Indole-3-glycerol Phosphate Synthetase of *Escherichia coli*,
an Enzyme of the Tryptophan Operon*

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SUMMARY

The indole-3-glycerol phosphate synthetase of *Escherichia coli* was purified to apparent homogeneity and was crystallized. The enzyme is a single polypeptide chain of 45,000 molecular weight with 1 N-terminal methionine residue. The amino acid composition of the protein was determined.

The rate of synthesis of this polypeptide chain in *E. coli* was found to be identical with the rate of synthesis of the polypeptide chains of the tryptophan synthetase α and β subunits.

I. P. Crawford. The A2 mutation results in the absence of any detectable tryptophan synthetase α subunit (formerly designated A protein) (6). This diploid strain was used as the source of wild type InGP synthetase, since it produced 60 to 100 times as much enzyme as wild type *E. coli* K-12.

The method of growth and the preparation of cell-free extracts have been described previously (7). The procedure used was different only in that sonic extracts were prepared in 0.1 M potassium phosphate buffer, pH 7.0.

Materials—CDRP was synthesized chemically by a previously described method (3), as modified by DeMoss. Guanidine hydrochloride was recrystallized three times from hot methanol by the method of Reithel and Sakura (8). Iodoacetic acid was recrystallized from boiling petroleum ether and stored in the dark. All other reagents were obtained commercially and used without further purification.

Enzyme Assays—Two assays have been used to detect InGP synthetase activity. The first is based upon measurement of the amount of InGP (after conversion to indole-3-aldehyde with periodate) formed from CDRP after incubation at 37° with the enzyme for 20 min. This assay was used as previously described (4), with the exception that the buffer was 0.05 M Tris-HCl, pH 7.8.

A second assay has been developed which takes advantage of differences in the ultraviolet absorption spectra of CDRP and InGP in 0.1 M Tris-HCl buffer, pH 7.8. The absorption spectrum of the CDRP preparation normally used for InGP synthetase assays is presented in Fig. 1A, along with the spectrum of the same mixture after conversion of the CDRP to InGP (3). Studies of the latter reaction indicated that it was catalyzed by a single enzyme (InGP synthetase) present in extracts of *E. coli* (4). As part of the characterization of the enzymes of the tryptophan operon of *E. coli*, the present paper describes the purification, crystallization, and properties of the enzyme InGP synthetase and examines certain aspects of its relationship to other enzymes of the tryptophan operon.

MATERIALS AND METHODS

Bacterial Strain and Growth Conditions—An *E. coli* K-12 tryptophan auxotroph with the A2 point mutation on both the chromosome and an F episome (5) was kindly supplied by Dr. J. A. DeMoss, personal communication.
complete conversion of CDRP to InGP by InGP synthetase).
In the spectrophotometric assay, the conversion of 1.0 mm
CDRP to InGP was found to lead to an increase in absorbance
at 280 mµ of 0.448 (Fig. 1B). One unit of enzyme activity is
defined as that activity which leads to the formation of 0.1
µmole of InGP in 20 min at 37°. One unit of enzyme is defined
as that amount of protein which has one unit of enzymatic
activity. The spectrophotometric assay was used in all the
experiments described in this paper. Protein was measured by
the method of Lowry et al. (9). Specific activity is expressed
as enzyme units per mg of protein.

NH₄-terminal Analyses—Analysis for NH₄-terminal amino
coids was carried out by two methods. The DFB method of
Sanger (10) was used as described by Fränkel-Conrat, Harris,
and Levy (11). DNP-amino acids were identified with the
tert-aryl alcohol-phthalate buffer, pH 6, chromatography
system of Blackburn and Lowther (12). The cyanate method
was used as described by Stark and Smyth (13). However,
pyrrolidonecarboxylic acid was not removed during the proce-
dure.

Ultracentrifugation Studies—All ultracentrifugal analyses were
performed with a Beckman/Spinco model E ultracentrifuge
with the use of schlieren optics. Sedimentation velocity exper-
iments were performed at 20° in a 4° single sector aluminum cell.
Sedimentation equilibrium studies were performed in either a
filled Epon double sector cell or a three-channel equilibrium cell
similar to that described by Yphantis (14). Fluorocarbon
FC-43 was used to form a false cell bottom. Initial concentra-
tions were determined with a double sector capillary type syn-
thetic boundary cell. Measurements were made from photo-
ographic plates with a two-dimensional Gaertner comparator.

Sedimentation equilibrium results were analyzed either by
Method II of Van Holde and Baldwin (15) or by determining
the concentration at each point from the initial concentration as
described by Schachman (16) to obtain the weight average
molecular weight.

The directions of Reithel, Robbins, and Gorin (17) were fol-
lowed with solutions of protein in 6 m guanidine hydrochloride.

RESULTS

Purification of InGP Synthetase

All operations (Table I) were carried out at 0-4°. Unless
otherwise stated, all potassium phosphate buffers were at pH 7.0
and contained 1.0 mm EDTA and 0.1 mm dithiothreitol (18).

To the cell-free extract were added EDTA and dithiothreitol
to 1 mm and antrhanilic acid (dissolved in the minimum quantity
of 95% ethanol) to 10 mm.

1. Treatment with Streptomycin Sulfate To each 100 ml of
crude extract were added 5 ml of a 20% solution of streptomycin
sulfate. The mixture was stirred for 15 min and then centrifuged
for 50 min at 16,000 × g. The precipitate was discarded.

2. Treatment with Acetic Acid—To the supernatant was added
slowly, with stirring, 1.0 M sodium acetate buffer, pH 3.2, to
lower the pH of the extract to 4.90 ± 0.05 (determined with a
glass electrode after 10-fold dilution with water). Approximately
9 to 10 ml of acetate buffer were required for 100 ml of
streptomycin sulfate supernatant. The suspension was im-
mediately centrifuged at 16,000 × g for 25 min. The pH of the
supernatant was rapidly raised to 7.0 ± 0.2 (narrow range pH
paper, pH 6.0 to 8.0) by addition of concentrated NH₄OH. The
precipitate was discarded.

3. Ammonium Sulfate Precipitation—To each 100 ml of acetic
acid supernatant were added slowly, with stirring, 28.0 g of solid
ammonium sulfate. After further stirring for 15 min, the
suspension was centrifuged for 25 min at 16,000 × g. The
precipitate was suspended in a small volume of 0.1 M potassium
phosphate buffer. The supernatant was discarded.

4. Sephadex G-100 Filtration—After dialysis of the suspended
ammonium sulfate precipitate against 0.1 M potassium phosphate
buffer for 3 to 6 hours, the slightly turbid and brown solution was
applied to the top of a Sephadex G-100 column (5 × 60 cm)

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total volume</th>
<th>Total enzyme</th>
<th>Total protein</th>
<th>Specific activity</th>
<th>Yield</th>
<th>Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>865</td>
<td>2.85</td>
<td>79.5</td>
<td>35.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin sulfate supernatant</td>
<td>760</td>
<td>2.62</td>
<td>—</td>
<td>92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid supernatant</td>
<td>640</td>
<td>1.90</td>
<td>—</td>
<td>67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dialyzed ammonium sulfate precipitate</td>
<td>71</td>
<td>1.86</td>
<td>8.52</td>
<td>218</td>
<td>65</td>
<td>5.8</td>
</tr>
<tr>
<td>Sephadex G-100 eluate</td>
<td>28</td>
<td>1.35</td>
<td>2.44</td>
<td>554</td>
<td>48</td>
<td>15</td>
</tr>
<tr>
<td>DEAE-Sephadex eluate</td>
<td>28</td>
<td>1.30</td>
<td>1.12</td>
<td>1160</td>
<td>45</td>
<td>31.5</td>
</tr>
<tr>
<td>Hydroxylapatite eluate</td>
<td>14</td>
<td>0.48</td>
<td>0.66</td>
<td>1380</td>
<td>23</td>
<td>37.5</td>
</tr>
</tbody>
</table>

* Not determined because of presence of streptomycin sulfate.
* Dialyzed ammonium sulfate precipitate.
0.1 M potassium phosphate buffer. The supernatant was dis-

The protein was eluted with the same buffer at the maximum flow

rate (80 ml per hour, 12-min fractions). Those fractions con-
taining greater than 1,000 units of InGP synthetase per ml
(these fractions usually comprised 130 to 170 ml) were combined. The protein was precipitated by the addition of 35 g of solid ammonium sulfate for each 100 ml of solution. The resulting precipitate was collected by centrifugation at 16,000 × g for 25 min. The precipitate was suspended in a small volume of 0.1 M KC1 containing 0.01 M potassium phosphate buffer. The supernatant was dis-
carded.

5. DEAE-Sephadex Chromatography—After dialysis of the suspended precipitate from Step 4 against 0.1 M potassium phos-
hosphate buffer for 12 to 16 hours, the clear yellow solution was applied to a column (3 × 50 cm) of DEAE-Sephadex A50 equilibrated with the same buffer. The protein was washed on with a small amount (50 to 100 ml) of buffer and eluted with a 2,000-ml linear gradient of 0.1 to 0.7 M potassium phosphate buffer. The flow rate was 80 ml per hour and fractions of 15 min were collected. Those fractions containing greater than 1,000 units of InGP synthetase per ml were pooled, and the protein was precipitated by addition of solid ammonium sulfate (40 g/100 ml). These fractions comprised 200 to 250 ml. After collecting the precipitate by centrifugation at 16,000 × g for 25 min, the precipitate was suspended in a small volume of 0.1 M KC1 containing 0.01 M potassium phosphate buffer.

6. Hydroxylapatite Chromatography—The suspended precipi-
tate from Step 5 was dialyzed 12 to 16 hours against 0.1 M KC1 in 0.01 potassium phosphate buffer (without EDTA). The clear, slightly yellow solution was placed on a column (44 × 20 cm) of hydroxylapatite (Bio-Gel HT, Bio-Rad) equilibrated with the same buffer. The protein was washed on with approxi-
mately 100 ml of buffer. Elution was carried out with a 1000-ml linear gradient of 0.01 to 0.08 M potassium phosphate buffer in 0.1 M KC1 (without EDTA). The flow rate was 20 ml per hour, and 1-hour fractions were collected. The InGP synthetase activity emerged as the first major protein peak (Fig. 2).

The enzyme of the peak tubes (Fractions 38 to 45, Fig. 2) was precipitated by addition of solid ammonium sulfate (40 g/100 ml), and the precipitate was suspended in 0.1 M potassium phos-
hosphate buffer. After dialysis for 12 to 16 hours against the same buffer, the protein was either stored at −15° or crystallized and stored as a crystalline suspension at 4°.

FIG. 2. Elution of InGP synthetase from column of hydroxyl-
apatite. Conditions are given in the text. ———, protein; ———, enzyme activity.

An ammonium sulfate solution saturated at 4° was adjusted to pH 7.0 (determined with a glass electrode after 50-fold dilution with water) by addition of concentrated NH4OH. This solution was added dropwise to the purified InGP synthetase (at least 20 mg per ml) until a distinct turbidity was observed. After addition of a small quantity of seed crystals, the suspension was stored at 4°. Within a few hours, crystallization had proceeded to the point where a definite silkiness of the suspension could be observed. Crystallization appeared to be complete within several days. The first crystalline preparation was obtained after 4 days at 4°.

For recrystallization, the crystals were centrifuged at 12,000 × g for 10 min and washed once with 50% saturated ammonium sulfate (obtained by dilution of the saturated solution used above, with water). The crystals were then dissolved to a concentration of approximately 20 mg per ml in 0.1 M potassium phosphate buffer containing 1 mM EDTA and 1 mM dithiothreitol. Crystallization was induced by the addition of a saturated solution of ammonium sulfate and seed crystals as described above. A second crop of crystals could usually be obtained from the mother liquor by the addition of ammonium sulfate to produce again a distinct turbidity.

A photomicrograph of InGP synthetase crystallized once is shown in Fig. 3. One preparation of purified InGP synthetase was crystallized three successive times as described above. The specific activity of the final crystalline material was not signifi-
cantly different from that of the initial purified material (1380 units per mg).

Electrophoresis

The electrophoretic patterns obtained with a Perkin-Elmer electrophoresis apparatus with a 2-ml cell are shown in Fig. 4. At either pH 7.0 or 7.4, the purified InGP synthetase migrated as a single symmetrical component in both the ascending and descending limbs of the cell.

Ultracentrifugation

Purified InGP synthetase exhibited a single symmetrical boundary in the analytical ultracentrifuge in 0.03 M potassium phosphate buffer (Fig. 5). The sedimentation coefficient (s20,w) was found to be 3.3 s at a concentration of approximately 10 mg per ml. Sucrose gradient centrifugation of the InGP synthetase in crude extracts at much lower concentrations yield the same sedimentation coefficient.

Sedimentation equilibrium studies on purified InGP synthetase in 0.1 M potassium phosphate buffer at an initial concentra-
tion of approximately 10 mg per ml were performed at 11,272 rpm. Analysis of the data by Method II of Van Holde and Baldwin (15) indicated that the protein was homogeneous with respect to molecular weight, as evidenced by the curve of Fig. 6. With the use of a value of 0.736 for the partial specific volume, estimated (19) from the amino acid composition of the protein, a molecular weight of 45,000 was calculated. Later studies on the same preparation yielded indications of aggregation and slightly higher z average molecular weights.

Sedimentation equilibrium studies on the InGP synthetase in 6 M guanidine hydrochloride and 10 mM dithiothreitol gave strong indications of nonideality at concentrations of approxi-
Fig. 3. InGP synthetase crystallized once. X 62

Fig. 4. Electrophoresis pattern of purified InGP synthetase. Conditions: protein (15 mg per ml) in 0.02 M sodium phosphate buffer, pH 7.4, and 0.15 M NaCl; 2'; photographs taken after 200 min. Upper curve, ascending boundary, movement from left to right; lower curve, descending boundary, movement from right to left.

Fig. 5. Sedimentation of purified InGP synthetase. Protein (10 mg per ml) in 0.03 M potassium phosphate buffer, pH 7.0, at 50,100 rpm and 20°. The photograph was taken 80 min after start of the sedimentation. Sedimentation is from right to left.

approximately 2.5 to 10 mg per ml. Such nonideality has been noticed under these conditions with other systems (17, 20–22). Following the procedure of Reithel, Robbins, and Gorin (17), the reciprocal of the weight average molecular weight was plotted against the concentration (Fig. 7). Extrapolation to zero protein concentration yields the reciprocal of the actual molecular
A sample of InGP synthetase crystallized three times was used for determination of its amino acid composition. The protein was dialyzed 75 hours against 2 liters of distilled water with three changes. The weight of the protein was determined after drying under reduced pressure at room temperature over sodium hydroxide and sulfuric acid. Samples of the protein were then dissolved in 5.7 N HCl, hydrolyzed at 105°C in sealed evacuated tubes for 24, 48, and 72 hours, and analyzed for amino acid content. Cysteine or cystine or both were determined as cysteic acid after performic acid oxidation of the protein for 2½ hours at 0°C followed by acid hydrolysis (26).

The results of these analyses are presented in Table II. Because of the partial destruction of serine and threonine upon acid hydrolysis, the concentrations of these amino acids were estimated by linear extrapolation to zero time of hydrolysis. There was also evidence of slight destruction of tyrosine. Incomplete liberation of isoleucine and valine was observed at 24 and 48 hours, so the 72-hour values were used for these two amino acids. Approximately 96% of the dry weight of the protein was recovered as amino acid residues.

Carboxymethylation was employed to determine the number of cysteine residues in the protein. A sample of protein was incubated for 90 min at 37°C in 8 M urea, 0.05 M iodoacetate, and 0.1 M ammonium carbonate buffer, pH 8.3. The carboxymethylation reaction was stopped by the addition of mercaptoethanol to 0.7 M, followed by extensive dialysis against 0.01 M ammonium carbonate buffer. The protein was then hydrolyzed for 48 hours in 5.7 N HCl at 105°C in sealed evacuated tubes. Amino acid analysis of the hydrolyzed protein indicated the presence of 6.1 moles of carboxymethylcysteine per mole of protein. There was no significant loss of any other amino acid. This result indicates that all half-cystine residues of purified InGP synthetase are present as cysteine residues.

Analysis of NH₂-terminal Amino Acid Residues

NH₂-terminal residues were analyzed by the cyanate method of Stark and Smyth (13). The results (Table III) indicated the presence of nearly 1 mole of NH₂-terminal methionine per 45,000 g of protein. The presence of glutamic acid is not considered significant because pyrrolidonecarboxylic acid was not

**Fig. 6.** Analysis of sedimentation equilibrium of purified InGP synthetase after 23 hours at 11,272 rpm by Method II of Van Holde and Baldwin (15). The values of the concentration (c) at each point minus the concentration at the meniscus (CM) and of the concentration gradient (dc/dz) are given in units as measured from the photographic plate. The initial protein concentration was approximately 10 mg per ml; the temperature was 4°C; and the column height was 2 mm.

**Fig. 7.** Plot of the reciprocal of the weight average molecular weight (MW) obtained from sedimentation equilibrium studies of purified InGP synthetase in 6 M guanidine-hydrochloride and 10 mM dithiothreitol with respect to the protein concentration (c). The values of c (one-half the sum of the concentrations at the meniscus and the cell bottom (15)) are given in units as measured from photographic plates. The highest initial concentration used corresponds to approximately 10 mg per ml. The rotor speed was 24,630 rpm; the temperature was 4°C; and the column height was 2 mm in each case.

**Ultraviolet Absorption and Fluorescence Spectra**

The ultraviolet absorption spectrum (Fig. 8) of purified InGP synthetase is characteristic of a protein of the tyrosine plus tryptophan type (23). Its absorption spectrum in 0.1 N NaOH (Fig. 8) clearly indicated (24) the presence of tryptophan in the protein (Table II). The fluorescence emission maximum (Fig. 9) at 300 mp and the excitation maximum at 290 mp are also indicative of the presence of tryptophan in the protein (25).

**Fig. 8.** Ultraviolet absorption spectra of purified InGP synthetase in 5 mM potassium phosphate buffer, pH 7.0 (--), and in 0.1 N NaOH (- - -). Measurements were made with a Gilford spectrophotometer at a protein concentration of 1.0 mg per ml.
removed during the procedure (13). The amount of glutamic acid found is approximately the amount that would be expected on the basis of the glutamic acid content of the protein and the approximate equilibrium in the cyanate procedure between glutamic acid and pyrrolidonecarboxylic acid (13). The only other amino acid detected was a small amount of glycine. The purified \( \text{InGP synthetase, which also has 1 NH}_2\text{-terminal methionine residue (27), was analyzed at the same time and yielded very similar results.}

Treatment of crystalline \( \text{InGP synthetase with DFB (11) yielded only DNP-methionine, in addition to the usual dinitrophenol and dinitrosoamine. There was no indication of any DNP-glutamic acid or DNP-glycine. No attempt was made to determine quantitatively the DNP-methionine detected in this experiment. However, the results support the finding of NH}_2\text{-terminal methionine by the cyanate method and rule out the existence of NH}_2\text{-terminal glutamic acid or glycine. This evidence and the molecular weight data indicate that the enzyme is a single polypeptide chain.}

**Peptide Patterns**

Crystalline \( \text{InGP synthetase was carboxymethylated with } ^{14} \text{C-iodoacetate (see “Amino Acid Composition”) and digested with trypsin plus chymotrypsin, and the peptides were separated on two-dimensional peptide patterns as previously described (28). There were approximately 60 major ninhydrin-staining spots, of which 14 stained for arginine and 9 for tyrosine (28). In addition, two spots were fluorescent under near ultraviolet light (indicating the presence of tryptophan) and four were radioactive (indicating the presence of } ^{14} \text{C-carboxymethylcysteine). The origin was also radioactive and stained for}

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**Table II**

**Amino acid composition of \( \text{InGP synthetase**}

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Amino acid residues per 100 g of protein</th>
<th>Calculated number of amino acid residues for molecular weight 45,000</th>
<th>Average or extrapolated value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>11.13</td>
<td>43.9 ± 0.5</td>
<td>43.5 ± 0.7</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.92</td>
<td>12.5 ± 0.2</td>
<td>13.0 ± 0.2*</td>
</tr>
<tr>
<td>Serine</td>
<td>4.35</td>
<td>21.2 ± 0.2</td>
<td>22.5 ± 0.3*</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>14.83</td>
<td>52.0 ± 0.7</td>
<td>51.7 ± 0.5</td>
</tr>
<tr>
<td>Proline</td>
<td>3.26</td>
<td>15.1 ± 0.2</td>
<td>15.1 ± 0.2</td>
</tr>
<tr>
<td>Glycine</td>
<td>3.66</td>
<td>28.9 ± 0.2</td>
<td>28.9 ± 0.3</td>
</tr>
<tr>
<td>Alanine</td>
<td>8.72</td>
<td>55.5 ± 1.1</td>
<td>55.2 ± 0.7</td>
</tr>
<tr>
<td>Valine</td>
<td>7.54</td>
<td>30.8 ± 0.3</td>
<td>34.1 ± 0.3*</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.31</td>
<td>4.5 ± 0.1</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>5.48</td>
<td>20.2 ± 0.3</td>
<td>21.8 ± 0.3</td>
</tr>
<tr>
<td>Leucine</td>
<td>11.40</td>
<td>45.6 ± 0.8</td>
<td>45.3 ± 1.1</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>5.19</td>
<td>14.6 ± 0.2</td>
<td>14.3 ± 0.2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.60</td>
<td>11.1 ± 0.1</td>
<td>11.1 ± 0.2</td>
</tr>
<tr>
<td>Lysine</td>
<td>4.64</td>
<td>10.1 ± 0.2</td>
<td>10.0 ± 0.2</td>
</tr>
<tr>
<td>Histidine</td>
<td>3.05</td>
<td>20.8 ± 0.3</td>
<td>21.1 ± 0.3</td>
</tr>
<tr>
<td>Arginine</td>
<td>7.32</td>
<td>10.0 ± 0.2</td>
<td>6.2 ± 0.1*</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>1.41</td>
<td>6.2 f 0.1</td>
<td>2.1 ± 0.2*</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.87</td>
<td>4.5 f 0.2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>101.65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* In calculating the residues, the determination for half-cystine and tryptophan was employed.

* Extrapolated value.

* The 72-hour value only.

* Determined as cysteic acid.

* Determined spectrophotometrically.

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**Table III**

**NH\(_2\)**-terminal residues of \( \text{InGP synthetase**}

The cyanate method of Stark and Smyth (33) was used with 0.5 \( \mu \text{ mole of crystalline InGP synthetase. Only those amino acids are presented for which more than 0.05 mole was found per mole of protein.}

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Amino acid-protein ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamic acid*</td>
<td>1.42</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.17</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.79</td>
</tr>
</tbody>
</table>

* Not removed as pyrrolidonecarboxylic acid.
tyrosine and arginine. Since the carboxymethylated protein contains 21 arginine, 14 tyrosine, 2 tryptophan, and 6 carboxymethylcysteine residues (see Table II), these results suggest that the protein is not composed of identical polypeptide chains.

**Michaelis Constant for Substrate**

The purified enzyme exhibited normal kinetic properties when the $K_m$ for the substrate CDRP was determined (Fig. 10). The apparent $K_m$ was found to be $5 \times 10^{-4}$ M with the use of initial rates of catalysis measured by the spectrophotometric assay. This $K_m$ value is somewhat lower than the value previously found (4). The difference is probably due to previous inability to measure the initial reaction rates accurately with the less convenient assay system that was available.

**Relative Synthesis of Polypeptide Chains**

The purification and characterization of InGP synthetase allows comparison of the synthesis of this polypeptide chain and the $\alpha$ and $\beta$ polypeptide chains of the $\alpha$ and $\beta_2$ subunits of the tryptophan synthetase of E. coli. Other studies (29) have shown that the $\alpha$ and $\beta$ polypeptide chains are synthesized in equimolar quantities. The synthesis of the $\alpha$ and $\beta_2$ subunits of tryptophan synthetase and InGP synthetase have been shown to be coordinately regulated in wild type E. coli (30). Thus, analysis of a single extract is sufficient to permit estimation of the relative synthesis of the three different polypeptide chains. The analysis of such an E. coli crude extract is presented in Table IV. The molar concentrations of the polypeptide chains have been calculated from the specific activities of the purified proteins and the molecular weights of the polypeptide chains. The results confirm the conclusion (29) that the $\alpha$ and $\beta$ polypeptide chains are normally present in equimolar concentrations. They further indicate that the polypeptide chain of InGP synthetase is present at the same molar concentration.

**TABLE IV**

Relative synthesis of polypeptide chains of tryptophan synthetase and InGP synthetase

The activities of the tryptophan synthetase subunits were assayed in the conversion of indole to tryptophan as previously described (31). 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 $\alpha$ and $\beta$ polypeptide chains are 30,000 (7) and 49,500, respectively. The specific activities of the purified $\alpha$ and $\beta_2$ subunits are 5,000 and 3,000 (32) units per mg, respectively. The extract used was from a strain carrying an $R_{-\text{amp}}$ mutation (regulatory mutation) (33) and a mutationally altered $\alpha_1$ subunit. It was grown in minimal medium (34) and harvested in log phase.

<table>
<thead>
<tr>
<th>Enzyme activity measured</th>
<th>Enzyme concentration of polypeptide chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan synthetase $\alpha$ subunit</td>
<td>350 units/ml 2.3 $\mu$M</td>
</tr>
<tr>
<td>Tryptophan synthetase $\beta_2$ subunit</td>
<td>352 units/ml 2.2 $\mu$M</td>
</tr>
<tr>
<td>InGP synthetase</td>
<td>137 units/ml 2.2 $\mu$M</td>
</tr>
</tbody>
</table>

**Fig. 10. Determination of $K_m$ for CDRP and InGP synthetase.**

The reciprocal of the CDRP concentration is plotted against the reciprocal of the initial velocity of the enzyme reaction ($V$). The velocity is expressed as the increase in absorbance at 280 m$\mu$ per min.

**Discussion**

The purified InGP synthetase described in this paper is a homogeneous protein on the basis of its electrophoretic behavior, sedimentation characteristics, and constant specific activity upon repeated crystallization. It appears to consist of a single polypeptide chain because its molecular weight in 6 M guanidine hydrochloride is no lower than in phosphate buffer, it contains only 1 NH$_2$-terminal methionine residue, and its trypsin plus chymotrypsin peptide pattern contains nearly the number of peptide spots expected on the basis of its amino acid content. No requirements for cofactors or metals for enzymatic activity have been observed. The crystalline enzyme does not contain any appreciable amount of nonprotein material, since its dry weight can be accounted for by its amino acid content.

Recent observations have suggested that in E. coli InGP synthetase might be related in some manner to the N-5'-phosphoribosylanthranilate isomerase of the tryptophan biosynthetic pathway. The latter enzyme catalyzes the conversion of N-5'-phosphoribosylanthranilate to CDRP, the substrate of InGP synthetase (Fig. 11). DeMoss (35) observed that the InGP synthetase and N-5'-phosphoribosylanthranilate isomerase activities sedimented together during sucrose gradient centrifugation. It was subsequently noticed that many single site mutants lacking InGP synthetase activity accumulated anthranilic acid rather than 1-(o-carboxyphenylamino)-1-deoxyribulose (36). This behavior suggests that both InGP synthetase and N-5'-phosphoribosylanthranilate isomerase activities are absent from these mutants. Anthranilic acid accumulates rather than N-5'-phosphoribosylanthranilate because the latter is very unstable and spontaneously breaks down to anthranilic acid (37). DeMoss$^3$ has subsequently found that the InGP synthetase crystallized three times prepared in this investigation had the same ratio of InGP synthetase and N-5'-phosphoribosylanthranilate isomerase activities sedimented together during sucrose gradient centrifugation. It therefore seems likely that the InGP synthetase of E. coli, a single polypeptide chain, is also the N-5'-phosphoribosylanthranilate isomerase of tryptophan biosynthesis.

A similar situation exists in Neurospora crassa, in which the product of a single gene possesses the same two enzymatic activities (38). However, the structure of the N. crassa enzyme is unknown. It is of interest that these two activities are

from E. coli, is a single polypeptide chain. Genetic and biochemical analyses with S. typhimurium mutants lacking InGP synthetase activity have revealed the same mutant fiZ subunits are present in crude extracts at the same molar concentration. Assuming that the enzymatic activity present in a crude extract is a measure of the amount of each polypeptide chain that is synthesized, this result indicates that equal numbers of polypeptide chains are synthesized. A similar conclusion has been reached concerning the synthesis of two enzymes of the galactose operon of E. coli (42). However, such equimolar synthesis of polypeptide chains has not been observed with the proteins of the lactose operon of E. coli (43). This question has also been investigated with the histidine operon of S. typhimurium (44). Other investigators (45, 46) have also concluded that in certain cases there must be some differential regulation at the level of translation of the genetic message. However, such regulation of translation is clearly not necessary for the three last genes of the tryptophan operon and those of the galac-

tose operon mentioned above. The basis of the difference between the systems described and its significance for regulation of translation remains to be determined.

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