Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 9391–9395, December 1990
Biochemistry

p-Aminobenzoate synthesis in *Escherichia coli*: Purification and characterization of PabB as aminodeoxychorismate synthase and enzyme X as aminodeoxychorismate lyase

(folate biosynthesis/chorismate)

QI-ZHUANG YE, JUN LIU, AND CHRISTOPHER T. WALSH

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115

Contributed by Christopher T. Walsh, August 23, 1990

ABSTRACT **The *Escherichia coli pabA* and *pabB* genes have been overexpressed separately and in tandem. Using purified PabB, we have confirmed the recent suggestion that PabB needs an additional protein, enzyme X, to convert chorismate and NH_3 to *p*-aminobenzoate (PABA). With chorismate and NH_3, pure PabB generates an intermediate presumed to be 4-amino-4-deoxychorismate based upon UV/visible spectroscopy and enzymatic and nonenzymatic transformations. The PabB-catalyzed interconversion of chorismate and isolated aminodeoxychorismate is readily reversible. With pure PabB as a stoichiometric assay reagent, enzyme X was purified ≈800-fold to near homogeneity as an apparent homodimer of 50 kDa from *E. coli*. Enzyme X shows no activity on chorismate but quantitatively converts the preformed aminodeoxychorismate into *p*-aminobenzoate and pyruvate, acting thereby as an aminodeoxychorismate lyase.**

In the bacterial biosynthesis of folate coenzymes, the *p*-aminobenzoate (PABA) moiety derives from chorismate via as yet poorly characterized enzymatic steps, in part because the enzyme activity in wild-type *Escherichia coli* has been difficult to detect in crude extracts and has been refractory to purification (1–4). At the genetic level two genes, *pabA* and *pabB*, have been identified and sequenced (5–7). The *pabA* gene encodes a 21-kDa protein (PabA) with high sequence homology to the TrpG component in *o*-aminobenzoate (anthranilate) biosynthesis (6). PabA and TrpG each encode a glutaminase activity, providing nascent NH_3 for the two regiospecific chorismate aminations. The *pabB* gene product (7), 51 kDa, has substantial homology (26% sequence identity) to the *trpE* gene product, 60 kDa, the large subunit of the anthranilate synthase TrpEG complex (8). The TrpE protein catalyzes NH_3-dependent chorismate amination to 2-amino-2-deoxyisochorismate and its subsequent aromatization by syn elimination of the elements of pyruvate (C_2-H,C_3-enolpyruvyl loss) (9, 10). It was anticipated that PabB catalyzes similar regiospecific amination and then aromatization of 4-amino-4-deoxychorismate, which was shown to be processed to PABA in crude bacterial extracts (11). A third enzyme with substantial homology to TrpE and PabB is the recently sequenced EntC (12, 13), isochorismate synthase, interconverting chorismate and its dihydroaromatic isomer isochorismate without aromatization (14), at the start of the enterobactin biosynthesis pathway (15).

Nichols *et al.* (16) have deconvoluted some of the PABA synthesis difficulty by demonstrating that in extracts overproducing PabA or PabB, still no PABA formation could be detected until soluble extract from a *pabA pabB* double mutant was added. This activity, dubbed enzyme X, was proposed to act on a diffusible intermediate generated by PabAB action and to convert it to the aromatic amino acid product PABA. In this report we describe the tandem overproduction of PabA and PabB and the purification and initial characterization of PabB, which enabled the subsequent 800-fold purification of enzyme X and preliminary characterization of the intermediate generated by PabB from chorismate and NH_3.

MATERIALS AND METHODS

Materials. Chorismate as free acid or barium salt, isopropyl β-D-thiogalactopyranoside (IPTG), NADH, and lactate dehydrogenase were purchased from Sigma. Anthranilate synthase subunit I was partially purified from a TrpE overproducer (J.L. and C.T.W., unpublished results). The *E. coli* strain XA90 and expression vector pHN1+ were gifts from Greg Verdine (Harvard University Chemistry Department, Cambridge, MA). The total genomic DNA of wild-type *E. coli* K-12 was a gift from Stan Tabor (Harvard Medical School, Boston). The polymerase chain reaction (PCR) primers used to amplify *pabA* and *pabB* genes were synthesized by Alex Nausbaum (Harvard Medical School, Boston). Their sequences are as follows (for each primer, the restriction sites are underlined and the coding sequences are in boldface): 5′ primer for *pabA*, 5′-GCGTGT<u>GAATTC</u>**AGGAGGAAAAACATATGATCCTGCTTATAGATAAC**-3′; 3′ primer for *pabA*, 5′-GGCACT<u>GAATTC</u>**AGCGATGCAGGAAATTAGC**-3′; 5′ primer for *pabB*, 5′-GCGTGT<u>GAATTC</u>**AGGAGGAAAAACATATGAAGACGTTATCTCCCGCT**-3′; 3′ primer for *pabB*, 5′-GGCACT<u>AAGCTT</u>**ACTTCTCCAGTTGCTTCAG**-3′.

Assays for PabB and Enzyme X. The NH_3-dependent transformation from chorismate to PABA was measured by two independent methods. One method was to detect PABA by its fluorescence, while the other was to detect pyruvate by a lactate dehydrogenase-coupled assay. For the fluorescence assay, a 200-μl assay mixture contained 50 mM Tris·HCl (pH 8.6), 5 mM $MgCl_2$, 50 mM $(NH_4)_2SO_4$, 1 mM chorismate, and various concentrations of PabB with or without enzyme X supplemented. The mixture was incubated at 37°C for 30 min, acidified with 20 μl of 2 M HCl, and extracted with 800 μl of ethyl acetate. After a brief centrifugation, the concentration of PABA was determined by measuring the fluorescence of the ethyl acetate solution at 290 nm (excitation)/340 nm (emission). To determine enzyme X activity, 8 μM purified PabB was included in the assay mixture. The unit of activity was defined as nmol of PABA formed in 30 min at 37°C. In the lactate dehydrogenase-coupled assay, the 1-ml assay mixture consisted of 50 mM Tris·HCl (pH 8.6), 5 mM $MgCl_2$, 50 mM $(NH_4)_2SO_4$, 1 mM chorismate, 0.2 mM NADH, 25 μg of lactate dehydrogenase, and various amounts of PabB and

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PABA, *p*-aminobenzoate; IPTG, isopropyl β-D-thiogalactopyranoside; PCR, polymerase chain reaction.

enzyme X. The reaction was followed by absorbance of NADH at 340 nm at 37°C.

Construction of PabA, PabB, and PabA-B Overproducers. The PCR mixture was made as described (17) except that 2 μg of genomic DNA was used as template in each reaction. The PCR was performed in 25 cycles, each cycle including 1 min at 94°C, 1 min at 50°C, and 2.5 min at 72°C. At the end of the last cycle, each reaction mixture was incubated sequentially at 72°C for 7 min and at 25°C for 30 min. The subsequent treatment of the reaction mixture with DNA polymerase I Klenow fragment, precipitation of DNA, and subcloning of the DNA fragments into pHN1+ were as described (17). This gave two overproducer constructs, pNPA and pNPB. To construct the tandem expression vector pNPAB (Fig. 1A), the *Eco*RI–*Eco*RI PCR fragment containing *pabA* gene was subcloned into pNPB that had been digested with *Eco*RI and treated with alkaline phosphatase (18). After transformation into *E. coli* XA90, the IPTG-dependent expression of PabA and PabB from the three overproducers was detected by PAGE (10%) of the whole cell lysate.

Purification of PabB. A 2-liter culture of pNPAB/XA90 in 2× TY medium (18) containing ampicillin (50 μg/ml) was incubated at 37°C until OD_{595} reached 0.6. IPTG was added to a final concentration of 1 mM and the incubation was continued for an additional 6 hr. The cells were harvested by centrifugation at 10,000 × *g* for 10 min. The cell pellet (6.5 g) was resuspended in 30 ml of 50 mM potassium phosphate buffer (pH 7.4) containing 0.2 mM dithiothreitol and 0.1 mM EDTA and passed through a French pressure cell twice at 12,000 psi (1 psi = 6.89 kPa). The lysate was centrifuged at 15,000 × *g* for 20 min. The nucleic acids in the supernatant (35 ml, 861 mg of protein) were removed by adding 7 ml of 2% protamine sulfate (pH 7.2). The protein was precipitated by ammonium sulfate (25–60% saturation) and the pellet was dissolved in 10 ml of 50 mM potassium phosphate (pH 7.4). The protein solution (13 ml, 416 mg of protein) was applied to a Sephacryl S-200 column (2.6 × 90 cm) and eluted with 50 mM potassium phosphate (pH 7.4). Active fractions were combined (48 ml, 235 mg of protein) and loaded directly to a DEAE-Sephacel column (2.6 × 35 cm). The proteins were eluted with a 0–0.6 M KCl gradient in 10 mM potassium phosphate buffer. The active fractions were combined (120 ml, 116 mg protein) and the proteins were concentrated by precipitation with 60% ammonium sulfate. The protein was >95% pure at this stage (Fig. 1*C*, lane 2). Portions of the precipitate were dissolved in 10 mM potassium phosphate buffer and, after dialysis against the same buffer, applied to a dye-agarose column (reactive yellow no. 3, from Sigma). The flowthrough fractions were combined and concentrated by precipitation with 60% ammonium sulfate. The precipitate was dissolved in 10 mM Tris·HCl buffer (pH 7.4) and dialyzed against 10 mM Tris·HCl (pH 7.4) containing 50% glycerol. The final protein solution was kept at −20°C.

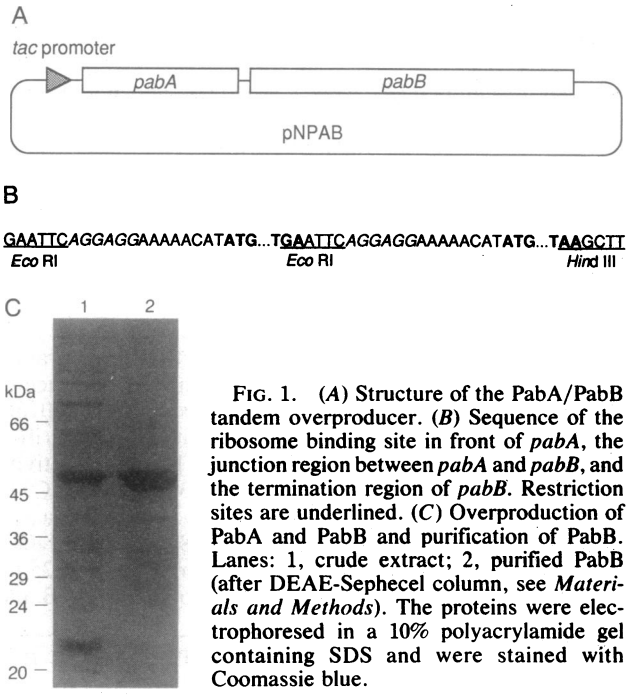

FIG. 1. (*A*) Structure of the PabA/PabB tandem overproducer. (*B*) Sequence of the ribosome binding site in front of *pabA*, the junction region between *pabA* and *pabB*, and the termination region of *pabB*. Restriction sites are underlined. (*C*) Overproduction of PabA and PabB and purification of PabB. Lanes: 1, crude extract; 2, purified PabB (after DEAE-Sephecel column, see *Materials and Methods*). The proteins were electrophoresed in a 10% polyacrylamide gel containing SDS and were stained with Coomassie blue.

Purification of Enzyme X. *E. coli* SG-5 cells (80 g, from a 10-liter culture) were resuspended in 240 ml of 100 mM Hepes buffer (pH 7.5) and lysed by passage through a French press at 12,000 psi. The 25–75% ammonium sulfate fraction was dissolved in 25 mM Tris·HCl buffer (pH 8.0) with 1 mM EDTA and 5 mM 2-mercaptoethanol and dialyzed against the same buffer. The protein solution was loaded onto a DEAE-Sepharose CL-6B column and eluted with a gradient of 0–0.5 M KCl in the 25 mM Tris buffer over 1200 ml. Ammonium sulfate was added (30%) to a combined active fraction, and the solution was loaded on a phenyl-Sepharose column and eluted with a gradient of 30–0% ammonium sulfate in 10 mM Tris·HCl buffer (pH 7.4). The active fractions were combined and concentrated with 60% ammonium sulfate. The precipitate was dissolved in 2 ml of 10 mM Tris·HCl (pH 8.0) and loaded on a Superose 6 gel filtration column. After elution with 10 mM Tris·HCl, the active fraction (30 ml) was loaded directly onto a Mono Q column (Pharmacia) and eluted with 0–0.6 M KCl in the 10 mM Tris buffer. The active fractions were combined and concentrated by ammonium sulfate precipitation. A portion of the precipitate was dissolved in 10 mM Tris·HCl, loaded onto a Superose 12 column, and eluted with 10 mM Tris·HCl containing 0.1 M KCl.

RESULTS

PabB Overproduction, Purification, and Activity Assay. Because PabA and PabB proteins had not been purified to homogeneity even though their substantial overproduction has been reported (2, 3, 16), we constructed overproducers of PabA, PabB, and PabA/PabB (*Materials and Methods*; Fig. 1*A*). Fig. 1*C* (lane 1) documents good overproduction of the 21-kDa PabA and 51-kDa PabB proteins from the tandem PabAB overproducer pNPAB. While the PabB overproducer pNPB likewise gave good yields of PabB, PabA overproduction was only modest from the PabA overproducer pNPA, consistent with other reports that *pabB* expression affects that of pabA (3, 19). Purification of PabB component from the tandem overproducer pNPAB proceeded via protamine sulfate, ammonium sulfate, and Sephacryl S-200. On the gel filtration column the PabB peak contained no associated PabA, suggesting only very weak interaction between the two components. PabB was further purified on DEAE-Sephacel to give >95% homogeneous PabB (Fig. 1*C*, lane 2) in a yield of 116 mg from a 2-liter culture. The N-terminal sequence of pure PabB was determined to be Met-Lys-Thr-Leu-Ser-Pro-Ala-Val-Ile-Thr-Leu-Pro, as predicted by the gene sequence with the exception of Pro-12, which was predicted to be Leu from the DNA sequence (7). To date, we have focussed on PabB without pursuing the purification of the PabA glutaminase subunit any further.

Initial assays of purified PabB with NH_3 and chorismate did show some PABA synthesis activity both by a fluorescence assay to detect PABA formation and by a lactate dehydrogenase-coupled assay to detect pyruvate release. However, the activity was marginal, ≈1 nmol/min per mg, which corresponds to 0.05 turnover/min for the purified PabB, an exceedingly low turnover number. Mindful of the experiments of Nichols *et al.* (16), when we passed the

purified PabB through a dye-agarose column (reactive yellow no. 3), the specific activity for PABA formation from the PabB that emerged fell by a factor of 10, to less than 1 molecule of PABA per minute per 200 molecules of pure PabB enzyme. In this condition PabB is indeed effectively incompetent as a PABA synthase.

Although PabB is incapable of catalyzing NH_3-dependent PABA formation from chorismate after the dye-agarose column, it is fully functional when purified enzyme X is supplied as described later. It thus appears that the low activity of PabB before the dye-agarose column was probably due to contaminating enzyme X.

Purification of Enzyme X. While Nichols *et al.* (16) used a *pabA pabB* double mutant as a source for enzyme X, we observed that with pure PabB prepared as above, extracts from several *E. coli* strains stimulated PABA production 40- to 100-fold (Fig. 2*A*) and have used this assay to process crude extract of *E. coli* SG5 (20) for enzyme X purification. Sequential application of DEAE-Sephacel, phenyl-Sepharose, Superose 6, Mono Q, and Superose 12 column chromatography yielded a 400- to 800-fold purification of enzyme X as assessed by specific activity increase in an assay using NH_3, chorismate, and a fixed amount of pure PabB to produce PABA. As shown in Fig. 2 *B* and *C*, the most highly purified fractions from the Superose 12 column were enriched in a 25-kDa polypeptide on SDS/PAGE analysis. Since the gel filtration results suggest a native molecular mass of 50 kDa, also seen by Nichols *et al.* (16) with crude enzyme X, enzyme X may be a homodimer. Under our assay conditions, the PABA synthase activity of enzyme X, normalized to 8 μM PabB in the assay, is $\approx 30\ min^{-1}$.

Catalytic Activities of PabB and Enzyme X. With highly purified PabB and enzyme X available, we could further examine the requirements to produce PABA by a variation of the dialysis experiments of Nichols *et al.* (16). We used a two-stage assay with Centricon filtration at the end of stage 1 to separate enzyme and small molecules before addition of the second enzyme component to the small molecule fraction and continuation of incubation. In these studies we corroborated that PabB must act first (Fig. 3 *A* and *B*) and that enzyme X can act second on a diffusible intermediate at about two-thirds the yield of both enzymes together (Fig. 3*C*). PabB requires Mg^{2+}, NH_3, and chorismate (Fig. 3 *E–G*) to yield the intermediate, whereas enzyme X has no requirement for Mg^{2+} to produce PABA from the PabB reaction product (Fig. 3 *D*). These results confirm that PabB yields an aminated intermediate from chorismate. Assays for pyruvate release also confirmed that the PabB product still retains the enolpyruvyl group, which is released only subsequently, on enzyme X-catalyzed generation of PABA.

When pure PabB was incubated with chorismate, Mg^{2+}, and NH_3 and the small molecule fraction was subsequently separated on HPLC, a new UV-absorbing species was detected that was eluted early in the gradient, well separated from chorismate, PABA, and *p*-hydroxybenzoate. The kinetics of formation of the intermediate and its isolation and identification will be described elsewhere (K. Anderson, W. Kati, K. Johnson, Q.-Z.Y., J.L., and C.T.W., unpublished data). This material was converted quantitatively to PABA on addition of purified enzyme X, and so the PabB-generated product is clearly a substrate for enzyme X. The UV/visible spectrum of the PabB product is almost indistinguishable from that of chorismate (Fig. 4), indicating integrity of the conjugated cyclohexadiene system. This PabB product must be an aminated cyclohexadiene carboxylate still containing its enolpyruvyl group (see above), i.e., an aminodeoxychorismate.

The competence of the intermediate as a precursor to PABA was verified by a nonenzymic decomposition experiment. Thus, HPLC-isolated intermediate was incubated in 50 mM NH_4OAc (pH 4.0) at room temperature for 5 days. When the mixture was reinjected into HPLC, a small but significant amount of PABA was detected in addition to the intact intermediate. In contrast, when chorismate was incubated under the same condition, only *p*-hydroxybenzoate was observed in addition to chorismate. It is therefore very likely that the intermediate is aminodeoxychorismate and the nonenzymic decomposition is due to the loss of the enolpyruvate side chain to form the aromatic PABA.

To test directly whether the PabB-catalyzed reaction might be reversible, the HPLC-isolated aminodeoxychorismate was reincubated with PabB and anthranilate synthase as a coupling enzyme. Anthranilate formation proceeded after a lag phase (Fig. 5), presumably due to chorismate build-up to the K_m for the second enzyme. Isochorismate is not a substrate for PabB in the backward direction. Clearly the PabB-mediated interconversion of chorismate and NH_3 to aminodeoxychorismate and H_2O is fully and readily reversible. A more complete study of this transformation will be detailed elsewhere.

DISCUSSION

We have purified the *E. coli* PabB protein from an overproducing construct, yielding $\approx$25% of the soluble cell protein as the desired enzyme, in 100-mg quantities. PabA appears to be produced in equivalent amounts from the tandem construct

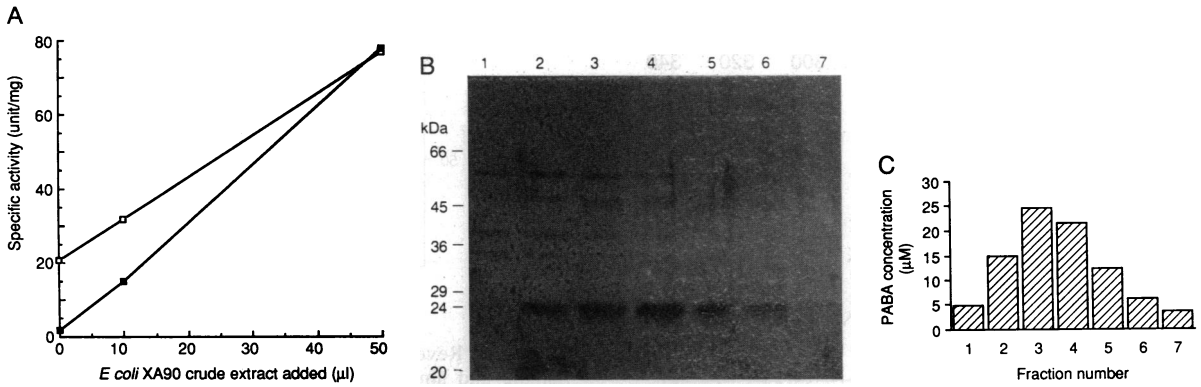

FIG. 2. (*A*) Stimulation of the PabB-catalyzed PABA formation by enzyme X. Crude extract (10 mg of protein per ml) from *E. coli* strain XA90 was added to an assay mixture containing chorismate and PabB before (□) and after (■) passage through the dye-agarose column. (*B*) SDS/PAGE analysis of Superose 12 fractions in enzyme X purification. The proteins were electrophoresed in a 10% polyacrylamide gel containing SDS and stained with $AgNO_3$. Fraction numbers are shown above the gel. (*C*) Activity of the Superose 12 fractions as assayed by PABA formation.

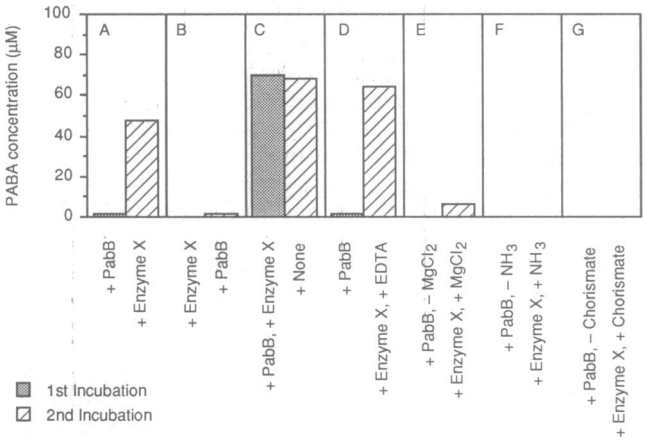

FIG. 3. Two-stage incubation experiments with Centricon-30 separation of protein components after the first incubation. First incubation was carried out at 37°C for 30 min with an assay mixture containing 1 mM chorismate, 50 mM Tris·HCl (pH 8.6), 5 mM $MgCl_2$, and 50 mM $(NH_4)_2SO_4$. Plus sign indicates that PabB (4 μM) or enzyme X (0.5 μM) was added to the common assay mixture; minus sign indicates that the component ($MgCl_2$, NH_3, or chorismate) was omitted from the mixture. After filtration through Centricon-30, PabB or enzyme X and other components as indicated were added and the incubation was continued for 30 min at 37°C. The PABA concentration in the assay mixture was determined before (stippled bars) and after (hatched bars) the second incubation.

but the two proteins, if associated, do not survive a gel filtration step and we have not yet turned to further purification of the PabA glutaminase subunit. With highly purified PabB we could then confirm the observation of Nichols *et al.* (16) that PabB-enriched extracts cannot yield PABA from chorismate and ammonia, in sharp contrast to the homologous anthranilate synthase large subunit, TrpE. We established an upper limit of less than 1 PABA per 200 PabB enzyme molecules per minute. The pure PabB protein can be used as a reagent for the purification of enzyme X, the additional component needed to produce PABA from NH_3

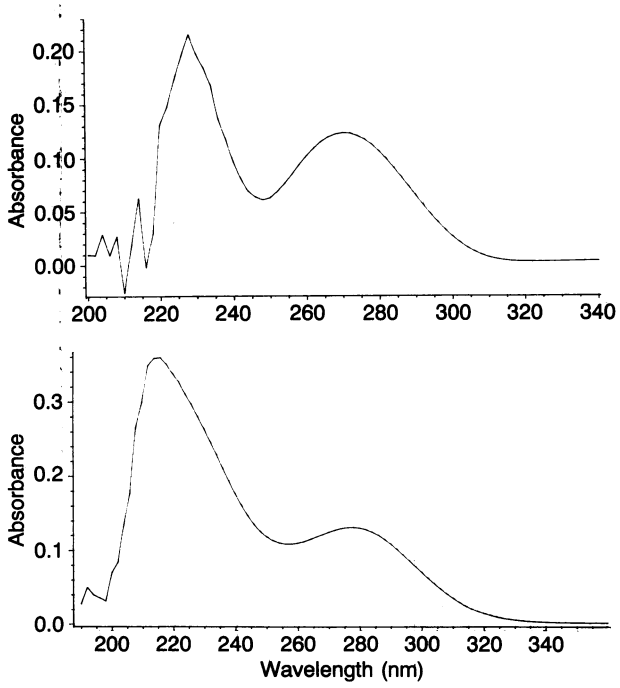

FIG. 4. UV spectra of HPLC-separated intermediate generated by PabB (*Upper*) and chorismate (50 μM) (*Lower*). The compounds were dissolved in 50 mM Tris·HCl (pH 7.8).

and chorismate. After 400- to 800-fold purification from wild-type *E. coli*, enzyme X preparations are enriched in a 25-kDa subunit along with some minor bands. Cloning, expression, and overproduction will be needed to validate the identity of the 25-kDa species as enzyme X. It is not clear how strong an association enzyme X and PabB have *in vivo*. Both proteins have a molecule mass of ≈50 kDa in native form and the overproduced, purified PabB does have a small amount of enzyme X associated before passage through a dye-agarose column.

With purified enzyme X we were able to validate the proposition that PabB and enzyme X act sequentially and enzyme X works on an intermediate that is generated by amination of chorismate by pure PabB. Enzyme X shows no activity on chorismate itself. The most likely situation is that PabB generates an aminodeoxychorismate, probably the 4-amino-4-deoxychorismate species (11). In that event enzyme X would be a 4-amino-4-deoxychorismate lyase, in analogy to chorismate lyase, the *ubiC* gene product (21), which is involved in *p*-hydroxybenzoate formation in ubiquinone biogenesis. UbiC and enzyme X must be distinct, since enzyme X does not convert chorismate to *p*-hydroxybenzoate.

On further examination of the activity of pure PabB protein by chorismate utilization and by product isolation and UV/visible analysis, it is clear that PabB has aminase activity, converting chorismate to a product incorporating NH_3 and retaining the diene system and the enolpyruvyl side chain. While definitive structural proof awaits isolation of the intermediate in quantities for NMR analysis, it is reasonable that the PabB product is the 4-amino-4-deoxychorismate, previously prepared and characterized by Teng *et al.* (11). Thus it is quite likely that PABA biosynthesis requires the three enzymes PabA, PabB, and enzyme X (4-amino-4-deoxychorismate lyase) acting as depicted in Fig. 6, with the PabB-catalyzed reaction fully reversible.

PabB, which is structurally homologous to EntC and TrpE, behaves functionally as a hybrid. Like EntC (isochorismate synthase), it generates a dihydroaromatic product that it releases prior to aromatization. TrpE, the anthranilate synthase large subunit, aminates chorismate, like PabB, but catalyzes aromatization via pyruvate elimination before release. It may be that PabB lacks a competent aromatization domain, requiring the enzyme X component just as chorismate requires UbiC for aromatization to *p*-hydroxybenzoate. Or release of aminodeoxychorismate may be necessary for other biosyntheses, since in *Streptomyces venezuelae* arylamine synthase can convert 4-amino-4-deoxychorismate to

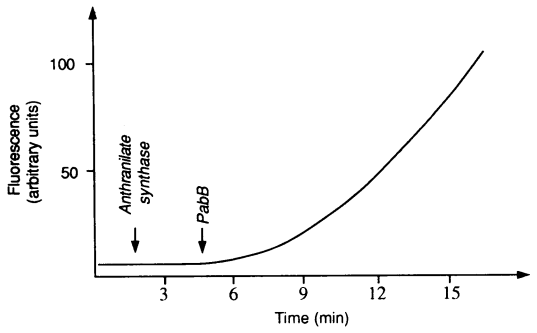

FIG. 5. Reversibility of PabB-catalyzed interconversion of chorismate and aminodeoxychorismate. To an assay mixture (1 ml) consisting of 200 mM Tris·HCl, 5 mM $MgCl_2$, 50 mM $(NH_4)_2SO_4$, and 100 μl of the HPLC fraction of the intermediate, 15 μg of partially purified anthranilate synthase was added. After 3 min, 3 μl of 200 μM PabB was added. The generation of anthranilate was followed by fluorescence (excitation at 325 nm, emission at 400 nm) with a Perkin/Elmer LS-3 fluorescence spectrometer.

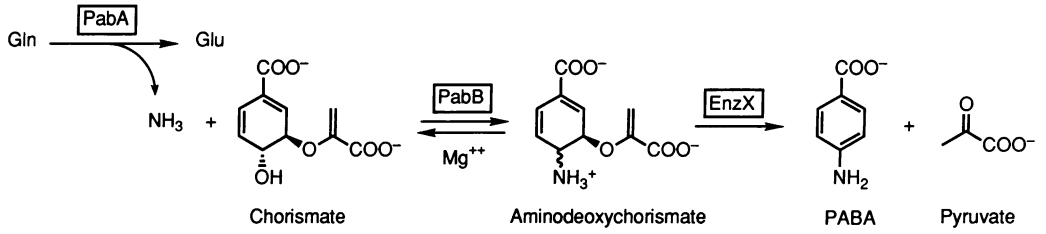

FIG. 6. Proposed action of PabA, PabB, and enzyme X (EnzX) in biosynthesis of PABA.

4-amino-4-deoxyprephenate on the way to chloramphenicol (11). The availability of pure PabB (aminodeoxychorismate synthase) and enzyme X, which we now name as aminodeoxychorismate lyase, should facilitate further deconvolution of PABA biogenesis, including how net NH_3-for-OH replacement is effected at C-4 of chorismate. This may facilitate inhibitor design for blockade of PABA and, thereby, folate biosynthesis.

We thank Professors Ken Johnson and Karen Anderson and Dr. W. Kati for isolation of the intermediate by HPLC. We are grateful to Dr. Francis X. Sullivan for a crude extract of *E. coli* SG-5 and to Dr. Chih-Ming Chen for help in enzyme X purification. This work was supported in part by National Institutes of Health Grant GM20001.

1. Zalkin, H. (1985) *Methods Enzymol.* **113,** 293–297.
2. Walsh, C. T., Erion, M. D., Walts, A. E., Delany, J. J. & Berchtold, G. A. (1987) *Biochemistry* **26,** 4734–4745.
3. McLeish, M. J., Wookey, P. J. & Mortimer, K. G. (1988) *Biochem. Int.* **16,** 727–735.
4. Kane, J. F. & O'Brien, H. D. (1975) *J. Bacteriol.* **123,** 1131–1138.
5. Huang, M. & Gibson, F. (1970) *J. Bacteriol.* **102,** 767–773.
6. Kaplan, J. B. & Nichols, B. P. (1983) *J. Mol. Biol.* **168,** 451–468.
7. Goncharoff, P. & Nichols, B. P. (1984) *J. Bacteriol.* **159,** 57–62.
8. Yanofsky, C., Platt, T., Crawford, I. P., Nichols, B. P., Christie, G. E., Horowitz, H., VanCleemput, M. & Wu, A. M. (1981) *Nucleic Acids Res.* **9,** 6647–6668.
9. Policastro, P. P., Au, K. G., Walsh, C. T. & Berchtold, G. A. (1984) *J. Am. Chem. Soc.* **106,** 2443–2444.
10. Teng, C.-Y. P. & Ganem, B. (1984) *J. Am. Chem. Soc.* **106,** 2463–2464.
11. Teng, C.-Y. P., Ganem, B., Doktor, S. Z., Nichols, B. P., Bhatnagar, R. K. & Vining, L. C. (1985) *J. Am. Chem. Soc.* **107,** 5008–5009.
12. Elkins, M. F. & Earhart, C. F. (1988) *FEMS Microbiol. Lett.* **56,** 35–40.
13. Ozenberger, B. A., Brickman, T. J. & McIntosh, M. A. (1989) *J. Bacteriol.* **171,** 775–783.
14. Liu, J., Quinn, N., Berchtold, G. A. & Walsh, C. T. (1990) *Biochemistry* **29,** 1417–1425.
15. Walsh, C. T., Liu, J., Rusnak, F. & Sakaitani, M. (1990) *Chem. Rev.*, in press.
16. Nichols, B. P., Seibold, A. M. & Doktor, S. Z. (1989) *J. Biol. Chem.* **264,** 8597–8601.
17. Liu, J., Albers, M. W., Chen, C.-M., Schreiber, S. L. & Walsh, C. T. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 2304–2308.
18. Ausubel, F. M., Brent, R., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1989) *Current Protocols in Molecular Biology* (Wiley, New York).
19. Kane, J. F. (1977) *J. Bacteriol.* **132,** 419–425.
20. Greer, S. & Perham, R. N. (1986) *Biochemistry* **25,** 2736–2742.
21. Lawrence, J., Cox, G. B. & Gibson, F. (1974) *J. Bacteriol.* **118,** 41–45.