81 filter circles and a Partisil-10 SAX HPLC column.

E. coli [*Pan*-^{3}H]ACP was prepared biosynthetically (6) and was purified as described previously (11). Biosynthetically prepared [*Pan*-^{3}H]CoA was purified by DEAE-cellulose chromatography (6). A 4′-phospho[^{3}H]pantetheine standard was generated by incubating [*Pan*-^{3}H]CoA (10 mM), dithiothreitol (1 mM), Tris hydrochloride (0.1 M, pH 8.0), and nucleotide pyrophosphatase (0.68 U) in a volume of 600 μl for 90 min at 37°C. The preparation was passed through a YMT ultrafiltration membrane, and 4′-phosphopantetheine was purified by DEAE-cellulose chromatography (16).

Bacterial strains and growth conditions. The bacterial strains used in this work were derivatives of *E. coli* K-12 and are listed in Table 1. Minimal medium was medium E salts (22) supplemented with glucose (0.4%), thiamine (0.001%), and required amino acids (0.01%). Bacteriophage P1 was propagated in medium containing tryptone (10 g/liter), NaCl (5 g/liter), and yeast extract (1 g/liter). The concentration of tetracycline hydrochloride was 10 μg/ml. Cell number was monitored during growth by using a Klett-Summerson colorimeter with a blue filter. The colorimeter was calibrated with strain SJ16 by determining the number of viable bacteria in the range of colorimeter readings encountered.

Strain SJ135 was a derivative of strain UB1005 constructed to contain the Δ(*aroP-aceEF*) deletion from strain SJ7, the *panD2* mutation from strain SJ16, and the *panF11* allele from strain DV14 (Table 1). Each of the alleles was introduced by transduction mediated by P1 bacteriophage and selection for an adjacent Tn*10* element (Tet^r). At the intermediate steps, the Tn*10* element was removed by the

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TABLE 1. Bacterial strains

Strain	Genotype	Source
DV5	*metB1 panD2 relA1 spoT1* λ^- λ^r *gyrA216* F^-	20
DV14	*metB1 panD2 panF11 zhc-12*::Tn*10* F^-	20
DV53	*metB1 panD2 coaA15*(Ts) F^-	EMS[a] mutagenesis of DV5 (21)
DV62	*metB1 panD2 coaA15*(Ts) *zij*::Tn*10* F^-	21
DV79	*metB1 panD2 coaA16*(Fr) *zij*::Tn*10* F^-	Spontaneous revertant of DV62
KΔ15	Δ(*aroP-aceEF*) *met thy*	13
SJ7	*met thy* Δ(*aroP-aceEF*) *zac*::Tn*10*	Derivative of KΔ15
SJ16	*metB1 relA1 spoT1* λ^- λ^r *gyrA216 panD2 zad-220*::Tn*10* F^-	6
SJ105	*metB1 relA1 spoT1* λ^- λ^r *gyrA216 zac*::Tn*10* Δ(*aroP-aceEF*) F^-	P1(SJ7) × UB1005
SJ121	*metB1 relA1 spoT1* λ^- λ^r *gyrA216* Δ(*aroP-aceEF*) F^-	Tet^s of SJ105
SJ128	*metB1 relA1 spoT1* λ^- λ^r *gyrA216* Δ(*aroP-aceEF*) *panD2 zad-220*::Tn*10* F^-	P1(SJ16) × SJ121
SJ134	*metB1 relA1 spoT1* λ^- λ^r *gyrA216* Δ(*aroP-aceEF*) *panD2* F^-	Tet^s of SJ128
SJ135	*metB1 relA1 spoT1* λ^- λ^r *gyrA216* Δ(*aroP-aceEF*) *panD2 panF11 zhc-12*::Tn*10* F^-	P1(DV14) × SJ134
UB1005	*metB1 relA1 spoT1* λ^- λ^r *gyrA216* F^-	3

[a] EMS, Ethyl methanesulfonate.

method of Bochner et al. (2) as modified by Maloy and Nunn (14).

Spontaneous revertants of the strain DV62 [*coaA15*(Ts)] growth phenotype (21) were obtained after a 2-day incubation at 42°C on minimal medium agar plus 4 μM β-alanine.

Identification and quantitation of β-alanine-derived metabolites. Cell samples from β-[3-^{3}H]alanine-labeled cultures were lysed by either the 2-propanol (8) or Triton X-100 (12) procedure. The lysates were centrifuged in a Beckman Instruments Microfuge, and the supernatants were treated with 10 mM dithiothreitol prior to analysis. The supernatants were analyzed by thin-layer chromatography on Silica Gel H layers developed with either butanol-acetic acid-water (5:2:4, vol/vol/vol) or ethanol–28% ammonium hydroxide (4:1, vol/vol) to 14 cm from the origin (6). The distribution of radioactivity on the thin-layer plate was determined either by scraping 0.5-cm sections of the Silica Gel into scintillation vials and counting in 3 ml of scintillation solution or by using a Bioscan imaging detector. The absolute amount of each metabolite (picomoles per 10^8 cells) was calculated by multiplying the total picomoles of β-[3-^{3}H]alanine-derived label recovered in the sample (cells, media, or both) by the percentage of the total of each metabolite determined by HPLC. Phosphorylated compounds were separated on a Whatman Partisil-10 SAX HPLC column eluted with a 30-min linear gradient of 0.05 to 0.9 M monobasic potassium phosphate containing 1 mM β-mercaptoethanol (8). Fractions were collected every 30 s, and 150-μl samples were counted in 3 ml of scintillation solution. The identities of the tritium-labeled metabolites were confirmed by comparisons with the retention times of standards (8).

Quantitation of intracellular CoA thioesters. Bacterial strains were labeled with β-[3-^{3}H]alanine as described in the figure legends, and the CoA pool composition was determined by using a modification of the method of DeBuysere and Olson (5) as described in detail by Vallari et al. (18). The identity of the tritium-labeled CoA species was confirmed by comparisons with the retention times of standards, and by cochromatography with ^{14}C-labeled CoA thioesters. β-Alanine eluted in the void volume of the chromatographic system, and ACP did not elute from the column under these gradient conditions. Recovery of CoA derivatives from the column was approximately 70%.

Pantothenate kinase assays. Cytosolic fractions were prepared and standard pantothenate kinase assays were performed as described previously (18). Substrate concentrations were 100 μM pantothenate and 2.5 mM ATP. Spontaneous revertants of strain DV62 [*coaA*(Ts)] were screened as follows. Cells were grown overnight at 30°C in 2 ml of glucose minimal medium containing 4 μM β-alanine, centrifuged for 10 min in a Beckman Microfuge, and suspended in 40 μl of a 10-mg/ml lysozyme concentration in 100 mM Tris hydrochloride (pH 7.4)–10 mM EDTA. The suspensions were incubated on ice for 30 min, and the cells were lysed by two cycles of freezing and thawing. Pantothenate kinase activity at 30, 42, and 42°C plus 0.5 mM CoA was determined by using 10 μl of lysates in the standard assay. Protein concentrations were measured by the microbiuret method, using bovine serum albumin as a standard (15).

Preparation and assay of [ACP]phosphodiesterase. [ACP] Phosphodiesterase was purified through the ammonium sulfate step as described by Vagelos and Larrabee (17) and dialyzed against 50 mM Tris hydrochloride (pH 7.5). Phosphodiesterase assays contained 0.15 M Tris hydrochloride (pH 9.0), 40 μM $MnCl_2$, 40 μM $MgCl_2$, 200,000 cpm of [*Pan*-^{3}H]ACP (specific activity, 120 Ci/mmol), and 40 μg of protein in a final volume of 50 μl. At the end of 10 min at 37°C, the reaction was cooled on an ice slush, and 5 μl of 5% bovine serum albumin and 10 μl of 10% perchloric acid were added. The mixture was vortexed and centrifuged in a Beckman Microfuge for 10 min to sediment precipitated protein. Two 15-μl aliquots of the supernatant were then removed and counted to determine the amount of 4′-phospho[^{3}H]pantetheine liberated.

RESULTS

Isolation of a feedback inhibition-resistant pantothenate kinase mutant. Temperature-sensitive pantothenate kinase mutants [*coaA*(Ts)] were recently isolated and characterized (21). Cytosolic extracts from strain DV62 [*coaA15*(Ts)] phosphorylated pantothenate at 1% of the wild-type rate (0.633 nmol/min per mg at 42°C), and this strain would not grow at 42°C. Revertants of the *coaA15*(Ts) growth phenotype were isolated, and the pantothenate kinase activities in these revertants were characterized. Strain DV62 spontaneously reverted to growth at 42°C at a frequency of approximately 1 in 10^7 cells plated on minimal medium agar plus 4 μM β-alanine. Cultures from 20 isolated colonies were screened. One group of isolates had no increase in enzyme activity at 42°C over that from strain DV62 (not shown), indicating the possible presence of a second site mutation(s) that was not investigated further. A different class of isolates recovered the wild-type level of pantothenate kinase (1.01

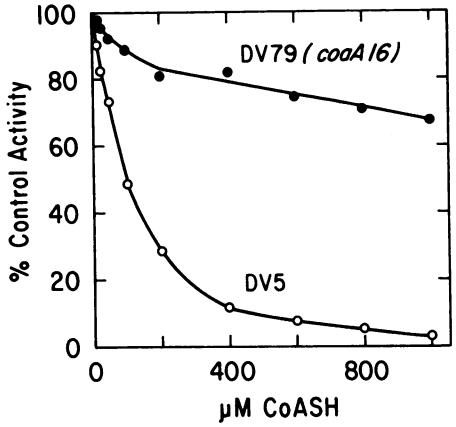

FIG. 1. Nonesterfied CoA (CoASH) inhibition of pantothenate kinase from strains DV79 [*coaA16*(Fr) *panD*] and DV5 (*panD*). Pantothenate kinase activity in cytosolic fractions from either strain DV79 or strain DV5 was assayed at 42°C in the presence of 2.5 mM ATP, 100 μM pantothenate, and the indicated concentrations of CoASH. Specific activity of pantothenate kinase in extracts from strain DV79 was 0.29 nmol/min per mg compared with 1.01 nmol/min per mg in wild-type strain DV5.

nmol/min per mg at 42°C), which was stable to increased temperature and was also inhibited by CoA. A third class of revertants was represented by strain DV79 (*coaA16*), which contained a pantothenate kinase that was only 29% as active as the wild-type enzyme (0.29 nmol/min per mg at 42°C). Further kinetic analysis showed that the enzyme extracted from strain DV79 had an increased affinity for ATP (K_m = 0.6 mM) compared with the wild-type pantothenate kinase at 42°C (K_m = 1.7 mM) and had lost sensitivity to the competitive inhibitor CoA (Fig. 1). Whereas pantothenate kinase from the wild-type strain DV5 was inhibited 50% by 100 μM CoA and 95% in the presence of 1 mM CoA, these concentrations of CoA inhibited the activity in strain DV79 lysates by only 12 and 33%, respectively (Fig. 1). These in vitro data suggested that pantothenate kinase was not efficiently regulated by CoA in strain DV79 [*coaA16*(Fr); Fr indicates feedback resistance] and that this property compensated for the reduced catalytic capacity at 42°C.

β-Alanine metabolism by feedback inhibition-resistant pantothenate kinase mutants. The CoA content in *panD* strains containing wild-type pantothenate kinase increases as a function of the β-alanine growth supplement up to 10 μM β-alanine. Growth in higher β-alanine concentrations does not result in higher CoA levels due to the reduced activity of the kinase (6). The importance of the CoA-binding site on pantothenate kinase to the regulation of the intracellular CoA content was tested by comparing the metabolism of a high β-[3-^{3}H]alanine concentration (24 μM) by strains DV79 [*coaA16*(Fr) *panD*] and DV5 (*coaA*$^+$ *panD*) (Table 2). An analysis of the labeled metabolites by thin-layer chromatography revealed larger CoA and 4′-phosphopantetheine pools in the strain DV79 culture. Further fractionation of the phosphorylated metabolites by HPLC (data not shown) showed that other phosphorylated CoA precursors were absent from both cultures. The pantothenate pool was smaller in the strain DV79 culture, whereas pantetheine, shown to be derived from 4′-phosphopantetheine (8), increased from below detectable levels to nearly 10% of the total. The elevated synthesis of CoA was consistent with the loss of feedback inhibition of pantothenate kinase that was observed in extracts from strain DV79 (Fig. 1). The presence

TABLE 2. β-[3-^{3}H]alanine-derived metabolites in cultures of strains DV79 [*coaA16*(Fr) *panD*] and DV5 (*panD*)[a]

Strain	pmol/10^8 cells					
	β-Alanine	Pantothenate	CoA	4′-Phospho-pantetheine	Pantetheine	ACP
DV5	33	775	100	9	ND[b]	43
DV79	21	460	232	81	99	67

[a] Strains DV79 and DV5 were grown in minimal medium and depleted of CoA at 37°C (6). Approximately 6 × 10^6 cells per ml were used to inoculate minimal medium containing 24 μM β-[3-^{3}H] alanine (specific activity, 0.33 Ci/mmol). The cultures were incubated for 12 h at 37°C, and samples (cells and medium combined) were extracted with 2-propanol and analyzed by thin-layer chromatography as described in Materials and Methods to determine the distribution of radioactivity and amount of each β-alanine-derived metabolite.
[b] ND, Not detected.

of large, predominantly extracellular (data not shown) 4′-phosphopantetheine and pantetheine pools in the strain DV79 culture supports the view that regulation of 4′-phosphopantetheine conversion to CoA is a second control site in the biosynthetic pathway (8) and comes into play in the absence of efficient pantothenate kinase regulation.

CoA degradation. 4′-Phosphopantetheine can arise from either the biosynthesis or the degradation of CoA, and the existence of a CoA degradative pathway was examined in strain SJ135 [*panD* Δ(*aroP-aceEF*)] in which extracellular acetate is the only route to the synthesis of acetyl-CoA. This point was addressed in an acetate starvation experiment. Strain SJ135 growing in glucose medium supplemented with 0.4% acetate and β-[3-^{3}H]alanine contained levels of CoA and ACP within the normal range for *E. coli* (Fig. 2) (8, 10, 18). At the midlogarithmic phase of growth (ca. 5 × 10^8 cells per ml), the cells were filtered and suspended in warm growth medium without acetate or β-alanine and samples were removed at 5-min intervals. The CoA pool composition was not altered by filtering the cells and suspending them in complete growth medium (see also reference 18). The composition of the CoA thioester pool in strain SJ135 during exponential growth was similar to that in other *E. coli* strains growing on glucose medium (Fig. 3A) (18), but immediately following acetate removal, acetyl-CoA dropped from 58% of the total pool to 17.5% and there was an increase in nonesterified CoA (Fig. 3B). Succinyl-CoA increased from 12.5 to 24.8% of the total pool, and malonyl-CoA was a minor component both in the presence and absence of acetate. Concomitant with the disappearance of acetyl-CoA, the intracellular level of β-alanine-derived metabolites dropped by almost 50% from 72 to 39 pmol/10^8 cells. Analysis of the intracellular pool showed that the decrease was due to the disappearance of CoA, a 64% reduction from 60.5 to 21.8 pmol/10^8 cells after starvation. The ACP level remained relatively constant, averaging 10 pmol/10^8 cells, and this observation was consistent with earlier work showing that the ACP prosthetic group turnover cycle functions to maintain ACP as the active holoenzyme (7). There was also a sharp increase in intracellular 4′-phosphopantetheine from barely detectable levels (<0.7 pmol/10^8 cells) to 7.4 pmol/10^8 cells (Fig. 2). The decreased tritium label in the cells was recovered in the medium (32 pmol/10^8 cells) as 4′-phosphopantetheine and pantetheine (Fig. 4), illustrating that most of the degraded CoA was excreted from the cell as 4′-phosphopantetheine. All of these changes were evident at the first time point (5 min), and there was no change in the CoA pool composition, CoA/ACP ratio, or total intracellular metabolite concentrations at subsequent time points up to 30

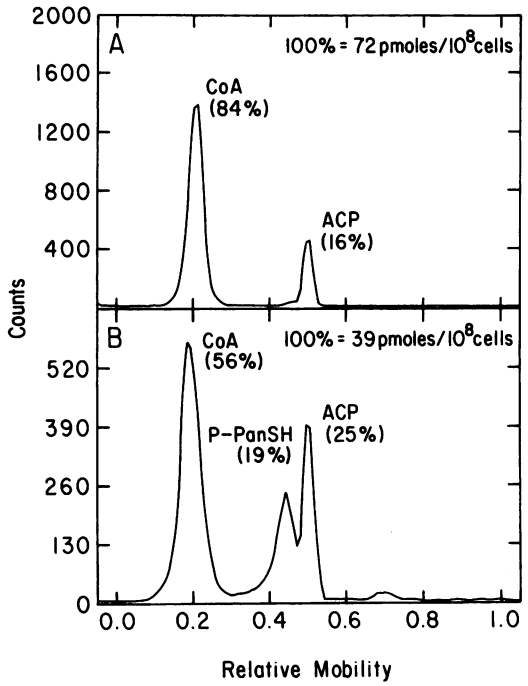

FIG. 2. Constriction of intracellular CoA pool following removal of acetate from cultures of strain SJ135 [*panD* Δ(*aroP-aceEF*)]. Strain SJ135 was grown in glucose minimal medium containing 0.4% acetate and 4 μM β-[3-^{3}H]alanine (specific activity, 2 mCi/mol). At a density of 5 × 10^8 cells per ml, samples were removed (A). CoA and ACP contents were 60.5 and 11.5 pmol/10^8 cells, respectively. The cells were then washed and suspended in medium lacking acetate and β-alanine, and samples were withdrawn after 5 min (B). CoA, ACP, and 4′-phosphopantetheine (P-PanSH) levels were 21.8, 9.8, and 7.4 pmol/10^8 cells, respectively. Washed cells were extracted by the Triton X-100 lysis procedure, the total amount of label was determined, and the size and composition of the intracellular metabolite pools were assessed by thin-layer chromatography on Silica Gel H layers developed with butanol-acetic acid-water (5/2/4, vol/vol/vol). The data are representative of three independent experiments.

min. At this time, acetate was added back to the culture and samples that were removed 5 min later showed an increase in acetyl-CoA to 75% of the total CoA pool as well as disappearance of intracellular 4′-phosphopantetheine due to its conversion to CoA (not shown).

CoA inhibition of [ACP]phosphodiesterase. CoA degradation to 4′-phosphopantetheine via ACP prosthetic group turnover in the acetate starvation experiments seemed unlikely since our previous metabolic labeling studies indicated that prosthetic group turnover is rapid at low CoA levels and decreases at higher intracellular CoA concentrations (9). These earlier data suggested that [ACP]phosphodiesterase activity was inhibited by high CoA levels, and this point was investigated further by using an in vitro assay. Physiological concentrations of CoA inhibited the generation of 4′-phosphopantetheine from ACP (Fig. 5). Significantly, acetyl-CoA was as potent as CoA, suggesting that the conversion of acetyl-CoA to CoA in vivo would not serve to activate this enzyme. Further evidence that the adenine portion of the CoA molecule was the key structural element required for enzyme inhibition was illustrated by the potency of 3′,5′-ADP as an [ACP]phosphodiesterase inhibitor (Fig. 5). Adenosine and AMP did not inhibit ACP hydrolysis in the standard assay. ATP and ADP were inhibitors; however, inhibition by these compounds was due to the chelation of Mn^{2+} and Mg^{2+} in the assay since they did not affect enzymatic activity in assays performed at higher divalent cation concentrations. In contrast, CoA, acetyl-CoA, and 3′,5′-ADP (500 μM) were still inhibitory when the concentration of Mn^{2+} or Mg^{2+} was increased to 600 μM in the standard assay, indicating that these agents were not affecting enzymatic activity due to chelation of metals.

DISCUSSION

The inability of cells possessing the *coaA16*(Fr) mutation to regulate CoA biosynthesis substantiates the role of feedback inhibition of pantothenate kinase as an essential component of the system that governs CoA content (Fig. 6). Pantothenate kinase controls the bifurcation between pantothenate incorporation and efflux (6). Extracellular pantothenate constitutes the largest CoA precursor pool (6), and a catalyzed efflux process (20) coupled with the pantothenate permease activity (19) mediate transmembrane pantothenate flux. Pantothenate kinase is regulated through feedback inhibition primarily by the level of nonesterified CoA and secondarily by the total CoA thioester pool (18). Nonesterified CoA is a more potent inhibitor of pantothenate kinase compared with its thioesters and reduces catalytic activity by competing with ATP within the physiological concentration range of both nucleotides (18). The simultaneous alteration of the CoA and ATP affinities in the *coaA16*(Fr) mutant kinase is consistent with the biochemical data indicating that the nucleotides bind to kinetically indistinguishable sites

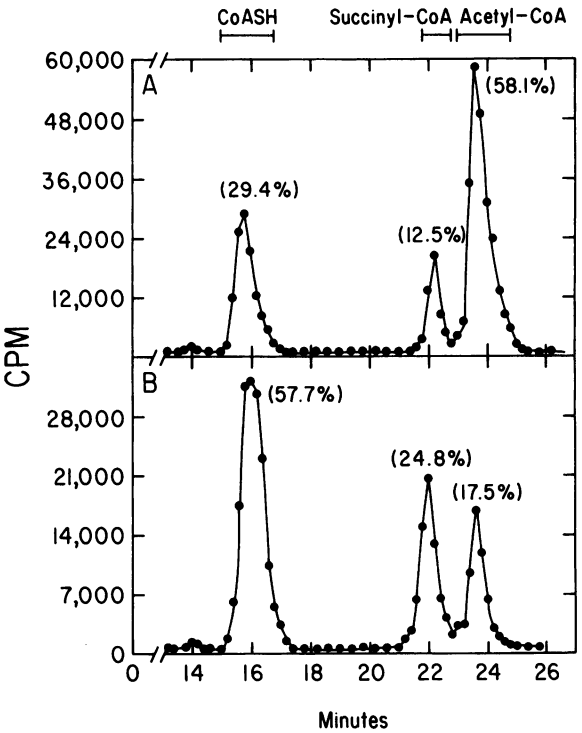

FIG. 3. CoA pool composition of strain SJ135 [*panD* Δ(*aroP-aceEF*)] either in the presence (A) or 5 min after removal (B) of the acetate growth supplement. Strain SJ135 was grown as described in the legend to Fig. 2, and cell samples were processed for separation of CoA species by reverse-phase HPLC as described in Materials and Methods. CoA content before starvation was 60.5 pmol/10^8 cells; it was 21.8 pmol/10^8 cells after starvation.

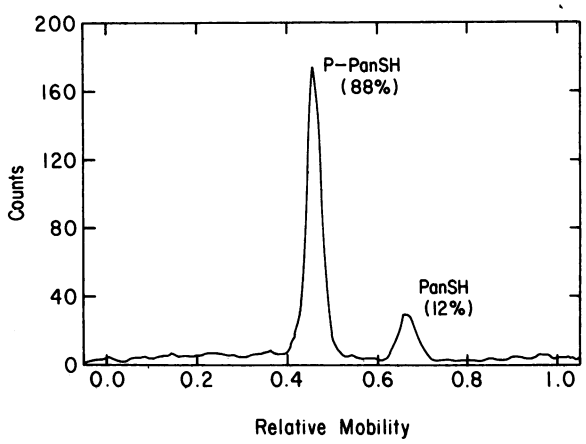

FIG. 4. CoA degradation products excreted from strain SJ135 [*panD* Δ(*aroP-aceEF*)] following removal of acetate from growth medium. Media from the cell samples analyzed in Fig. 2 were collected, and the amount of label excreted from the cells was determined to be 32 pmol/10^8 cells. The identities of the extracellular metabolites were assessed by thin-layer chromatography on Silica Gel H layers developed with butanol-acetic acid-water (5/2/4, vol/vol/vol). The distribution of radiolabel was determined with a Bioscan imaging detector. P-PanSH, 4′-Phosphopantetheine.

(18). The elevated intracellular CoA level in the pantothenate kinase mutants that are insensitive to CoA inhibition [*coaA*(Fr)] (Table 2) illustrates that modulation of the synthetic pathway is a primary determinant of the CoA content in *E. coli*. These results suggest that the *coaA16*(Fr) mutation will provide an opportunity to examine the physiological consequences of abnormally high intracellular CoA concentrations on cellular processes that require and are regulated by CoA.

Our work indicates that there is a direct pathway for the degradation of CoA to 4′-phosphopantetheine in addition to the ACP prosthetic group turnover cycle (8). This degradative pathway becomes operative when the acetyl-CoA/CoA ratio decreases and the majority of the generated 4′-phosphopantetheine is excreted (Fig. 2 to 4). This process is

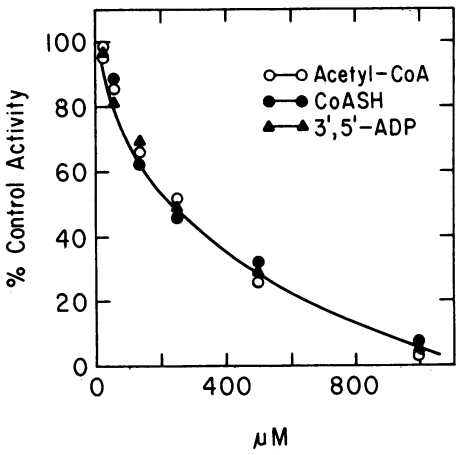

FIG. 5. Inhibition or [ACP]phosphodiesterase by CoA and acetyl-CoA. [ACP]phosphodiesterase activity was assayed as described in Materials and Methods in the presence of the indicated concentration of either CoASH or acetyl-CoA. Control activity corresponded to the release of 22,000 cpm of 4′-phospho-[^{3}H]pantetheine from [*Pan*-^{3}H]ACP per assay.

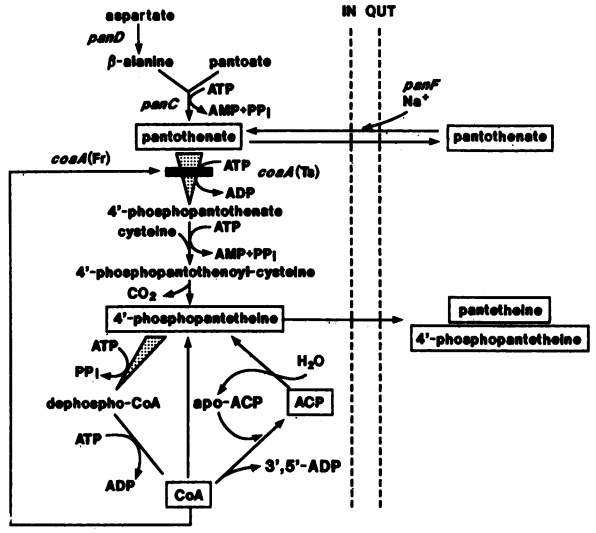

FIG. 6. Regulation of CoA content in *E. coli*. Pantothenate kinase is the principal regulatory site in CoA biosynthesis. This enzyme is the product of the *coaA* gene, and its activity is governed by feedback inhibition primarily by nonesterified CoA and secondarily by CoA thioesters. CoA inhibits pantothenate kinase catalytic activity by competing with ATP. More pantothenate is produced than is used for CoA synthesis, and the unphosphorylated pantothenate is released into the medium. Extracellular pantothenate re-enters the cell via a sodium-stimulated permease (*panF*). CoA levels are reduced by degradation to 4′-phosphopantetheine either directly or from ACP prosthetic group turnover. Conversion of 4′-phosphopantetheine to CoA is regulated by 4′-phosphopantetheine adenylyltransferase, and unutilized 4′-phosphopantetheine exits the cell.

essentially irreversible since, unlike pantothenate (19), extracellular 4′-phosphopantetheine is not assimilated by *E. coli* (8). Two characteristics of [ACP]phosphodiesterase indicate that the activation of this enzyme is not responsible for the production of 4′-phosphopantetheine in our experiments (Table 2 and Fig. 4). First, in vivo measurements show that prosthetic group turnover is lowest at the elevated CoA concentrations present during normal growth and prior to acetate starvation (9). Second, [ACP]phosphodiesterase activity is equally inhibited by acetyl-CoA and CoA (Fig. 5), which is not consistent with a change in the ratio of these metabolites triggering an increase in ACP hydrolysis. Further experiments are required to determine whether there is a CoA-specific pyrophosphorylase that increases in activity in response to the acetyl-CoA/CoA ratio. These data suggest that CoA degradation has a significant role in reducing the intracellular CoA concentration following abrupt alterations in cell metabolism.

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