JOURNAL OF BACTERIOLOGY, Dec. 1987, p. 5795–5800
0021-9193/87/125795-06$02.00/0

Vol. 169, No. 12

Isolation and Characterization of Temperature-Sensitive Pantothenate Kinase (*coaA*) Mutants of *Escherichia coli*

DAVID S. VALLARI† AND CHARLES O. ROCK*

Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee 38101, and Department of Biochemistry, The University of Tennessee, Memphis, Memphis, Tennessee 38163

Received 1 June 1987/Accepted 18 September 1987

Escherichia coli **mutants conditionally defective in the conversion of pantothenate to coenzyme A were isolated and characterized. The gene was designated *coaA* and localized between *argEH* and *rpoB* near min 90 of the chromosome. The *coaA15*(Ts) mutation caused a temperature-sensitive growth phenotype and temperature-dependent inactivation of pantothenate kinase activity assayed both in vivo and in vitro. At 30°C, *coaA15*(Ts) extracts contained less than 20% of the wild-type pantothenate kinase activity; the kinase had near normal kinetic constants for the substrates ATP and pantothenate and was inhibited by coenzyme A to the same degree as the wild-type enzyme. These data define the *coaA* gene as the structural gene for pantothenate kinase.**

Coenzyme A (CoA) is synthesized by a series of reactions beginning with the phosphorylation of the vitamin pantothenic acid (1). In *Escherichia coli*, pantothenate kinase catalyzes the rate-controlling step in the pathway (9, 10). Metabolic labeling studies illustrate that down regulation of CoA biosynthesis is accomplished by decreased pantothenate phosphorylation coupled with efflux and accumulation of pantothenate in the culture medium (9, 10). Pantothenate kinase is inhibited by nonesterified CoA (CoASH) and to a lesser extent by CoA thioesters (16). Inhibition of pantothenate kinase by CoASH is competitive with ATP, indicating that CoA production is coordinated with the energy state of the cell. The correspondence between the effective concentrations of these compounds in vitro and their intracellular concentrations supports the hypothesis that the size of the CoA pool is a primary determinant of pantothenate kinase activity (16). Pantothenate kinase is also thought to govern CoA production in animal cells (14, 15). Like *E. coli* pantothenate kinase, the mammalian enzyme is inhibited by CoA in vitro (2, 6–8, 11), but the potency of CoA versus that of its thioesters and the interaction between CoA and ATP are less clear. *Salmonella typhimurium* mutants possessing temperature-sensitive pantothenate kinase activity (*coaA*) have been isolated; the gene is located near min 89 of the chromosome (5). In the present work, *E. coli* pantothenate kinase (*coaA*) mutants were isolated as a first step toward the genetic analysis of this key regulatory enzyme in this organism.

MATERIALS AND METHODS

Materials. Sources of supplies were as follows: Amersham Corp., ACS scintillation solution; Analabs Inc., 250-μm silica gel H plates; Boehringer Mannheim Biochemicals, Tris; Millipore Corp., type HA filters (pore size, 0.45 μm); New England Nuclear Corp., 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, ATP and CoA; Research Organics, Inc., dithiothreitol; Sigma Chemical Co., β-alanine, D-pantothenate, $MgCl_2$, ethyl methanesulfonic acid, ampicillin, chloramphenicol, rifampin, tetracycline hydrochloride, and bovine serum albumin; and Whatman, Inc., DE81 filter circles. D-[3-^{3}H]pantothenic acid (specific activity, 5.0 Ci/mmol) was synthesized and purified as previously described (19).

Bacterial strains and growth conditions. The bacterial strains used in this work were derivatives of *E. coli* K-12 (Table 1). Minimal medium was medium E salts (20) 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 concentrations of antibiotics were as follows: ampicillin, 20 μg/ml; chloramphenicol, 40 μg/ml; rifampin, 100 μg/ml; and tetracycline hydrochloride, 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 CFU in the range of colorimeter readings encountered.

Isolation of pantothenate kinase (*coaA*) mutants. Cell growth does not stop after the cessation of CoA biosynthesis but continues until the intracellular reserve of CoA is depleted by continued cell division (9). Therefore, strain DV5 (*panD*) was used so that the CoA content could be depleted to ensure that mutants unable to synthesize CoA would not grow during the ampicillin enrichment procedure. A logarithmic-phase culture of DV5 grown at 37°C in minimal medium plus 10 μM β-alanine was mutagenized with 1.5% ethyl methanesulfonate for 2 h as described (12). Surviving cells were grown to the stationary phase (2.5×10^9 cells per ml) at 30°C in minimal medium supplemented with 10 μM β-alanine. The cells were washed and suspended to 5×10^7 cells per ml in minimal medium minus β-alanine and incubated at 42°C until growth stopped because of depletion of intracellular CoA (9). The β-alanine-starved cells were subcultured at 42°C in minimal medium containing 10 μM β-alanine, and cells that were unable to grow were enriched by using ampicillin and recovered after removal of the ampicillin by subculturing in minimal medium plus 10 μM β-alanine at 30°C. The cycle of CoA depletion at 42°C followed by ampicillin enrichment of temperature-sensitive mutants was repeated except that casein amino acids (0.1%) were included in the minimal medium and pantothenate (4 μM) was added to the ampicillin selection medium. After the recovery of cells at 30°C, the CoA depletion step was

* Corresponding author.
† Present address: Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN 37831.

TABLE 1. Bacterial strains used

Strain	Genotype	Construction or source
AB468	Δ(*gpt-proA*)*62 lacY1 galK2* λ⁻ *hisG4 xyl-5 thi-1 purD13* F⁻	B. Bachmann (CGSC)[a]
AB1932	*argH1 metA28 thi-1 lac galK2 xyl tsx-6 supE44* F⁻	B. Bachmann (CGSC)
DV4	*metB1 panD2 panF11 relA1 spoT1* λ⁻ λ^r *gyrA216* F⁻	18
DV5	*metB1 panD2 relA1 spoT1* λ⁻ λ^r *gyrA216* F⁻	18
DV12	*zhc-12*::Tn*10*	18
DV15	*panD2 panF11 zij*::Tn*10*	P1 (W1485::Tn*10*) × DV4
DV29	*panC4 zad-220*::Tn*10 his-4 argE3 thi-1 lacY1 galK2 xyl-5 mtl-1 tsx-29* λ⁻ *supE44* F⁻	18
DV51	*metB1 panD2 coaA14*	EMS[b] mutagenesis of DV5
DV53	*metB1 panD2 coaA15*	EMS mutagenesis of DV5
DV56	*metB1 panD2 coaA15 zij*::Tn*10*	P1 (DV15) × DV53
DV58	*panD2 coaA15 zij*::Tn*10*	P1 (DV15) × DV53
DV59	*metB1 panD2 zij*::Tn*10*	P1 (DV15) × DV53
DV60	*argE3 panD2 coaA15*	P1 (DV29) × DV53
DV62	*metB1 panD2 coaA15 zij*::Tn*10*	P1 (DV56) × DV5
HS4004	Δ(*gpt-lac*)*5 rpsL150 rpoB324 metA28* λ⁻	B. Bachmann (CGSC)
SJ16	*panD2 metB1 relA1 spoT1* λ⁻ λ^r *gyrA216* F⁻ *zad-220*::Tn*10*	9

[a] CGSC, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.
[b] EMS, Ethyl methanesulfonic acid.

omitted and a third ampicillin enrichment of temperature-sensitive cells was performed by using minimal medium plus casein amino acids, β-alanine (10 μM), and pantothenate (4 μM). Survivors were spread on minimal medium agar plus 1 μM β-alanine at 30°C, and single colonies were tested for a temperature-sensitive growth phenotype. Temperature-sensitive colonies were then grown overnight at 30°C in minimal medium plus 0.5 μM β-alanine. Samples of the cultures were mixed with an equal volume of minimal medium containing 2 μM D-[3-^{3}H]pantothenate (specific activity, 5.0 Ci/mmol) and incubated at 42°C, and the uptake of the radiolabel was measured after 40 min as described previously (17). Strains that demonstrated deficient D-[3-^{3}H]pantothenate incorporation were isolated for further analysis.

Pantothenate kinase assays. Lysates were prepared from overnight cultures grown in minimal medium plus 1 μM β-alanine as previously described (16), and cytosolic proteins were dialyzed overnight at 4°C in 25 mM Tris hydrochloride (pH 7.4). Standard assay mixtures contained D-[1-^{14}C]pantothenate (50 μM; specific activity, 62,500 dpm/nmol), ATP (2.5 mM), $MgCl_2$ (2.5 mM), Tris hydrochloride (0.1 M, pH 7.4), and dialyzed protein (60 to 80 μg per assay) in a total volume of 40 μl. The incubation time was 10 min, and 4′-phospho[1-^{14}C]pantothenate was quantitated as label bound to DE81 filter circles (16). Protein concentrations were measured by the microbiuret method with bovine serum albumin as a standard (13).

Preparation of cell extracts and chromatography. Samples from β-[3-^{3}H]alanine-labeled cultures were pipetted into an equal volume of ice-cold 2-propanol, and the cells were lysed by sonication with four 10-s bursts in a Heat Systems cup horn sonicator at the maximum output setting. The extracts were centrifuged in a Beckman Microfuge, and the supernatants were treated with 10 mM dithiothreitol before analysis (9). The supernatants were analyzed by thin-layer chromatography on silica gel H layers developed with ethanol–28% ammonium hydroxide (4:1 [vol/vol]) to 14 cm from the origin (9). The distribution of radioactivity on the thin-layer plate was determined by scraping 0.5-cm sections of the silica gel into scintillation vials and counting in 3 ml of scintillation solution.

Localization of *coaA*. The *zij*::Tn*10* element was isolated from a random Tn*10* insertion pool by selecting tetracycline-resistant *metB*$^+$ recombinants as described previously (18) and was tested along with transposons *zad-220*::Tn*10* (near *panBCD*) (9) and *zhc-12*::Tn*10* (near *panF*) (18) for cotransduction with *coaA15*(Ts) by using bacteriophage P1 (12). Transductants were selected for tetracycline resistance or other chromosomal markers (*metB*, *argEH*, *rpoB*, *purD*, and *metA*) at 30°C on minimal medium agar containing 10 μM β-alanine and scored for growth at 42°C [*coaA*(Ts)]. The orientation of the *coaA* gene with respect to nearby loci was determined from three-factor crosses. Cotransduction frequencies were converted to map distances by using the formula of Wu and assuming the length of the P1 transducing fragment to be 2 min (3, 21).

RESULTS

Isolation of *E. coli* mutants with deficient D-[^{3}H]pantothenate incorporation. Selection conditions for mutants defective in CoA synthesis were optimized by depleting intracellular CoA in the mutagenized β-alanine-requiring strain DV5 (*panD*) at 42°C before the addition of ampicillin. In the absence of the pantothenate precursor, β-alanine, strain DV5 underwent two doublings before growth ceased. Temperature-sensitive mutants were enriched to 28% of the cells after the three cycles of ampicillin selection described in Materials and Methods. Isolates defective in pantothenate utilization were identified by incubating cultures from 48 temperature-sensitive colonies with D-[3-^{3}H]pantothenate for 40 min at 42°C and measuring the extent of pantothenate incorporation. Twenty-five percent of the cultures were labeled with D-[3-^{3}H]pantothenate to the same extent as the wild-type strain DV5 (approximately 14 pmol/10^8 cells), whereas the remaining cultures incorporated radioactivity at less than 10% of the wild-type level. Strains DV53 and DV51 were representative of the latter group. A chromosomal fragment causing temperature-sensitive growth was moved from the mutagenized strain DV53 into strain DV5, yielding strain DV62, to characterize the defect in vivo and confirm that temperature-sensitive pantothenate kinase activity correlated with the growth phenotype. This was accomplished by bacteriophage P1 transduction in two steps. The *zij*::Tn*10* element was first inserted near the mutation, yielding strain DV56 (*coaA zij*::Tn*10*), and then the mutant allele was introduced into strain DV5 by using the transposon as a positive selectable marker (Table 1; see below). This defective allele shared by strains DV53 and DV62 was designated *coaA15*.

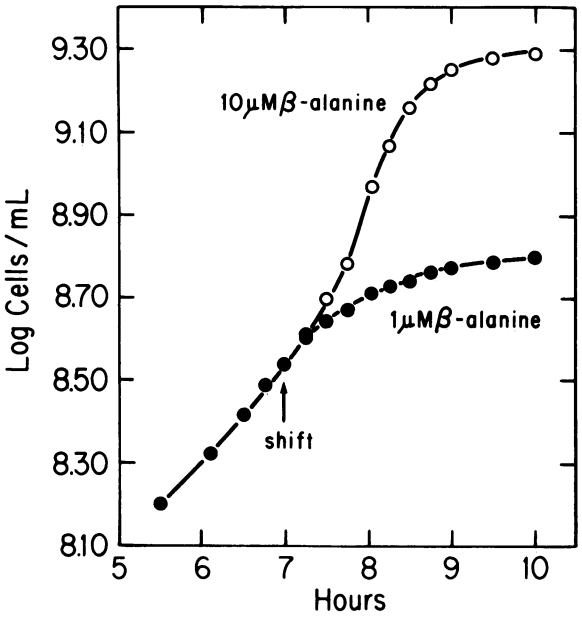

FIG. 1. Growth phenotype of strain DV62 (*coaA15 panD*). Strain DV62 was grown in minimal medium and subcultured to deplete CoA (9) at 30°C. Culture flasks containing minimal medium plus either 1 or 10 μM β-alanine were inoculated with 10^7 cells per ml and incubated at 30°C. Seven hours after the start of the incubation, the cultures were transferred to 42°C. Growth was monitored as described in the text.

β-Alanine-dependent, temperature-sensitive growth of strain DV62 (*panD coaA15*). The intracellular CoA content depends on the β-alanine supplement in *panD* mutants and increases approximately 10-fold between 1 and 10 μM β-alanine (9). If *coaA15* mutants were defective in CoA biosynthesis at the nonpermissive temperature, then the number of cell doublings after the temperature shift would increase as the β-alanine growth supplement at 30°C increased. Strain DV62 grew at the same rate as strain DV5 at 30°C, and the doubling time for the strains in minimal medium containing 1 μM or more β-alanine was 1.25 h (Fig. 1). When cultures supplemented with 1 μM β-alanine were shifted to 42°C during logarithmic growth, strain DV62 (*panD coaA15*) stopped growing after one division (Fig. 1), whereas the doubling time of strain DV5 (*panD coaA*$^+$) decreased to 1.1 h and the cells reached their normal stationary-phase cell density (data not shown). The onset of growth stasis at 42°C was delayed when strain DV62 was grown before the temperature shift in medium containing a higher β-alanine concentration. The doubling time of strain DV62 at 42°C decreased to 0.9 h and the culture grew to the same density as did strain DV5 when the CoA content in strain DV62 was maximized at 30°C in medium containing 10 μM β-alanine (Fig. 1). Thus, the growth of strain DV62 at 42°C could be extended for a few cell divisions by high intracellular CoA concentrations, consistent with the temperature-sensitive-growth phenotype of strain DV62 being due to the inability to synthesize CoA.

Defective pantothenate phosphorylation in strain DV62. The metabolism of β-[3-^{3}H]alanine by logarithmic-phase cultures of strains DV62 (*coaA15 panD*) and DV5 (*panD*) was compared to determine whether the CoA synthesis defect in strain DV62 was due to the lack of pantothenate phosphorylation. At 30°C, the strains demonstrated identical abilities to convert radioactive β-alanine into pantothenate and phosphorylated pantothenate metabolites (primarily CoA; data not shown). When strain DV5 cultures were shifted to 42°C and then labeled with β-[3-^{3}H]alanine, [3-^{3}H]pantothenate rapidly reached a steady-state concentration of 2 pmol/10^8 cells and the level of labeled phosphorylated metabolites increased throughout the monitoring period to 12.8 pmol/10^8 cells after 1 h (Fig. 2, upper panel). In strain DV62 cultures at 42°C, the accumulation of labeled phosphorylated metabolites stopped after 30 min at approximately 6.5 pmol/10^8 cells, whereas conversion of β-[3-^{3}H]alanine to [3-^{3}H]pantothenate increased to 12.8 pmol/10^8 cells after 1 h (Fig. 2, lower panel). Therefore, the inability of strain DV62 (*coaA15*) to synthesize CoA at 42°C was due to the lack of pantothenate phosphorylation.

Temperature-sensitive pantothenate kinase activity in vitro. The pantothenate kinase activity in extracts prepared from the wild-type strain DV5 increased with temperature to a maximum at 37°C, and 95% of the maximum activity was retained at 42°C (Fig. 3A). In contrast, extracts of strain DV53 (*coaA15*) demonstrated maximum activity at 30°C which was less than 20% of the wild-type rate and which decreased to less than 1% of the wild-type rate at 42°C (Fig. 3A and Table 2). The enzyme activity from strain DV53 was

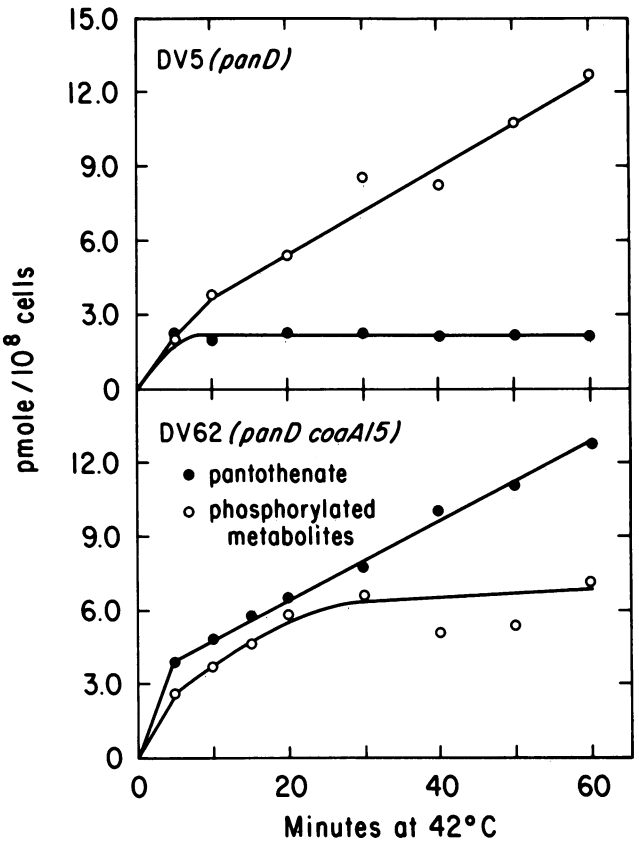

FIG. 2. Pantothenate synthesis and phosphorylation in strains DV62 (*coaA15 panD*) and DV5 (*panD*). Strains DV62 and DV5 were grown in minimal medium plus 1 μM β-alanine to the mid-logarithmic phase (3.6 × 10^8 cells per ml) at 30°C. The cultures were filtered, the cells were washed and suspended in minimal medium minus β-alanine at 30°C, and chloramphenicol was added. The suspensions were transferred to 42°C, and after 1 min, β-[3-^{3}H]alanine (1.0 μM; specific activity, 8.0 Ci/mmol) was added. Samples were removed at 5- or 10-min intervals and analyzed by thin-layer chromatography as described in the text.

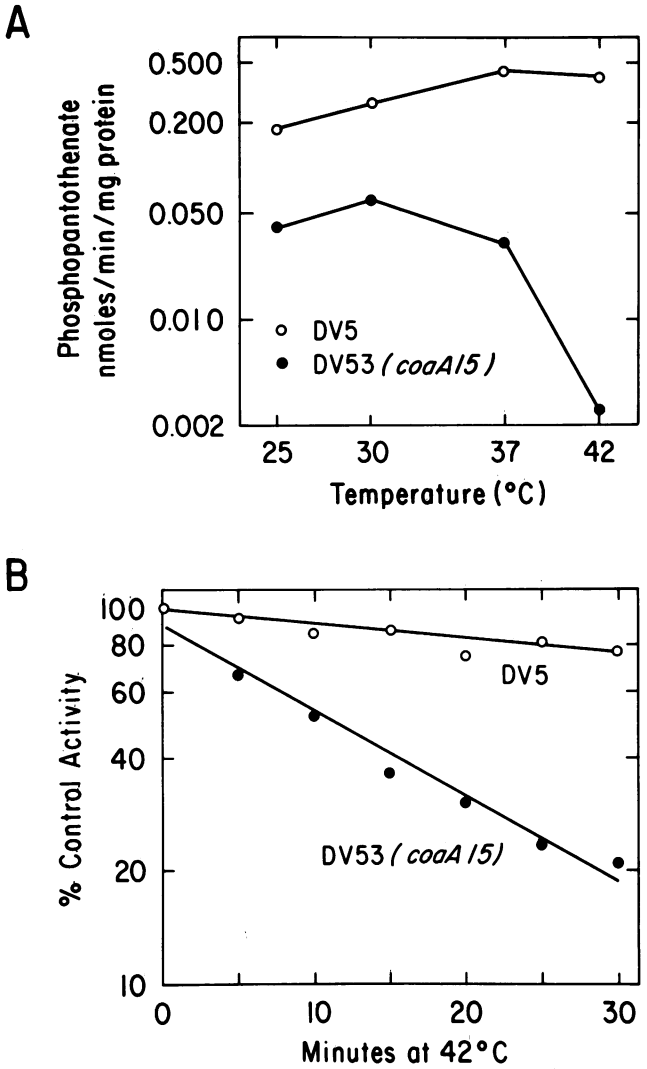

FIG. 3. Heat inactivation of pantothenate kinase in vitro. (A) Samples (80 μg of protein) of a cell lysate from either strain DV53 or strain DV5 were incubated for 10 min in 34 μl of 117 mM Tris hydrochloride (pH 7.4) at the indicated temperature and then assayed at the same temperature for pantothenate kinase activity. (B) A cell lysate (800 μg of protein per 340 μl) from either strain DV53 or strain DV5 was heated in 117 mM Tris hydrochloride (pH 7.4) at 42°C. At the indicated times, samples were cooled in an ice bath and immediately assayed for pantothenate kinase activity at 30°C. The results shown in panel B are the averages from two experiments and are expressed as percentages of the reaction rates in assays in which the heating step was omitted.

inactivated with a 15-min half-life at 42°C (Fig. 3B). Neither pantothenate nor 4'-phosphopantothenate was degraded by strain DV53 extracts at 42°C (data not shown). At 30°C, the temperature-sensitive pantothenate kinase had the same apparent K_m for pantothenate (25 μM) but a 43% higher apparent K_m for ATP (1.0 mM) compared with that of the wild-type enzyme (0.7 mM). CoASH (100 μM) inhibited the wild-type and temperature-sensitive enzymes to the same extent (Table 3). Pantothenate kinase from a strain DV62 extract exhibited temperature sensitivity identical to that of the strain DV53 enzyme (Table 2). These data confirm that the defect in pantothenate phosphorylation in *coaA15* mutants was due to temperature-sensitive pantothenate kinase

TABLE 2. Pantothenate kinase activities in extracts prepared from *coaA* mutants and wild-type cells

Strain	nmol of 4'-phosphopantothenate/min per mg of protein at[a]:	
	30°C	42°C
DV5	0.431	0.633
DV51 (*coaA14*)[b]	0.007	ND[c]
DV53 (*coaA15*)[b]	0.069	0.003
DV59[b]	0.438	0.593
DV62 (*coaA15*)	0.063	0.004

[a] Assays were performed at the indicated temperature with dialyzed lysates, and the assay mixtures contained D-[1-^{14}C]pantothenate (50 μM; specific activity, 62,500 dpm/nmol), ATP (5 mM), $MgCl_2$ (5 mM), and Tris hydrochloride (0.1 M, pH 7.5).

[b] Extracts were incubated at the indicated temperature in 117 mM Tris hydrochloride (pH 7.4) for 10 min before the assay.

[c] ND, Not detected.

activity. In addition to strain DV53, strain DV51 (*coaA14*) was isolated as a mutant with defective pantothenate incorporation but represented a class of pantothenate kinase mutants having nearly undetectable activity in vitro (Table 2).

Chromosomal location of *coaA*. To test whether *coaA* was near previously mapped chromosomal loci affecting pantothenate synthesis in *E. coli* (9, 18) or the *coaA* mutation at min 89 in *S. typhimurium* (5), strain DV53 was transduced with bacteriophage P1 stocks grown on strains harboring a tetracycline resistance element, Tn*10*, near each site. Tetracycline-resistant recombinants were obtained, and only those selected for the *zij*::Tn*10* insertion gained the ability to grow on minimal medium agar at 42°C, indicating that *coaA15* is located in the same area as the *coaA* gene in *S. typhimurium*. The 28% cotransduction frequency between *zij*::Tn*10* and either *coaA14* or *coaA15* indicated that both alleles mapped to the same location close to *metB* near min 89 on the *E. coli* chromosome. The *coaA*$^+$ recombinants (represented by strain DV59) possessed wild-type pantothenate kinase activity in vitro (Table 2). The position of *coaA15* between the *argEH* and *rpoB* loci was defined by three-factor crosses (Table 4), and the results were consistent with the map positions calculated from cotransduction frequencies between *coaA15* and known genes in the 89- to 90.5-min region of the chromosome (Fig. 4).

DISCUSSION

These data define a new gene (*coaA*) encoding the first enzyme in the CoA biosynthetic pathway. The temperature-dependent inactivation of pantothenate phosphorylation in *coaA* mutants both in vivo (Fig. 1 and 2) and in vitro (Fig. 3) establishes this locus as the structural gene for pantothenate

TABLE 3. CoASH inhibition of temperature-sensitive and wild-type pantothenate kinase

Strain	nmol of 4'-phosphopantothenate/min per mg of protein with[a]:		
	No addition	DTT	DTT + CoASH
DV5	0.32	0.34	0.20
DV53 (*coaA15*)	0.083	0.072	0.048

[a] Assays were performed at 30°C with dialyzed lysates and contained D-[1-^{14}C]panthothenate (50 μM; specific activity, 62,500 dpm/nmol), ATP (5 mM), $MgCl_2$ (5 mM), and Tris hydrochloride (0.1 M, pH 7.4). Some assay mixtures also contained CoASH (100 μM) dithiothreitol (DTT; 2 mM) or both.

TABLE 4. Three-factor analyses

Cross (P1 donor × recipient)	Selection (no.)	Recombination class (no.)	Implied gene order
DV29 (*argE*) × DV53 (*metB coaA15*)	*metB*$^+$ (100)	*argE coaA*$^+$ (17) *argE coaA* (25) *argE*$^+$ *coaA*$^+$ (1) *argE*$^+$ *coaA* (57)	*metB argE coaA*
DV58 (*coaA15*) × HS4004 (*metA rpoB*)	*metA*$^+$ (131)	*rpoB*$^+$ *coaA* (67) *rpoB*$^+$ *coaA*$^+$ (15) *rpoB coaA* (3) *rpoB coaA*$^+$ (46)	*coaA rpoB metA*
DV58 (*coaA15*) × AB1932 (*argH metA*)	*argH*$^+$ (94)	*metA*$^+$ *coaA* (6) *metA*$^+$ *coaA*$^+$ (4) *metA coaA* (38) *metA coaA*$^+$ (46)	*argH coaA metA*
	metA$^+$ (70)	*argH*$^+$ *coaA* (7) *argH*$^+$ *coaA*$^+$ (0) *argH coaA* (16) *argH coaA*$^+$ (47)	
HS4004 (*rpoB*) × DV60 (*argE coaA15*)	*argE*$^+$ (157)	*rpoB coaA*$^+$ (52) *rpoB coaA* (0) *rpoB*$^+$*coaA*$^+$ (5) *rpoB*$^+$ *coaA* (100)	*argE coaA rpoB*

kinase. The data also verify the results of metabolic labeling experiments (9, 10) and enzymatic analyses (16) showing that the phosphorylation of pantothenate is the only route to CoA in *E. coli*. The *coaA* gene is located at min 90 of the *E. coli* chromosome (Table 4 and Fig. 4) and is not linked to the genes responsible for the pantothenate synthetic enzymes (*panBCD*) at min 3 (4) or the pantothenate permease (*panF*) at min 72 (18). Mutants expressing a temperature-sensitive pantothenate kinase (*coaA*) in *S. typhimurium* map to chromosomal min 89 (5), and our mutants probably represent the *coaA* counterpart in *E. coli*.

Allosteric regulation of pantothenate kinase activity by the CoA pool is a major determinant of the CoA biosynthetic rate, and there is no evidence for regulation of enzyme synthesis as a mechanism to control pantothenate phosphorylation (16). The in vitro kinetic properties of pantothenate kinase suggest that this regulatory enzyme expresses only 20 to 25% of its maximum catalytic capacity in vivo because of feedback inhibition by CoASH and its thioesters (16). The mutant pantothenate kinase (*coaA15*) retained its sensitivity to CoA inhibition at 30°C (Table 3) and had near normal kinetic constants for ATP and pantothenate. The reduced enzyme activities found at 30°C in the *coaA14* and *coaA15* mutants (Table 2) support the conclusion that wild-type pantothenate kinase is normally produced at levels higher than that required for growth. Pantothenate kinase mutants with altered feedback inhibition characteristics are needed to determine the contribution of allosteric regulation of pantothenate phosphorylation to the overall control of intracellular CoA concentrations.

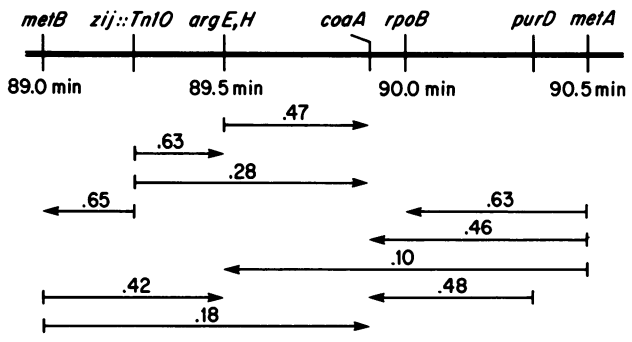

FIG. 4. Location of *coaA* on the *E. coli* chromosome. The map position of *coaA* was computed as described in the text. The number above each arrow indicates the cotransduction frequency, and for each gene pair, the arrowhead is directed toward the unselected marker of the cross.

ACKNOWLEDGMENTS

This work was supported by grant-in-aid 851165 from the American Heart Association, Public Health Service grant GM 34496 from the National Institutes of Health, Cancer Center (CORE) support grant CA 21865, and the American Lebanese Syrian Associated Charities.

LITERATURE CITED

1. **Abiko, Y.** 1975. Metabolism of coenzyme A, p. 1–25. *In* D. M. Greenburg, (ed.), Metabolic pathways, 3rd ed., vol. 7. Academic Press, Inc., New York.
2. **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.
3. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
4. **Cronan, J. E., Jr., K. J. Little, and S. Jackowski.** 1982. Genetic and biochemical analyses of pantothenate biosynthesis in *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. **149**:916–922.
5. **Dunn, S. D., and E. E. Snell.** 1979. Isolation of temperature-sensitive pantothenate kinase mutants of *Salmonella typhimurium* and mapping of the *coaA* gene. J. Bacteriol. **140**:805–808.
6. **Fisher, M. N., and J. R. Neely.** 1985. Regulation of pantothenate kinase from various tissues of the rat. FEBS Lett. **190**:293–296.
7. **Fisher, M. N., J. D. Robishaw, and J. R. Neely.** 1985. The properties and regulation of pantothenate kinase from rat heart. J. Biol. Chem. **260**:15745–15751.

8. **Halvorson, O., and S. Skrede.** 1982. Regulation of the biosynthesis of CoA at the level of pantothenate kinase. Eur. J. Biochem. **124**:211–215.
9. **Jackowski, S., and C. O. Rock.** 1981. Regulation of coenzyme A biosynthesis. J. Bacteriol. **148**:926–932.
10. **Jackowski, S., and C. O. Rock.** 1984. Metabolism of 4′-phosphopantetheine in *Escherichia coli*. J. Bacteriol. **158**:115–120.
11. **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.
12. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Munkres, K. D., and F. M. Richards.** 1965. The purification and properties of *Neurospora* malate dehydrogenase. Arch. Biochem. Biophys. **109**:466–479.
14. **Robishaw, J. D., D. Berkich, and J. R. Neely.** 1982. Rate-limiting step and control of coenzyme A synthesis in cardiac muscle. J. Biol. Chem. **257**:10967–10972.
15. **Smith, C. M., and C. R. Savage, Jr.** 1980. Regulation of coenzyme A biosynthesis by glucagon and glucocorticoid in adult rat liver parenchymal cells. Biochem. J. **188**:175–184.
16. **Vallari, D. S., S. Jackowski, and C. O. Rock.** 1987. Regulation of pantothenate kinase by coenzyme A and its thioesters. J. Biol. Chem. **262**:2468–2472.
17. **Vallari, D. S., and C. O. Rock.** 1985. Pantothenate transport in *Escherichia coli*. J. Bacteriol. **162**:1156–1161.
18. **Vallari, D. S., and C. O. Rock.** 1985. Isolation and characterization of *Escherichia coli* pantothenate permease (*panF*) mutants. J. Bacteriol. **164**:136–142.
19. **Vallari, D. S., and C. O. Rock.** 1986. Preparation of high specific activity D-[3-^{3}H]pantothenic acid. Anal. Biochem. **154**:671–675.
20. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.
21. **Wu, T. T.** 1966. A model for three-point analysis of random general transduction. Genetics **54**:405–410.