JOURNAL OF BACTERIOLOGY, Feb. 1969, p. 725-733
Vol. 97, No. 2
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Anthranilate Synthetase, an Enzyme Specified by the Tryptophan Operon of *Escherichia coli*: Purification and Characterization of Component I

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Received for publication 31 July 1968

A procedure employed in the purification of anthranilate synthetase component I of *Escherichia coli* is described. The purified component appears homogeneous by starch gel electrophoresis and by sedimentation analysis. A molecular weight of 60,000 was estimated by gel filtration on Sephadex G-100. This value is consistent with the molecular weight estimated from the sedimentation and diffusion coefficients. Purified anthranilate synthetase component I cannot use glutamine as substrate and thus has no activity in the reaction of chorismate + L-glutamine → anthranilate; however, it is active when ammonium sulfate is provided as amino donor. Sucrose density gradient analyses showed that ammonium sulfate does not affect the sedimentation velocity of component I. The ultraviolet absorption and fluorescence spectra of the purified component indicated that it contains tryptophan. Peptide pattern and extract complementation evidence suggested that the protein is a single polypeptide chain. Enzyme activity measurements indicated that wild-type *E. coli* produces equimolar amounts of at least four of the five polypeptides specified by the operon. Purified anthranilate synthetase component I is inhibited by L-tryptophan.

In a previous communication, we reported that the anthranilate synthetase of *Escherichia coli* consists of nonidentical subunits specified by the first two genes, *trpE* and *trpD*, of the tryptophan operon (16). This conclusion was based on the observation that nonsense mutants with alterations in either of these genes lacked anthranilate synthetase activity. It was also shown that anthranilate synthetase activity appeared when extracts of strains carrying *trpE* and *trpD* nonsense mutants were mixed. This activity was associated with a species with the same sedimentation coefficient as wild-type anthranilate synthetase, approximately 7.5*S*, while the contributing components of the *trpE* and *trpD* mutants sedimented at approximately 4.3*S* and 4.4*S*, respectively.

The component specified by the D gene, in addition to being required for anthranilate formation, catalyzes the subsequent step in tryptophan synthesis, the conversion of anthranilate to *N*-(5′-phosphoribosyl) anthranilate. Studies with the anthranilate synthetase of *Salmonella typhimurium* disclosed essentially the same subunit relationships (2), and investigations with the enzyme isolated from *Aerobacter aerogenes* also indicated that anthranilate formation and anthranilate conversion to *N*-(5′-phosphoribosyl) anthranilate were catalyzed by the same enzyme complex (8).

The present report is concerned with the purification and characterization of the *trpE* product, component I (CoI) of anthranilate synthetase. A preliminary report of some of these studies was presented at the Seventh International Congress of Biochemistry, Tokyo (Ito and Yanofsky, vol. 14, p. 669, 1967).

MATERIALS AND METHODS

Bacterial strains and growth conditions. Tryptophan auxotrophs of *Escherichia coli* K-12, carrying mutations *trpD9778* and *trpE5972*, were employed in this study. The strain carrying *trpD9778*, a nonsense mutation, lacks PR transferase [anthranilate synthetase component II (CoII)], and the strain carrying *trpE5972*, also a nonsense mutation, cannot produce anthranilate synthetase CoI. The culture medium used consisted of the salts mixture of Vogel and Bonner (30) supplemented with 0.4% glucose, 0.005%

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neutralized acid-hydrolyzed casein, and 5 μg of L-tryptophan per ml. Cultures were grown overnight with aeration at 37 C in 5-gallon (18.9 liters) carboys. Cells were harvested in a Sharples centrifuge and were washed once in 0.1 M tris(hydroxymethyl)-aminomethane (Tris)-hydrochloride buffer at *p*H 7.8. The yield was approximately 70 g (wet weight) per carboy.

Materials. Chorismic acid was isolated from culture filtrates of *A. aerogenes* strain 62-1 and crystallized according to the procedures of Gibson and Gibson (9; *personal communication*). All other chemicals were obtained from commercial sources and were used without further purification.

Enzyme assays. The assay procedure for anthranilate synthetase CoI was partly described previously (16). The reaction mixture contained 2×10^{-4} M chorismic acid, 4×10^{-3} M $MgSO_4$, 10^{-2} M L-glutamine, 10^{-2} M potassium phosphate buffer at *p*H 7.6, 10^{-3} M β-mercaptoethanol, extract of strain 5972 (5 to 10 units of anthranilate synthetase CoII) and anthranilate synthetase CoI in a final volume of 2 ml. The reaction was started by the addition of anthranilate synthetase CoI to the reaction mixture warmed to 37 C. Activity was followed by observing the increase in fluorescence at 390 nm in an Aminco-Bowman recording spectrophotofluorometer. Water (at 37 C) was circulated through the cuvette holder. One unit of anthranilate synthetase CoI or anthranilate synthetase CoII is defined as the amount of enzyme required to form 0.1 μmole of anthranilate in 20 min at 37 C. Specific activity is expressed as units of enzyme per milligram of protein. The relationship between enzyme concentration and the rate of anthranilate formation under the above assay conditions is presented in Fig. 1. The assay procedure for anthranilate synthetase CoII is essentially the same, except that 5 to 10 units of anthranilate synthetase CoI is substituted for anthranilate synthetase CoII (16).

The α subunit of tryptophan synthetase and indole-3-glycerol phosphate synthetase were assayed according to Smith and Yanofsky (27). Bacterial alkaline phosphotase was assayed according to the method of Schlesinger and Barrett (26). Protein was determined by the method of Lowry et al. (19).

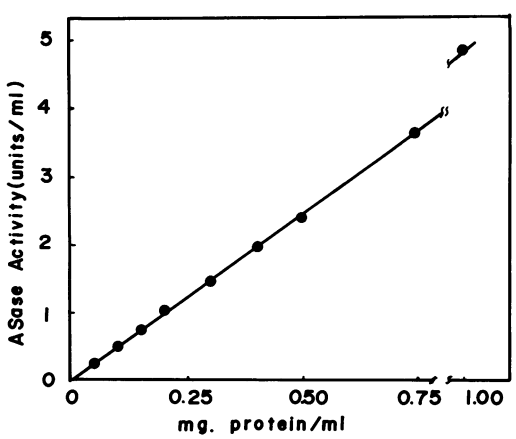

FIG. 1. *Activity as a function of the concentration of anthranilate synthetase CoI, in the presence of excess anthranilate synthetase CoII.*

RESULTS

Purification of anthranilate synthetase CoI. All operations were carried out in an ice bath, except where noted.

Crude extract preparation. Washed cells were taken up in 0.1 M Tris-hydrochloride buffer at *p*H 7.8 to give a 30% (w/v) suspension. Portions (500 ml) were subjected to sonic disruption with a Branson sonic oscillator for 30 min. The suspension was centrifuged at 16,000 × *g* for 30 min, and the supernatant solution was decanted. The pellet was suspended in a small volume of the same buffer and the sonic disruption was repeated. After centrifugation, the supernatant solutions were combined.

Treatment with streptomycin sulfate. To each 100 ml of crude extract, 5 ml of a 30% solution of streptomycin sulfate in water was added with stirring. After 15 min, the mixture was centrifuged for 60 min at 16,000 × *g* and the precipitate was discarded.

Ammonium sulfate precipitation. For each 100 ml of supernatant solution from the previous step, 25 g of solid ammonium sulfate was added slowly with stirring. The mixture was stirred for an additional 15 min and the precipitate was collected by centrifugation for 30 min at 16,000 × *g*. The precipitate was washed once with a 40% saturated ammonium sulfate solution at *p*H 6.8. The precipitate was suspended in 0.1 M Tris-hydrochloride buffer, *p*H 7.8, containing 10^{-3} M β-mercaptoethanol.

Sephadex G-100 gel filtration. The suspended ammonium sulfate precipitate was dialyzed for 4 hr against 0.05 M Tris-hydrochloride buffer, *p*H 7.8, containing 10^{-3} M β-mercaptoethanol. The dialysate was centrifuged for 30 min at 16,000 × *g* and the precipitate was discarded. The supernatant solution was loaded on a Sephadex G-100 column (5 by 60 cm) equilibrated with 0.05 M Tris-hydrochloride buffer. The protein was eluted with the same buffer at a maximum flow rate of 50 ml/hr. Fractions with more than 50 enzyme units per ml were pooled, and the protein was precipitated by the addition of 28 g of solid ammonium sulfate per 100 ml of eluate. The resulting precipitate was collected by centrifugation at 16,000 × *g* for 20 min. The precipitate was suspended in 0.01 M potassium phosphate buffer, *p*H 7.0, containing 10^{-2} M β-mercaptoethanol. The supernatant solution was discarded.

First chromatography on diethylaminoethyl

(DEAE)-Sephadex. The suspended ammonium sulfate precipitate was dialyzed for 8 to 10 hr against 1 liter of 0.01 M potassium phosphate buffer, *p*H 7.0, containing 10^{-3} M β-mercaptoethanol. A column of DEAE-Sephadex A-50 (3 by 100 cm) was prepared and equilibrated with the same buffer. The dialysate was centrifuged briefly and the precipitate was discarded. The supernatant solution was applied to the column and then washed on with 50 to 100 ml of 0.01 M potassium phosphate buffer. A linear gradient was employed for elution with 1 liter of 0.01 M potassium phosphate, *p*H 7.0, in the mixing flask and 1 liter of 0.6 M potassium phosphate buffer, at the same *p*H, in the reservoir flask. The column flow rate was adjusted to about 45 ml/hr and fractions were collected every 20 min. Those fractions containing more than 150 units of anthranilate synthetase CoI units per mg of protein were pooled, and the protein was precipitated by the addition of solid ammonium sulfate (42 g/100 ml). After collecting the precipitate by centrifugation at 16,000 × *g* for 30 min, the precipitate was suspended in a small volume of 0.01 M potassium phosphate buffer containing 10^{-3} M β-mercaptoethanol.

Second chromatography on DEAE-Sephadex. After dialysis of the suspended precipitate for a total of 10 to 12 hr against 1-liter volumes (changed twice) of 0.01 M potassium phosphate buffer, *p*H 7.0, the clear solution was applied to a DEAE-Sephadex column prepared as described above, and the previous DEAE-Sephadex step was repeated. The elution pattern showed coincident peaks of protein and activity; the specific activity was constant throughout the peak. The results of a typical purification run are presented in Table 1.

Evidence of homogeneity. The degree of homogeneity of purified anthranilate synthetase CoI preparations was determined by starch gel electrophoresis, sedimentation velocity in a Spinco model E ultracentrifuge, and sucrose density gradient centrifugation. Starch gel electrophoresis was performed as described by Smithies (28). Figure 2A shows the starch gel electrophoretic pattern of the final preparation obtained after the second chromatographic step on DEAE-Sephadex. Only trace impurities were detected.

TABLE 1. *Purification of anthranilate synthetase CoI of E. coli*

Fraction	Volume (ml)	Enzyme (units/ml)	Specific activity	Yield (%)
Crude extract	800	170	4.6	100
Streptomycin sulfate supernatant fluid	810	158	4.8	94
Dialyzed ammonium sulfate precipitate	80	1,105	28.0	65
Sephadex G-100 eluate	35	1,555	58.0	40
First DEAE-Sephadex eluate[a]	30	1,033	320.0	25
Second DEAE-Sephadex eluate[a]	12	1,133	510.0	10

[a] Dialyzed after ammonium sulfate precipitation.

An $S_{20,w}$ value of 4.04 was measured on a synthetase CoI preparation containing approximately 6 mg of protein per ml (specific activity, 500 units/mg) in 0.1 M potassium phosphate buffer, *p*H 7.0. A representative schlieren pattern is shown in Fig. 3, indicating a degree of homogeneity comparable to that shown by starch gel electrophoresis.

It was also demonstrated that the anthranilate synthetase CoI activity of the above preparations was associated with the main protein component. This was shown by comparing the activity and protein profiles following sucrose density gradient centrifugation (20).

Molecular weight determination. The molecular weight of anthranilate synthetase CoI was determined by measuring its elution volume on a calibrated column of Sephadex G-100, according to the method of Whitaker (31). The reference proteins and their molecular weights were: bacterial alkaline phosphatase, 80,000 (18, 26); *E. coli* indoleglycerolphosphate synthetase, 45,000 (5); *E. coli* tryptophan synthetase α subunit, 29,000 (13). The estimated molecular weight of the anthranilate synthetase CoI of strain 9778 was 60,000 (Fig. 4).

A diffusion coefficient was determined by a synthetic boundary analysis of the same anthranilate synthetase CoI preparation (at 6 mg of protein per ml, 0.1 M potassium phosphate buffer, *p*H 7.0) and was corrected to the viscosity of water at 20 C (25); the value found was 6.1×10^{-7} $cm^2 sec^{-1}$. This value, together with a sedimentation coefficient of 4.04*S* and an assumed partial specific volume of 0.74 cc/g, gave a molecular weight of 63,000. This calculated molecular weight is in good agreement with the value determined by Sephadex G-100 gel filtration.

Ultraviolet absorption and fluorescence spectra. The ultraviolet absorption spectrum of purified anthranilate synthetase CoI was determined in 0.01 M potassium phosphate buffer at *p*H 7.0. The spectrum was that of a simple protein with an absorbance ratio ($A_{280}:A_{260}$) of 1.54 (Fig. 5). The absorption spectrum of anthranilate synthetase CoI in 0.1 M NaOH indicated the presence of tryptophan in the protein (11). The presence

of tryptophan was confirmed by measuring the fluorescence spectrum of the protein. For comparison, the α subunit of *E. coli* tryptophan synthetase, which lacks tryptophan (13), and chymotrypsin, which contains 10 moles of tryptophan per mole of protein (24), were employed. Fluorescence maxima at 350 nm were obtained with

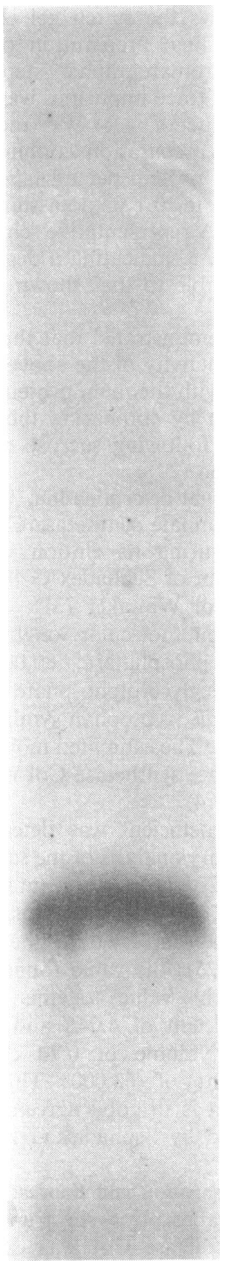

FIG. 2. *Starch gel electrophoretic pattern of purified anthranilate synthetase CoI. Approximately 200 μg of the anthranilate synthetase CoI was applied to a strip of Whatman no. 1 filter paper cut slightly smaller than the dimensions of the transversely slit starch gel. Electrophoresis was carried out at 4 C for 6 hr at 10 v/cm. The direction of migration is from bottom to top.*

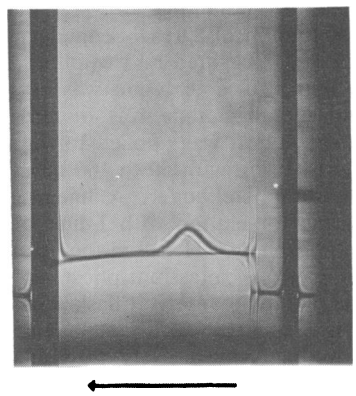

FIG. 3. *Schlieren pattern of purified anthranilate synthetase CoI. The photograph was taken 56 min after attaining a speed of 59,780 rev/min at 20.0 C. Protein, 5.7 mg/ml; 0.1 M potassium phosphate, pH 7.0.*

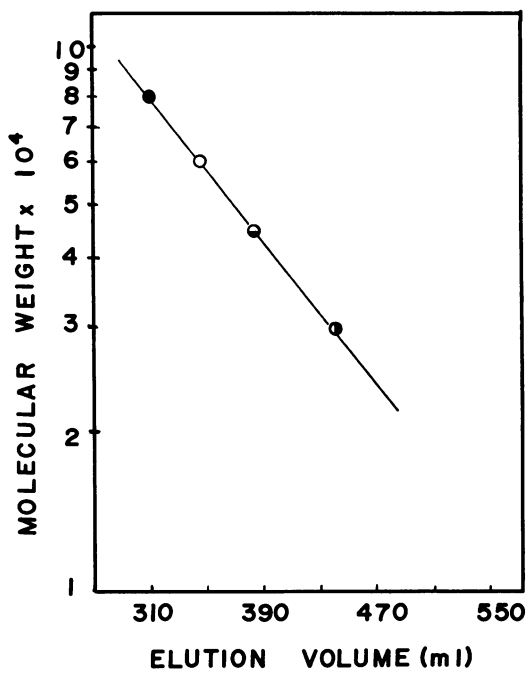

FIG. 4. *Molecular weight determination by gel filtration. A Sephadex G-100 column (3 by 100 cm) was equilibrated with 0.05 M potassium phosphate buffer, pH 7.0, containing 10^{-3} M β-mercaptoethanol. A solution (3 ml) containing anthranilate synthetase CoI and calibration proteins was layered on the column. Fractions of 10 ml were collected and assayed for activities. The reference points shown are (top to bottom): alkaline phosphatase, anthranilate synthetase CoI, indole-3-glycerol phosphate synthetase, and α subunit of tryptophan synthetase.*

chymotrypsin and anthranilate synthetase CoI, while the α subunit of tryptophan synthetase fluoresced weakly, with a maximum at 330 nm (Fig. 6).

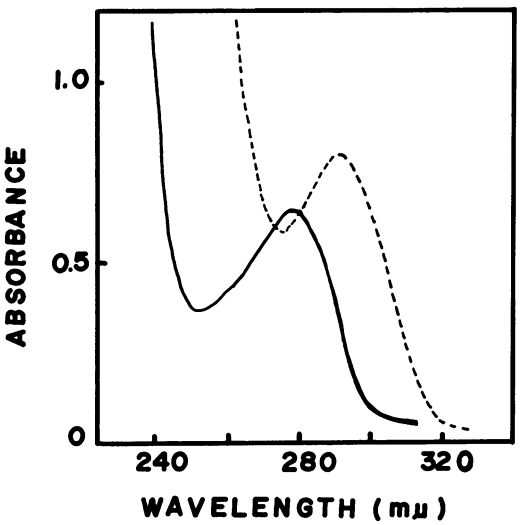

FIG. 5. *Ultraviolet absorption spectra of purified anthranilate synthetase CoI (1.65 mg/ml) in 0.05 M potassium phosphate buffer, pH 7.0 (solid line), and in 0.1 N NaOH (dashed line). Measurements were made with a Gilford spectrophotometer.*

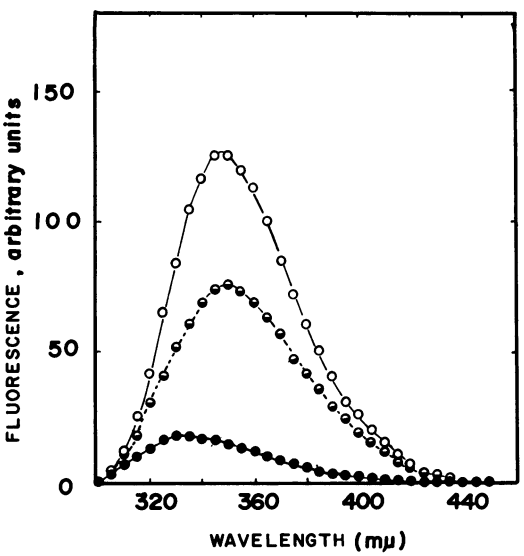

FIG. 6. *Fluorescence spectra. The proteins examined were dissolved in 2.0 ml of 0.01 M potassium phosphate buffer, pH 7.0, containing 0.1 M sodium chloride. The fluorescence spectra were determined with a 285 nm excitation wavelength. Top curve, chymotrypsin (0.25 mg); middle curve, anthranilate synthetase CoI (0.57 mg); bottom curve, α subunit of E. coli tryptophan synthetase (0.3 mg).*

Effect of pH on enzyme activity. The activity of anthranilate synthetase CoI was examined over the *p*H range 6 to 9 in potassium phosphate buffer and Tris-hydrochloride buffer. Maximal activity was observed between *p*H 7.2 and *p*H 7.8.

Effect of NH_4^+ ions on the activity of anthranilate synthetase CoI. During the purification of anthranilate synthetase CoI, it was noticed that ammonium ions activated the component. The effect of NH_4^+ ion concentration on component I activity was determined; a plot of the reciprocal of the reaction rate versus the reciprocal of the ammonium sulfate concentration can be seen in Fig. 7. From these data, a K_m value of 0.015 M for NH_4^+ can be calculated.

Table 2 shows that NH_4^+ ions, at the concentration chosen, are about 20% as effective as anthranilate synthetase CoII in activating anthranilate synthetase CoI. It is also evident that L-glutamine does not serve as amino group donor in anthranilate formation in the presence (Table 3) or absence (Table 2) of NH_4^+ when anthranilate synthetase CoI alone is catalyst. L-Asparagine and L-aspartic acid are also inactive as amino group donors.

Effect of NH_4^+ ions on the sedimentation of anthranilate synthetase component I. The possibility was considered (based on the *S* value of the anthranilate synthetase complex) that NH_4^+ ions promoted the dimerization of anthranilate synthetase CoI subunits and that the dimer is the enzymatically active species. It was found

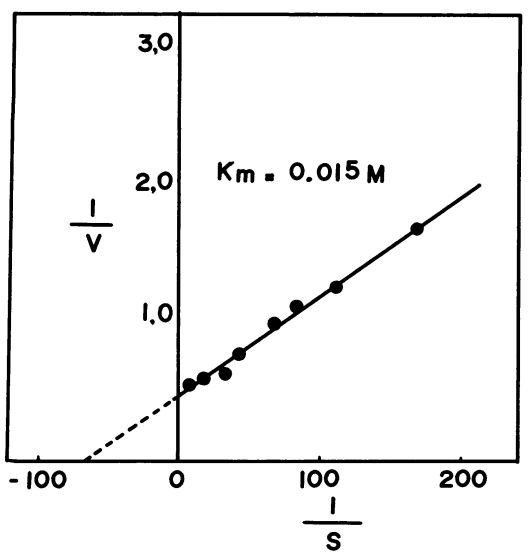

FIG. 7. *Lineweaver-Burk plot of anthranilate synthetase CoI activity (V) as a function of ammonium sulfate concentration(s). Assays were performed in 0.05 M Tris-hydrochloride buffer at pH 7.8.*

TABLE 2. *Effect of NH_4^+ ions on the activity of anthranilate synthetase CoI*[a]

Anthranilate synthetase component	Substrate and other additions	Activity (units/ml)
CoI + CoII	Chorismate + L-glutamine	90
CoI + CoII	Chorismate + L-glutamine plus $(NH_4)_2SO_4$	91
CoI + CoII	Chorismate + $(NH_4)_2SO_4$	18
CoI alone	Chorismate + L-glutamine	0
CoI alone	Chorismate + $(NH_4)_2SO_4$	18
CoI alone	Chorismate + L-glutamine plus $(NH_4)_2SO_4$	17
CoII alone	Chorismate + L-glutamine	0
CoII alone	Chorismate + $(NH_4)_2SO_4$	0
CoII alone	Chorismate + L-glutamine plus $(NH_4)_2SO_4$	0

[a] Crude extracts of *E. coli* strains carrying mutations *trpD 9778* and *trpE 5972* were used as sources of anthranilate synthetase CoI and CoII, respectively. Activities were determined under standard assay conditions in the presence or absence of anthranilate synthetase CoII, as indicated. Ammonium sulfate was added at a final concentration of 0.04 M.

TABLE 3. *Effect of L-glutamine on the activity of anthranilate synthetase component I*[a]

Ammonium sulfate (M)	Control	L-Glutamine added
	units/ml	*units/ml*
0.5×10^{-3}	3.5	3.2
0.75×10^{-3}	6.0	6.2
1.5×10^{-3}	11.0	10.6
3.0×10^{-3}	19.3	18.0
4.5×10^{-3}	26.5	29.5
6.0×10^{-3}	29.2	28.5
9.0×10^{-3}	37.3	34.2
12.0×10^{-3}	40.0	38.0

[a] The anthranilate synthetase CoI preparation used in this experiment was a crude extract of the strain of *E. coli* carrying the mutation *trpD 9778*. The reaction mixture contained: 5×10^{-4} M chorismate, 4×10^{-3} M magnesium acetate, 2×10^{-3} M β-mercaptoethanol, 5×10^{-2} M Tris-hydrochloride buffer, at *p*H 7.8, and the indicated amount of ammonium sulfate with or without L-glutamine (10^{-2} M) in a final volume of 2 ml.

that the sedimentation of anthranilate synthetase CoI in a sucrose gradient was unaffected by NH_4^+ ions. The $S_{20,w}$ value of component I relative to bacterial alkaline phosphatase, the reference marker, was 4.3*S* in the presence of NH_4^+ ions and 4.5*S* in their absence. This result does not eliminate the possibility that the active species is a weakly associated dimer.

Identification of the reaction product as anthranilic acid. In order to produce enough product of the anthranilate synthetase CoI reaction for identification, the reaction mixture was increased 10-fold. The mixture was incubated for 10 min at 37 C; the reaction was terminated by inactivating the enzyme by heating in a boiling-water bath for 1 min. The reaction product was extracted with ethyl acetate and the solution was concentrated by bubbling with nitrogen gas. The remaining solution was spotted on Whatman 3 MM paper, and the reaction product was separated by descending chromatography [developing solvent contained *n*-butyl alcohol-*n*-propanol-water (1:2:1) and 1% 1 N NH_4OH] for 14 hr at room temperature. Anthranilic acid was co-chromatographed as a reference compound. One strongly fluorescent spot was observed in the reaction product sample; this spot had the same R_F value as anthranilic acid. The absorption spectrum of the reaction product was identical with that of anthranilic acid.

Peptide patterns. In an attempt to obtain information on the number of identical or nonidentical polypeptide chains that constitute the 60,000 molecular weight species of anthranilate synthetase CoI, peptide patterns were examined. Digestion of the anthranilate synthetase CoI was carried out by a procedure similar to that described for the α subunit of tryptophan synthetase (12). A sample of 8 to 10 mg of anthranilate synthetase CoI was dissolved in 6 M urea buffered with 0.01 M NH_4HCO_3 at *p*H 8.2. This solution was allowed to stand for 15 min at room temperature and then diluted with two parts of distilled water. Trypsin was added at a protein-trypsin ratio of 40:1 (w/w) and the digestion was allowed to proceed for 3 hr at 25 C with gentle shaking. The digest was then diluted fourfold with distilled water and applied to a Dowex 50 x 2 (50–100 mesh, H^+ form) column. The column was washed with 150 ml of distilled water to remove the urea. The peptides were then eluted from the column with 4 M NH_4OH. The eluate was dried in vacuo at room temperature over sodium hydroxide pellets and sulfuric acid. The residue was dissolved in a small volume of distilled water (0.01 ml/mg of peptides) and the peptides contained were separated by two-dimensional chromatography-electrophoresis (12). An insoluble residue remained at the origin. Approximately 40 major ninhydrin-reacting soluble peptides were detected on the chromatogram, of which 24 contained arginine and 10 contained tyrosine (Fig. 8). These numbers agree roughly with the numbers expected on the basis of preliminary amino acid analyses (approximately 20 lysine, 35 arginine, and 13 tyrosine residues), assuming that the estimated molecular weight of 60,000 corresponds to a single polypeptide chain.

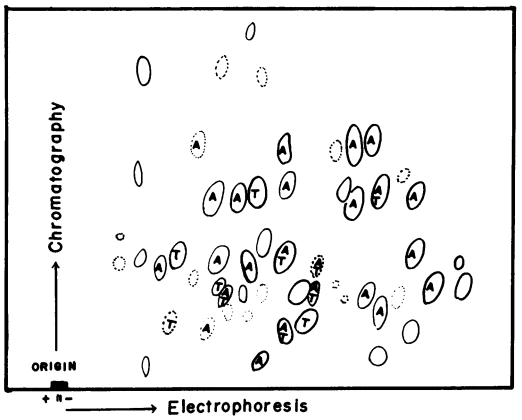

FIG. 8. *Tracing of a tryptic peptide pattern of anthranilate synthetase CoI. Major peptide spots are circled with a solid line and minor peptide spots with a dashed line. Positive color reactions for specific amino acids are indicated by A for arginine and T for tyrosine.*

Relative synthesis of polypeptide chains. It has been shown that the formation of all the enzymes of the tryptophan operon is coordinately regulated in wild-type *E. coli* (15). It has also been shown that the tryptophan synthetase α and β polypeptide chains (A and B gene products) and the indoleglycerol phosphate synthetase polypeptide chain (C gene product) are synthesized in equimolar amounts (5, 6, 10, 32). Since we obtained a highly purified preparation of the E gene product, anthranilate synthetase CoI, and since our data suggest that the 60,000 molecular weight species may be a single polypeptide chain (or composed of two or more nonidentical polypeptide chains), we can compare the molar ratios of the tryptophan synthetase α and β polypeptide chains and the anthranilate synthetase CoI polypeptide chain(s) in extracts of *E. coli*. Table 4 presents such a comparison, which suggests that there is equimolar production of these polypeptide chains in *E. coli*.

Effect of tryptophan. The anthranilate synthetases of several bacterial species (1, 2, 7, 8, 9, 16, 23) have been shown to be subject to feedback inhibition by L-tryptophan. Since it is generally observed that regulatory enzymes are oligomeric proteins (22), it was of considerable interest to determine whether anthranilate synthetase CoI (when NH_4^+-activated and not a component of the anthranilate synthetase complex) is inhibited by tryptophan. Figure 9 demonstrates that anthranilate synthetase CoI is inhibited by low concentrations of tryptophan. It can be seen from Table 5 that this inhibition is remarkably specific. The data presented in Fig. 10 indicate that tryptophan inhibition is competitive with respect to chorismate. Other experiments have

TABLE 4. *Relative synthesis of the polypeptide chains of tryptophan synthetase and anthranilate synthetase CoI*

Enzyme activity measured	Enzyme activity	Concn of polypeptide chain
	units/ml	*μmoles*
Tryptophan synthetase α subunit[b]	396[a]	2.68
Tryptophan synthetase β_2 subunit	352 (16)	2.70
Anthranilate synthetase CoI	88 (4)	2.88

[a] Specific activity.

[b] The tryptophan synthetase subunits were assayed according to Smith and Yanofsky (27). The molar concentrations of the polypeptide chains were calculated from their molecular weights and the specific activities of the purified proteins. The molecular weights of the α and β polypeptide chains are 29,000 (13) and 49,500 (10, 32), respectively. The specific activities of the purified α and β_2 subunits are 5,000 (4, 5, 13) and 2,700 units of protein (33), respectively. The extract used was obtained from a strain of *E. coli* carrying a mutation in *trpR* (repressor-constitutive). Culture conditions were described previously (34).

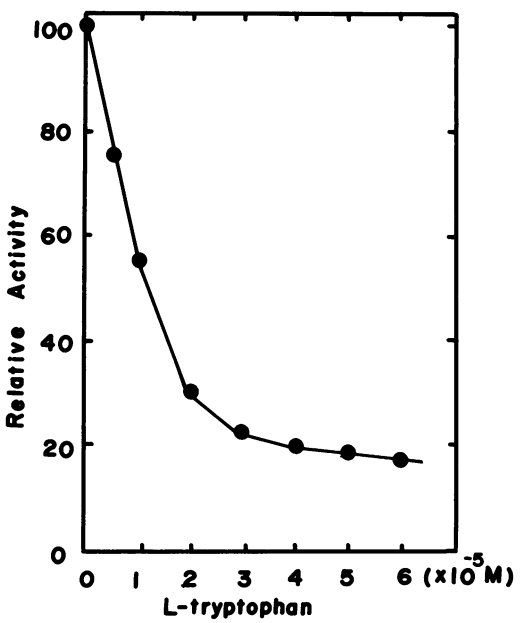

FIG. 9. *Progressive inhibition of E. coli anthranilate synthetase CoI activity by increasing L-tryptophan concentrations. Purified anthranilate synthetase CoI was used. Assay system: chorismate, 10^{-5} M; Mg acetate, 2×10^{-3} M; ammonium sulfate, 1.5×10^{-2} M; β-mercaptoethanol, 10^{-3} M; potassium phosphate buffer (pH 7.6), 10^{-2} M; and the indicated amount of L-tryptophan. Initial rates are plotted.*

TABLE 5. *Inhibition of anthranilate synthetase CoI by various compounds*[a]

Component tested	Concn	Enzyme activity	Inhibition
	M	*units/ml*	%
None		0.60	0
L-Tryptophan	1×10^{-4}	0.06	90
DL-Tryptophan	2×10^{-4}	0.08	87
D-Tryptophan	1×10^{-4}	0.58	4
Indole	2×10^{-4}	0.57	5
DL-Serine	2×10^{-4}	0.62	0
L-Tyrosine	2×10^{-4}	0.59	2
DL-Phenylalanine	5×10^{-4}	0.62	0
L-Histidine	5×10^{-4}	0.61	0

[a] The reaction mixture contained 0.04 ml of partially purified anthranilate synthetase CoI (specific activity of 400), 10^{-5} M chorismate, 2.5×10^{-3} M magnesium acetate, 1.5×10^{-2} M ammonium sulfate, 10^{-3} M β-mercaptoethanol, 10^{-2} M potassium phosphate buffer at *p*H 7.6, and the indicated concentration of test compound.

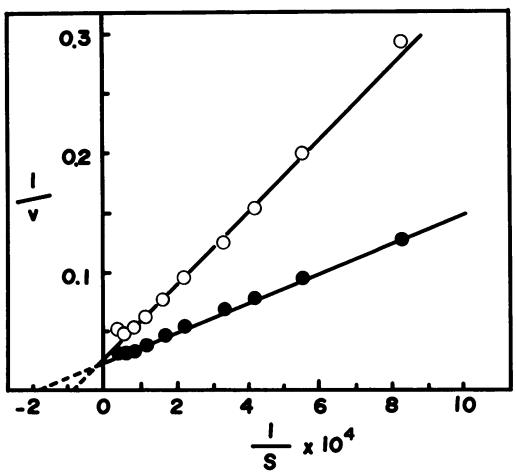

FIG. 10. *Reciprocal plots of the dependence of anthranilate synthetase CoI activity on chorismate concentration(s) in the presence and absence of* L-*tryptophan. The ammonium sulfate concentration was 1.5 × 10^{-2} M and the* L-*tryptophan concentration was 10^{-5} M. V is expressed in units of component I activity.* L-*Tryptophan present* (○); *control* (●).

shown that tryptophan inhibition is noncompetitive with respect to ammonium sulfate concentration. It is evident from Fig. 7 and 10 that the dependence of the initial velocity on chorismate and ammonium sulfate concentrations is noncooperative. The calculated Hill coefficients are n = 1.1 for chorismate and n = 1 for ammonium sulfate.

A comparison of other properties of the anthranilate synthetase complex and anthranilate synthetase CoI is presented in the accompanying paper (17).

DISCUSSION

The findings reported in this paper indicate that anthranilate synthetase CoI of *E. coli*, purified to near homogeneity, has a molecular weight of approximately 60,000 daltons and a specific activity, in the presence of excess anthranilate synthetase CoII, of 500. Peptide pattern studies with tryptic digests of purified anthranilate synthetase CoI suggest that it does not consist of two or more identical polypeptide chains; rather, the 60,000 daltons can be accounted for by unique amino acid sequences, probably in a single or two nonidentical polypeptide chains. The fact that extracts of strains carrying various nonsense mutations in *trpE* do not complement one another suggests that component I is a single polypeptide chain. On the basis of these conclusions, it was calculated that *E. coli* (a *trpR* mutant) produces equimolar amounts of component I and the α and β chains of tryptophan synthetase. It was previously shown that indoleglycerol phosphate synthetase was present at the same molar concentration as tryptophan synthetase α and β in extracts of a *trpR* mutant *E. coli*. Since the first, third, fourth and fifth gene products of the operon are produced in equimolar amounts, it is extremely likely that all five polypeptides specified by the polycistronic *trp* messenger (14) are produced in equal numbers. The same conclusion has been reached concerning the synthesis of two of the enzymes specified by the galactose operon of *E. coli* (33). With regard to the histidine operon of *S. typhimurium*, three of the histidine enzymes are produced at equimolar levels, whereas the enzyme specified by the first gene of the operon is synthesized in greater amounts (21). Studies of the induced levels of the enzymes specified by the lactose operon of *E. coli* also indicate that equimolar production is not obtained (3). It is relevant to these considerations that, under certain conditions, the concentrations of the tryptophan enzymes in *E. coli* are not equimolar (15, 29, 34).

Inhibition studies with purified anthranilate synthetase CoI (Fig. 9 and 10) showed that the activity of this component can be regulated by L-tryptophan. This finding indicates that this component has both a catalytic site and a feedback inhibitor site. In the accompanying paper (17), more detailed kinetic data are presented.

ACKNOWLEDGMENTS

This work was supported by grants from the Public Health Service (GM-09738) and from the National Science Foundation (GB-6790).

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