

Phosphopantothenoylcysteine Synthetase from *Escherichia coli*

IDENTIFICATION AND CHARACTERIZATION OF THE LAST UNIDENTIFIED COENZYME A BIOSYNTHETIC ENZYME IN BACTERIA*

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Phosphopantothenoylcysteine synthase catalyzes the formation of (R)-4'-phospho-N-pantothenoylcysteine from 4'-phosphopantothenate and L-cysteine: this enzyme, involved in the biosynthesis of coenzyme A (CoA), has not previously been identified. Recently it was shown that the NH₂-terminal domain of the Dfp protein from bacteria catalyzes the next step in CoA biosynthesis, the decarboxylation of (R)-4'-phospho-N-pantothenoylcysteine to form 4'-phosphopantetheine (Kupke, T., Uebele, M., Schmid, D., Jung, G., Blaesse, M., and Steinbacher, S. (2000) *J. Biol. Chem.* 275, 31838–31846). We have partially purified phosphopantothenoylcysteine decarboxylase from *Escherichia coli* and demonstrated that the protein encoded by the *dfp* gene, here renamed *coaBC*, also has phosphopantothenoylcysteine synthetase activity, using CTP rather than ATP as the activating nucleoside 5'-triphosphate. This discovery completes the identification of all the enzymes involved in the biosynthesis of coenzyme A in bacteria.

Coenzyme A (CoA)¹ fulfils a vital role in the normal metabolism of living organisms: it has been reported that ~4% of all enzymes utilize either CoA, its thioesters or 4'-phosphopantetheine (1). As an essential cofactor of fatty acid synthases, polyketide synthases, and nonribosomal peptide synthases, 4'-phosphopantetheine functions as an acyl group carrier, activating carboxylic acids for biological Claisen reactions and the formation of peptides and esters. Acetyl-CoA and succinyl-CoA

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¹ The abbreviations used are: CoA, coenzyme A; PPC-DC, phosphopantothenoylcysteine decarboxylase; PPC-S, phosphopantothenoylcysteine synthetase; PPanCys, (R)-4'-phospho-N-pantothenoylcysteine; PCR, polymerase chain reaction; IPTG, isopropyl-1-thio-β-D-galactopyranoside; DTT, dithiothreitol; DMF, dimethyl formamide; ESI/FTMS, electrospray ionization Fourier transform mass spectrometry; SWIFT, stored waveform inverse Fourier transform; HPLC, high performance liquid chromatography; FMN, flavin mononucleotide; PAGE, polyacrylamide gel electrophoresis.

are key intermediates in energy metabolism, taking part in the tricarboxylic acid cycle. It is therefore surprising that three of the CoA biosynthetic enzymes have only recently been identified in *E. coli* (2, 3).

Phosphopantothenoylcysteine decarboxylase (PPC-DC) catalyzes the decarboxylation of 4'-phosphopantothenoylcysteine (PPanCys) to form 4'-phosphopantetheine (Fig. 1). From a mechanistic perspective this reaction proves to be particularly interesting as it is unclear how the stabilization of the carbanion intermediate is achieved. To study this enzyme we have repeated, with major modifications, the published purification of PPC-DC from wild-type *E. coli* (4). Amino-terminal sequencing identified the *dfp* gene as coding for the protein having this activity. The flavin-containing Dfp protein was first identified from a temperature-sensitive mutant (*dfp-707*), believed to be involved in DNA and pantothenate metabolism (5, 6). However, the conditional lethality of the mutant was reported not to be a direct result of pantothenate auxotrophy, and its complete characterization was not achieved. While our work was in progress, Kupke *et al.* (7) also identified Dfp as having PPC-DC activity by sequence comparison with EpiD, a lantibiotic-synthesizing protein catalyzing the decarboxylation of peptidylcysteine intermediates.

We now report that the protein encoded by *dfp* also catalyzes the formation of PPanCys from 4'-phosphopantothenate and L-cysteine using cytidine 5'-triphosphate (CTP) as the activating nucleoside 5'-triphosphate. The protein releases CMP as the product of the coupling reaction, indicating the formation of an activated acyl-cytidylate intermediate. In addition, we show that the protein does not contain a covalently linked pyruvoyl group as cofactor as was reported previously (4). The identification of phosphopantothenoylcysteine synthase (PPC-S) completes the identification of all the enzymes involved in the biosynthesis of CoA in bacteria and prepares the way for its careful study and possible exploitation in the design of antimicrobial agents.

EXPERIMENTAL PROCEDURES

Materials and Methods—All chemicals were purchased from Aldrich or Sigma and were of the highest purity. Resins and columns were purchased from Amersham Pharmacia Biotech, except hydroxyapatite (Bio-Rad). Radiolabeled chemicals (L-1,1'-[¹⁴C]cystine (100 mCi/mmol, 1 mCi/ml) and [¹⁴C]NaHCO₃ (50 mCi/mmol, 1 mCi/ml)) were purchased from American Radiolabeled Chemicals (St. Louis, MO). Ecolume (ICN) was used for liquid scintillation counting, performed on a Beckman LS 1801 instrument. Oligonucleotide synthesis and automated DNA sequencing were performed at the Cornell Biotechnology Resource Center. ¹H NMR was performed on a Bruker INNOVA 400 MHz instrument.

Purification of the Native Protein—PPC-DC activity was partially purified from 38 g of frozen *E. coli* B cells (Fermentation Facility, University of Alabama, Birmingham, AL) using normal protein purification methodology (Table I). However, protein concentration was not accomplished by buffer removal through membranes as this led to large losses of enzymatic activity; instead, solutions were concentrated on short DEAE-Sepharose columns and the concentrated protein eluted in buffer containing 400 mM NaCl when necessary. Buffer exchanges and salt removal were performed by dialysis.

Cloning and Overexpression of the *coaBC* Gene—The *coaBC* gene was amplified by PCR using as the forward primer: 5'-CCTACAGGAAAA-ATCCATATGAGCCTGGCCGG-3', introducing an *Nde*I restriction site (underlined), and as the reverse primer: 5'-GCCAGTTCTACGGCGTC-CTCGAGACAGGCACGCAGG-3', introducing an *Xho*I restriction site (underlined). The resulting PCR product coding for Dfp from Met²⁵ to the end of the protein was cloned into *Nde*I/*Xho*I-digested pET28a ex-

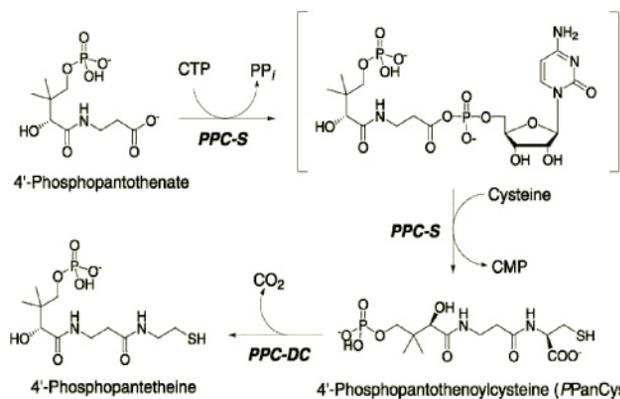


FIG. 1. Reactions catalyzed by the PPC-S and PPC-DC activities of Dfp.

pression vector (Novagen). The sequence of the resulting plasmid, designated *pCLK1210*, was verified by automated DNA sequencing. The expression plasmid was transformed into the *E. coli* Tuner(DE3) strain (Novagen).

Purification of Recombinant Protein—*E. coli* Tuner(DE3) *pCLK1210* was grown in LB broth supplemented with 15 μ g/ml kanamycin sulfate at 37 °C to $A_{595} \sim 0.6$, and induced with 200 μ M IPTG. After growing overnight at 37 °C, the cells were harvested, suspended in sonication buffer (5 mM imidazole, 0.5 M NaCl, and 20 mM Tris, pH 7.9, 10 ml/1 g of cell paste), disrupted by sonication, and centrifuged at 35,000 $\times g$ for 30 min to collect the crude cell extract. This was applied to a 5-ml His-Bind column (Novagen). Weakly bound proteins were removed by washing with sonication buffer, followed by sonication buffer containing 100 mM imidazole. The protein of interest was eluted as a yellow band by increasing the imidazole concentration to 0.5 M. Elution was monitored at A_{280} and A_{450} .

Synthesis of 4'-Phosphopantothenate and 4'-Phosphopantothenoylcysteine—Benzyl pantothenate was synthesized by alkylation of sodium pantothenate (1.00 g, 4.15 mmol dissolved in 15 ml of dry DMF) with benzyl bromide (494 μ l, 4.15 mmol) by heating at 70 °C overnight. DMF was removed *in vacuo*, ethyl acetate (100 ml) was added, the organic layer was washed with saturated aqueous NaCl (3 \times 20 ml), dried (Na₂SO₄), and the solvent removed. The product was purified by flash column chromatography (silica gel; ethyl acetate/hexane 2:1 to 4:1; 1.13 g, yield 88%). Dibenzylchlorophosphate was prepared *in situ* by reacting dibenzylphosphite (1.57 g, 6.0 mmol) and *N*-chlorosuccinimide in dry benzene (8 ml) for 2 h at 25 °C. The reaction mixture was filtered and added dropwise to benzyl pantothenate (615 mg, 2.0 mmol) dissolved in dry pyridine (9 ml) at -40 °C (dry ice/acetonitrile). After stirring at -40 °C for 2 h, the mixture was placed in a -20 °C freezer overnight. The reaction was warmed to 25 °C and then quenched with water (3 ml). The solvent was removed *in vacuo*, and ethyl acetate (25 ml) was added. The resulting suspension was washed with 1 M H₂SO₄ (2 \times 5 ml), 1 M NaHCO₃ (2 \times 5 ml), and saturated Na₂SO₄ (1 \times 5 ml), dried (Na₂SO₄), and the solvent removed. The resulting benzyl pantothenate 4'-(dibenzyl phosphate) was purified by flash column chromatography (silica gel; ethyl acetate/hexane 2:1), to give a clear oil (576 mg, yield 50%). Benzyl pantothenate 4'-(dibenzyl phosphate) (250 mg, 439 μ mol) was dissolved in 90% aqueous methanol and hydrogenated at atmospheric pressure for 2 h in the presence of 10% palladium/carbon. After filtering and solvent evaporation, the final product (131 mg, quantitative) was dissolved in H₂O, titrated to pH \sim 6.5 with 1 M NaOH, and stored as frozen aliquots of a stock solution (50 mM) at -20 °C. ¹H NMR (400 MHz, D₂O): δ 0.70 (s, ³H), 0.77 (s, ³H), 2.43 (t, 2H), 3.31 (t, 2H), 3.43–3.45 (m, 1H), 3.64 (d of d, 1H), 3.82 (s, 1H).

4'-Phosphopantothenoylcysteine was synthesized by adding *S*-propyl thiopantothenate 4'-(dibenzyl phosphate) (50 mg, 93 μ mol) (Ref. 8, with phosphorylation step modified as above) to L-cysteine (12.4 mg, 102 μ mol) dissolved in 102 μ l of 1 M NaOH and 900 μ l of methanol. After stirring for 4 h, the solvent was removed under a stream of argon, 500 μ l of 1 M HCl was added, and the product was extracted with ethyl acetate (3 \times 500 μ l). The combined organic extracts were dried (Na₂SO₄) and the solvent evaporated under a stream of argon. The resulting residue was dissolved in \sim 2.5 ml of liquid NH₃ and sodium pieces added until the blue color persisted. Methanol (two drops) was then added, and the ammonia was allowed to evaporate. Ice water (1 ml) was added, followed by a suspension of Dowex 50WX8-100 resin in

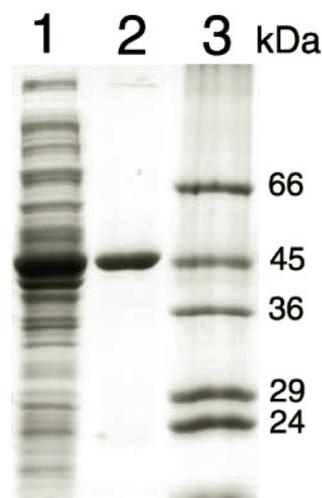


FIG. 2. SDS-PAGE analysis of the overexpression and purification of recombinant Dfp. Lane 1, cell-free extract. Lane 2, purified protein fraction. Lane 3, molecular weight markers.

deionized water, until the pH of the solution was acidic. The suspension was loaded on a prepared Dowex 50WX8-100 column (500 μ l) in a plastic pipette, the product was eluted with 2 ml of water, and the solvent was removed by lyophilization. The final product (30 mg, 80%) was dissolved in H₂O, titrated to pH \sim 6.5 with 1 M NaOH, and stored as frozen aliquots of a stock solution (18 mM) at -20 °C. ¹H NMR (400 MHz, D₂O): δ 0.64 (s, ³H), 0.79 (s, ³H), 2.40 (d of t, 2H), 2.70 (d, 2H), 3.22 (d of d, 1H), 3.34 (t, 2H), 3.55 (d of d, 1H), 3.91 (s, 1H), 4.19 (t, 1H).

The corresponding ¹⁴C-labeled substrate, PPan-1-[¹⁴C]Cys, was similarly synthesized on the same scale as above using L-1-[¹⁴C]cysteine (\sim 0.4 mCi/mmol), prepared from L-1,1'-[¹⁴C]cysteine and L-cysteine.

Assay Procedure—The CO₂ release assay was performed as described previously (4), with modifications. Assays were performed in unmodified microcentrifuge tubes, and all additions were made directly to the sample followed by immediate closure of the tube. Assay mixtures contained 10 mM DTT, 2 mM MgCl₂, 50 mM Tris, pH 7.6, enzyme (\sim 2 μ g) and either 3.0 mM PPan-1-[¹⁴C]Cys or 3.5 mM L-1-[¹⁴C]cysteine in a total volume of 60 μ l; if the synthetase activity was being assayed, mixtures also contained 2.5 mM 4'-phosphopantothenate and 3.5 mM CTP. The reaction was initiated by addition of the ¹⁴C-labeled substrate (10 μ l of stock solution, *i.e.* 21 mM cysteine or 18 mM PPanCys) to the rest of the assay components in 50 μ l and stopped after 10 min at room temperature by addition of 100 μ l of 5 M H₂SO₄. The amount of CO₂ released was determined as reported previously (4). Efficiency of ¹⁴CO₂ trapping was determined over the same concentration range as for the assay, using [¹⁴C]NaHCO₃ as standard. Assay results were adjusted using the resulting standard curve ($y = 0.70x - 0.08$, $R^2 = 0.99$).

High Resolution Mass Spectrometry—Protein samples were desalted by loading onto reverse-phase protein traps (Michrom Bioresources, Auburn, CA), washed with 2 ml of 2:96:2 methanol/H₂O/acetic acid, and step eluted with 70:26:4 methanol/H₂O/acetic acid. Mass spectra were acquired on a modified 6-T Finnigan FTMS with the Odyssey data system described previously (9), with nanospray sample injection (10). Specific ions were isolated using stored waveform inverse Fourier transform (SWIFT) (11), and assignment of the fragment masses and compositions was made using the computer program THRASH (12). Spectra were calibrated externally using bovine ubiquitin ($M_r = 8,564.64$).

Identification of the CTP Product—HPLC analysis was performed on reaction mixtures containing 1.5 mM 4'-phosphopantothenate, 3.5 mM CTP, 3.5 mM L-cysteine, 25 mM Tris, 2.5 mM MgCl₂, pH 7.6, and 20 μ l of purified enzyme (0.95 mg/ml in 25 mM Tris, 1 mM EDTA, 2 mM DTT, pH 7.6) in a total volume of 500 μ l. After standing at 25 °C for 4 h, protein was removed by centrifugation through a membrane (Vivaspin 500, Vivascience), and the reaction mixture was analyzed with a Supelcosil LC-18-T 3 μ m, 15 cm \times 4.6 mm inner diameter column (Supelco) on an HP series 1100 HPLC system eluting with 100 mM potassium phosphate buffer, pH 6.8, containing 4 mM tetrabutylammonium hydrogen-sulfate. Elution was monitored at 254 nm, and identification was performed by comparison to CMP, CDP, and CTP as standards.

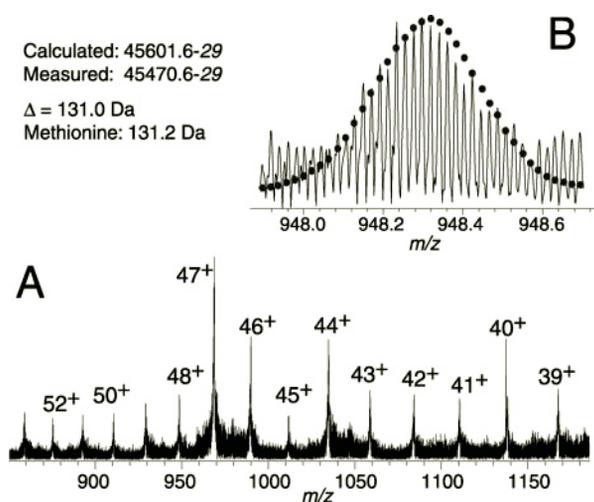


FIG. 3. ESI/FTMS analysis of recombinant Dfp. Spectrum A shows the broadband molecular ion spectrum of the protein (25 scans), with the SWIFT (16) isolation of the 48⁺ ions shown as spectrum B. The dots show the theoretical isotopic distribution. The calculated and measured mass values are shown, with the mass difference (in units of 1.0034 Da) between the most abundant isotopic peak and the mono-isotopic peak denoted in *italics* after each mass value. The difference of 131.0 Da between the calculated and measured values corresponds to the loss of NH₂-terminal methionine from the protein.

RESULTS AND DISCUSSION

Purification of the Native Protein and Identification of the Synthetase Activity—Our procedure for the purification of PPC-DC differed significantly from that previously published; it is summarized in Table I. Throughout the purification the protein exhibited a number of characteristics that hampered its isolation. These included copurification with proteins of high molecular weight and coagulation on concentrating membranes leading to large losses of activity. After solving these problems PPC-DC could be partially purified 1,280-fold as one of a small set of proteins (~15% purity, the major contaminant (~80%) being alcohol dehydrogenase (*adhE* gene product) as determined by NH₂-terminal sequencing), the final fraction having a specific activity of 370 nmol CO₂/min/mg.

During the purification trials we observed that substrate analogs like 4'-phosphopantothencysteine and 4'-phosphopantothencysteine did not inhibit PPC-DC activity in partially purified fractions. Furthermore, the high concentrations of PPanCys needed to saturate the enzyme, combined with the reported failure to detect PPanCys in cell extracts (13), suggested that PPanCys was not the physiological substrate for the protein catalyzing its decarboxylation. The most likely alternative was to consider close interaction between the PPC-S and PPC-DC activities, and subsequently we tested 4'-phosphopantothenate, CTP, and L-1-[¹⁴C]cysteine as substrates. We found that ¹⁴CO₂ was released from the reaction mixture, while cysteine was not decarboxylated in the absence of the other substrates. Our choice of CTP as activating nucleoside triphosphate rather than the more usual ATP was based on previous studies (13) that had demonstrated that crude extracts from *E. coli* preferentially used CTP for 4'-phosphopantothencysteine synthetase activity. However, it was not possible to determine whether these PPC-DC and PPC-S activities found in our partially purified fractions were located on the same protein, or on two distinct proteins which were copurifying, until the enzyme was purified to homogeneity.

Identification, Cloning, Overexpression, and Purification of Recombinant PPC-DC—The proteins from the purified fraction with the highest specific activity were separated by SDS-PAGE and subsequently electroblotted to a polyvinylidene difluoride membrane. Determination of the NH₂-terminal sequence of the

band at 45.0 kDa gave SLAGKKILVLGV, revealed by a BLAST (14) search to correspond to residues 26–36 of the protein coded for by the *dfp* gene, annotated as a flavoprotein affecting DNA and pantothenate metabolism in an unspecified way.

Amplification of the *dfp* gene (using ATG²⁵ as the start codon) from the *E. coli* genome by PCR and subsequent insertion of the product into pET28a gave *pCLK1210* as the resulting plasmid. Transformation of this plasmid into the Tuner(DE3) cell strain, a *lac* permease deletion mutant allowing uniform entry of IPTG into cells and providing strict concentration-dependent control of induction, allowed the production of soluble overexpressed Dfp following induction at 37 °C with 200 μM IPTG (Fig. 2, lane 1). Expression in the BL21(DE3) host strain failed to produce soluble protein under a variety of conditions. The presence of an NH₂-terminal 6 × His-tag made the purification of the recombinant protein possible by imidazole elution from a Ni²⁺-chelating affinity resin (Fig. 2, lane 2). Both the purification procedure and subsequent dialysis had to be performed at an ambient temperature as the protein precipitated at 4 °C. Solutions of the protein could be concentrated by centrifugation through a membrane if the procedure was carried out at 15 °C.

PPC-DC Is Not a Pyruvoyl-dependent Enzyme—The report of the first purification of PPC-DC from *E. coli* (4) claimed that the enzyme contained a base labile pyruvoyl group. We set out to determine whether we could identify such a post-translational modification by mass spectrometric analysis of the pure recombinant protein.

The analysis of Dfp by high resolution ESI/FTMS showed that the protein had a molecular mass of 45,470.6 Da (Fig. 3). This value corresponds exactly to the calculated mass of the recombinant protein with the NH₂-terminal methionine removed (45,470.4 Da) and excludes the possibility that PPC-DC contains a pyruvoyl group or any other covalent post-translational modification.

Identification of the Flavin Cofactor—The purified protein exhibited a UV-visible spectrum characteristic of flavoenzymes, with a λ_{max} at 455 and 378 nm. The identity of the flavin was previously found to be flavin mononucleotide (FMN) by TLC analysis of the denatured protein and comparison to standards (5). We confirmed this finding by isolating the flavin cofactor from heat-denatured enzyme and subjecting it to ESI/MS analysis. The isolated product had an *m/z* value of 457.2 (M + H)⁺ corresponding to FMN (M_r 456.3 for the free acid). Further MS/MS analyses of the molecular ion confirmed this identification. No peaks corresponding to FAD molecular ions could be observed.

4'-Phosphopantothencysteine Synthetase Activity of the Dfp Protein—Since our initial studies on partially purified mixtures of PPC-DC suggested that it was copurifying with PPC-S activity, we repeated our experiments with the purified Dfp protein. This enzyme catalyzes the release of ¹⁴CO₂ from L-1-[¹⁴C]cysteine in the presence of 4'-phosphopantothenate, CTP, and Mg²⁺, but not when any one of these cosubstrates was absent. Analysis of the reaction mixture by ESI/MS demonstrated the formation of 4'-phosphopantetheine.

None of the other nucleoside 5'-triphosphates, *i.e.* ATP, GTP, and UTP, could substitute for CTP in the coupling reaction even at concentrations as high as 10 mM. The function of cytidyltransferases in the formation of critical intermediates in the biosynthesis of lipids and complex carbohydrates has been studied extensively; these enzymes use CTP to form activated CDP-alcohols or CMP-acid sugars (Ref. 15 and references cited therein). However, the utilization of CTP by enzymes to activate acyl groups in a transient fashion has, to our knowledge, not been reported. Furthermore, it has been reported that members of the family of cytidyltransferases can be identified by the presence of certain conserved binding motifs, the HXGH

TABLE I
 Purification of PPC-DC activity from *E. coli* B cells

Purification step	Protein	Activity	Specific activity	Yield	Purification
	mg	nmol/min	nmol/min/mg	%	-fold
Crude extract ^a	2927	845	0.289	100	1
30–50% (NH ₄) ₂ SO ₄ fraction	1152	767	0.665	91	2.3
HiPrep 16/60 Sephacryl S-200	429	480	1.12	57	3.9
DEAE-Sepharose Fast Flow	15.7	240	15.3	28	53
Hydroxyapatite (CHT Type I)	3.32	103	31.0	12	107
HiTrap Blue	0.45	36.7	80.9	4.3	280
Superdex 200 prep grade	0.026	9.5	371	1.1	1280

^a The data were obtained from 38 g of frozen cell paste. Special precautions had to be followed during the purification, see "Experimental Procedures" for details.

 TABLE II
 K_m and k_{cat} values of the PPC-S and PPC-DC activities of Dfp

Substrate	K_m	k_{cat} ^a
	μM	min^{-1}
4'-Phosphopantothenate	290	53
CTP	290	
Cysteine	240	
PPanCys	780	50

^a k_{cat} values are for the coupled (synthetase and decarboxylation) and decarboxylation reactions, respectively.

and RTXGISTT motifs being the best examples (15). However, Dfp from *E. coli* does not contain such a motif, or any other motif that has been associated with NTP binding.

HPLC analysis of the products of the coupling reaction showed that CTP was converted exclusively to CMP. This indicates the formation of an activated acyl-cytidylate, which is subsequently attacked by cysteine (Fig. 1). This mode of action contrasts with that of the partially purified rat liver PPC-S (16), which was shown to produce ADP and phosphate as products of the coupling reaction, suggesting the formation of an acyl-phosphate intermediate for that system.

Steady State Kinetic Analysis—Time course assays based on the release of ¹⁴CO₂ by the Dfp protein demonstrated a linear increase of CO₂ production for the first 10 min of reaction. We have therefore assumed that the average rate of a reaction quenched within this period of time is a close approximation of the initial rates of reaction.

The accurate determination of the kinetic parameters was hampered by a number of factors. It was found that increasing the enzyme concentration above ~30 $\mu g/ml$ led to a rapid decrease in activity. Protein from which the His-tag had been removed by thrombin cleavage exhibited similar behavior (data not shown). Although leading to practical problems, the physiological significance of this observation is unclear, because the decrease in activity is only observed at levels of enzyme concentration above those expected to occur under normal cellular conditions. However, it does confirm the tendency of the enzyme to aggregate, a process hastened at lower temperatures. The enzyme also loses activity over time; this deterioration may be slowed by the presence of high (~200 μM) concentrations of NaCl in the enzyme sample, but its presence negatively influences the determination of the kinetic parameters.

Despite these problems, preliminary values for K_m and k_{cat} were determined at ~30 $\mu g/ml$ enzyme, while maintaining nonvariable substrates at concentrations of at least $5 \times K_m$ (Table II). 4'-Phosphopantothenate shows substrate inhibition at high concentrations. The 2.7-fold higher value of $K_{m(pPanCys)}$,

compared with $K_{m(4'-phosphopantothenate)}$, suggests that this intermediate is not the physiological substrate.

Conclusion—The Dfp protein from *E. coli* bears little resemblance to PPC-S and PPC-DC from mammalian sources: PPC-DC has been partially purified from both horse (17) and rat liver (18), and in the case of the former, it was shown that the enzyme is pyruvoyl-dependent (19). PPC-S from rat liver uses ATP as the activating nucleotide, instead of CTP, and forms an acyl-phosphate intermediate as suggested by the formation of ADP and phosphate (16). These differences between the key CoA biosynthetic enzymes in bacteria and higher organisms raise the possibility of exploiting the selective inhibition of the bacterial enzyme in the development of new antibiotics. Our identification of the Dfp protein from *E. coli* as having both PPC-S and PPC-DC activities leads us to suggest the renaming of the *dfp* gene as *coaBC*, in accordance with the nomenclature established for CoA biosynthetic enzymes and to accommodate the existence of separate orthologs, notably in *Klebsiella pneumoniae* and *Streptococci*, encoding for CoaB and CoaC. This finding completes the identification of all the enzymes involved in the biosynthesis of coenzyme A in bacteria.

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