Purification and Properties of the $\alpha_2\beta_2$ Complex of Tryptophan Synthetase of *Proteus mirabilis* *

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A procedure is described for the purification of the tryptophan synthetase $\alpha_2\beta_2$ complex from cell extracts of *Proteus mirabilis*. A 30-fold purification was achieved with an overall yield of about 23% and a specific activity of 1,600. The complex can be dissociated and the subunits isolated in a pure form. The complex can be reconstituted from the isolated subunits to regain the initial activity. The $\alpha$ and $\beta_2$ subunits of the tryptophan synthetase complex of *P. mirabilis* are not significantly different from those of *Escherichia coli* and other enteric bacteria as to their physical properties, amino acid compositions, and enzymic properties.

Complementation studies indicate that the $\alpha$ subunit of *P. mirabilis* hybridizes well with the $\beta_2$ subunit from *E. coli*. Similarly, the $\beta_2$ subunit of *P. mirabilis* readily complexes with the $\alpha$ subunits from *E. coli*, *Salmonella typhimurium*, and *Serratia marcescens*. The hybrids formed are all effective in catalyzing the conversion of indoleglycerol phosphate plus serine into tryptophan and glyceraldehyde 3-phosphate. However, these hybrids have reduced or no activity in the other reactions, namely, the condensation of indole and serine to form tryptophan or the aldolytic cleavage of indoleglycerol phosphate.

Tryptophan synthetase (tryptophan synthase or L-serine hydrolyase (adding indole), EC 4.2.1.20) has been isolated and characterized from a variety of organisms. In bacteria, the enzyme is known to exist as an $\alpha_2\beta_2$ complex in equilibrium with the individual $\alpha$ and $\beta_2$ subunits (1, 2). The three commonly known reactions catalyzed by this enzymic complex (3) are:

$$\text{Indole} + L\text{-serine} \rightarrow L\text{-tryptophan}$$  \hspace{1cm} (1)

$$\text{Indole-3-glycerol phosphate} \rightarrow \text{indole} + \text{d-glyceraldehyde 3-phosphate}$$  \hspace{1cm} (2)

$$\text{Indole-3-glycerol phosphate} + L\text{-serine} \rightarrow L\text{-tryptophan} + \text{d-glyceraldehyde 3-phosphate}$$  \hspace{1cm} (3)

The physiological reaction is Reaction 3. Reactions 1 and 2 are considered to be two halves of the physiological reaction and each subunit alone is able to catalyze one of the half-reactions, namely, the $\alpha$ subunit catalyzes Reaction 2 and the $\beta_2$ subunit catalyzes Reaction 1. However, the catalytic capabilities of the individual subunit represent less than 10% of those of the associated subunits.

Comparative studies on the structure of the tryptophan synthetase subunits from several enteric bacteria have been conducted in various laboratories. The amino acid sequences of the subunits from *Escherichia coli* (4), *Salmonella typhimurium* (5), and *Klebsiella aerogenes* (6) have been elucidated. The partial sequences of the $\alpha$ subunit from *Shigella dysenteriae* (7) and *Serratia marcescens* (8) and those of two species unrelated to the enteric bacteria, *Pseudomonas putida* (9) and *Bacillus subtilis* (10) have also been reported. The elucidation of the primary structures of these $\alpha$ subunits has contributed much information concerning the relatedness of these enzymes and their corresponding bacterial species. This phylogenetic relationship has also been corroborated by comparisons of nucleotide sequence homology between tryptophan operon messenger RNA and tryptophan operon DNA (11). Furthermore, immunological studies of the tryptophan synthetase $\alpha$ and $\beta_2$ chains of various enteric bacteria have contributed additional evidence in establishing the relatedness in the structure of these subunits (12, 13).

The present study deals with the purification and properties of the tryptophan synthetase enzyme from another enteric bacterial species, *Proteus mirabilis*. A comparison of the structure of the enzyme from this organism with those of other enteric bacteria could be revealing in view of the large difference in the DNA base composition between *P. mirabilis* (39% guanosine plus cytosine), *E. coli* or *S. typhimurium* (51%), *K. aerogenes* (56%), and *S. marcescens* (57%) (14). A prerequisite for such an investigation was the purification of the enzyme and a characterization of its basic properties. This communication reports the purification of the *P. mirabilis* enzyme as the $\alpha_2\beta_2$ complex. A procedure permitting a reversible dissociation of the complex has also been developed. Some properties of these subunits, such as their molecular weights,
tryptophan synthetase $\alpha$ subunit, 29,000; and trypsin, 23,000.

**Amino Acid Composition**—Protein samples were extensively dialyzed against ammonium bicarbonate buffer, 5 mM, pH 8.3, and 1-mg aliquots were hydrolyzed at 110° for 24, 48, and 72 hours in evacuated, sealed tubes with 1.0 ml of 6 N HCl. The analyses were performed using a Beckman model 120C amino acid analyzer. Values for threonine and serine were extrapolated to zero times of hydrolysis and valine and isoleucine values taken at 72 hours. Methionine and half cystine were determined on separate samples as methionine sulfoxide and cysteic acid, respectively, with oxidation with performic acid according to Hirs (20) and tryptophan was determined spectro-photometrically (21).

**RESULTS**

**Dissociation of Complex to Its $\alpha$ and $\beta_2$ Subunits**—As detailed in the supplement, the enzyme was purified as the native $\alpha_2\beta_2$ complex. The procedure developed for the dissociation of the complex and isolation of the subunits consisted in a chromatography on a DEAE-cellulose column using sodium acetate buffers of progressively lower pH as the eluting solution. A column (1.5 × 15 cm) was prepared and equilibrated with Buffer C (see miniprint supplement). The enzyme (two to three fractions obtained after Sephadex G-200 chromatography and representing about 20 mg of protein) was dialyzed for 6 hours against 1 liter of Buffer C with one change to remove the serine present and then applied onto the column. The column was washed with 100 ml of 0.1 mM sodium acetate buffer, pH 6.6, containing pyridoxal phosphate, 40 $\mu$M; 2-mercaptoethanol, 10 mM; and 10% glycerol (v/v). This step permitted the removal of all NaCl present in Buffer C used for washing the column and in the enzyme sample. Then a gradient consisting of 300 ml of this buffer in the mixing chamber and 300 ml of the same buffer but at pH 3.2 in the reservoir was applied. The flow rate was 20 ml/hour and 7-ml fractions were collected. The results are shown in Fig. 3. The $\alpha$ subunit eluted first at approximately pH 5.8 and was essentially free of $\beta_2$ subunit activity. The second peak represents the $\beta_2$ subunit activity which eluted at about pH 5.3. This peak was found to contain traces of $\alpha$ subunit activity but these were readily removed by heating the fractions for 1 min at 70°. The small precipitate formed was eliminated by centrifugation. During the chromatography or very soon thereafter 1 ml of 1 M Tris HCl buffer, pH 7.8, was added to all fractions in order to raise the pH to about 7.0. This pH adjustment was necessary to prevent the precipitation and denaturation of the $\beta_2$ subunit
which otherwise would occur after a few hours. The recoveries of each subunit were nearly quantitative, suggesting that little or no denaturation occurred during this chromatographic procedure. The purity of each of the subunits was demonstrated by polyacrylamide disc gel electrophoresis (Fig. 4, Gels B and C). The specific activity of the reconstituted αβ₂ complex was found to be identical with that of the original complex.

Interchange of α and β₂ Subunit from P. mirabilis with Those of Other Enteric Bacteria—The capacity of the P. mirabilis enzyme subunits to complement and form functional hybrids with the subunits from other enteric bacterial species was investigated. For these experiments different known amounts of the α subunit (Fig. 8) or the β₂ (Fig. 9) from P. mirabilis was mixed with constant amounts of the complementary subunit from another species. The activities of these hybrid preparations were measured for each of the three enzymatic reactions. Fig. 8A represents the activities measured in the physiological reaction (Reaction 3). As expected for the homologous Proteus system, the maximal activity is obtained when both subunits are present in approximately equal concentrations. It can also be seen that the β₂ subunit from E. coli can effectively replace the Proteus β₂ in this reaction even though a 10-fold excess of β₂ subunits was required to obtain the same activity. Paradoxically, this hybrid combination is totally inactive in the catalysis of Reaction 1 (Fig. 8B) indicating that the α subunit of Proteus is not able to activate the E. coli β₂ in this reaction. Fig. 8C represents the activities measured in Reaction 2. It can be seen that the β₂ subunit from E. coli can stimulate the subunit of Proteus but this stimulation represents only approximately 30% of the activities obtained with the homologous combination.

The interaction of the β₂ subunit from P. mirabilis with the α subunits isolated from several enteric bacterial species was also estimated. As shown in Fig. 9A the β₂ subunits from P. mirabilis readily formed hybrids with the α subunits of E. coli, S. typhimurium, and S. marcescens which are quite active in Reaction 3. Furthermore, this interaction was more efficient than in the inverse situation (see Fig. 8A) since only a 3-fold excess of the α subunit was required to achieve the maximum activity. In Fig. 9B, it is shown that the different α subunits do
not equally and rather poorly activate the Proteus \( \beta_2 \) subunit in the catalysis of Reaction 1. In all cases, a large excess of \( \alpha \) subunit was required. The most active hybrid was the Serratia \( \alpha \)-Proteus \( \beta_2 \) combination while with the \( \alpha \) subunits from E. coli and S. typhimurium maximal activities were about 30% of that obtained for the homologous control. Only the \( \alpha \) subunit of E. coli in combination with P. mirabilis \( \beta_2 \) subunit has been tested in Reaction 2. With this combination, the maximum stimulation of the \( \beta_2 \) subunit of Proteus by E. coli \( \alpha \) subunit reached 30% of the homologous control (results not shown). The concentration ratio of \( \alpha \) to \( \beta_2 \) subunit necessary to obtain this activity was 1 to 10.

DISCUSSION

The procedure developed for the purification of the tryptophan synthetase complex from P. mirabilis gave an homogeneous preparation with a relatively good recovery. The purification of the enzyme as the \( \alpha \beta_2 \) complex was possible provided all buffer solutions used in the course of the purification contained serine, NaCl, and pyridoxal phosphate; factors which are known to favor the association of the two components of the E. coli enzyme (22). The absence of these factors in the buffer solutions resulted in considerable dissociation of the enzyme into its subunits which are rather unstable. Indeed, our early attempts at the purification of the subunits as separate entities have met with limited success. The presence of glycerol was found to be essential during dialysis (Step 3) but it also had considerable stabilizing effects during the subsequent purification steps and upon storage. A procedure was also developed for the dissociation of the enzyme and the isolation of pure subunits which upon reassociation regain the activity of the isolated complex.

A comparison of the chemical and physical properties of the subunits of the enzyme from P. mirabilis and from E. coli revealed no striking differences. Their molecular weights and sedimentation coefficient values appear to be identical while their amino acid compositions are very similar. The subunit from Proteus is acid-stable and its \( \beta_2 \) subunit considerably heat-resistant; properties which are shared with the E. coli subunits. Furthermore, the enzyme from Proteus can catalyze the three reactions known for the E. coli enzyme and the individual subunits alone have some activity in one of the two half-reactions. Then, on the basis of these considerations, it appears that the tryptophan synthetase from P. mirabilis does not appreciably differ from that isolated from other enteric bacteria.

However, it is now well known that in spite of their apparently similar chemical, physical, and enzymatic properties the \( \alpha \) subunits from enteric bacteria may differ significantly from each other in their primary structures (6-8). But since these subunits readily interchange, the regions of the \( \alpha \) subunits' surface containing the \( \beta_2 \) subunit-binding site should have a common amino acid sequence. Indeed, common amino acid sequence regions exist not only among the different \( \alpha \) subunits from enteric species but also from the unrelated species Bacillus subtilis (10) and Pseudomonas putida (9). Furthermore, it has been observed that residues in positions at which inactivating amino acid changes have been noted in E. coli \( \alpha \) subunit are all conserved in the \( \alpha \) subunit of other enteric species (23). Since P. mirabilis is probably the most divergent enteric species as judged by the much lower guanosine plus cytosine content of its DNA, the amino acid sequence of the \( \alpha \) subunit from this species would be expected to provide valuable information on the structure-function relationships of this group of enzymes.

In the present study, it has been demonstrated that the \( \alpha \) or \( \beta_2 \) subunit of P. mirabilis forms catalytically active hybrids with the complementary subunit from a variety of enteric species but each hybrid does not equally catalyze all three reactions. For example, while the E. coli \( \beta_2 \)-Proteus-\( \alpha \) combination at the appropriate \( \alpha \) to \( \beta_2 \) ratio is fully active in the physiological reaction, it has no activity in the indole \( \rightarrow \) tryptophan reaction and exhibits only moderate activity in the catalysis of Reaction 2. That this hybrid combination is active in Reaction 3 provides conclusive evidence that the subunits can associate. The lack of activity in Reaction 1 simply indicates that association of subunits to form a stable complex is not the sole prerequisite to obtain activity in the half reactions but this association must also be enzymically favorable. In the reverse combination, the \( \beta_2 \) subunit of P. mirabilis also efficiently complements with the \( \alpha \) subunit from E. coli, S. marcescens, and S. typhimurium since all hybrids are highly effective in the catalysis of Reaction 3. However, they also show low activity in Reaction 1.

The behavior of these hybrids with respect to Reactions 1 and 3 is analogous to that observed for a class of E. coli mutants which have an alteration in the \( \beta_2 \) subunit (24). The altered \( \beta_2 \) subunit in association with the normal \( \alpha \) subunit is still active in the physiological reaction but is without activity in the conversion of indole plus serine to tryptophan (Reaction 1). That this class of mutants was isolated is not unexpected because the half-reactions are not essential nor normally implicated in the biosynthesis of tryptophan in vivo. Then, the selection pressure exerted on a bacterial population living in a tryptophan-free environment would favor the conservation of the physiological reaction in preference to the half reactions. This assumption is well supported by the present data since all hybrids are definitely more active in the physiological reaction. Thus, the structural changes which occurred in the course of evolution did not affect primarily this reaction.

Subunit interchange experiments to evaluate the evolutionary relatedness of tryptophan synthetase subunits and their corresponding organisms have been conducted for a variety of organisms (see Ref. 25 for a review). It is assumed that the affinity of these subunits obtained from different organisms might closely approximate the degree of sequence similarities. This approach, however, has not always lived up to its expectation. A possible reason for this may be derived from the fact that the affinities of the hybrids were in the majority of instances determined by measuring their activities in one of the half reactions rather than in the physiological reaction. The present results from heterologous hybrids show that there is not always a clear correlation between the capacities of subunits to form complexes and the efficiencies of these complexes to catalyze either one of the half-reactions. Furthermore if, on the one hand, the absence of reciprocal activation of the subunits in the catalysis of the half reactions is not indicative of dissimilarity, a limited activation, on the other hand, may provide only a weak proof of relatedness. When it is considered that one of the subunits does not actively participate in the half-reactions but only assists the other subunit and that assays are conducted with an excess of one of the subunits, then it is possible that activation through nongeneric interactions between protein molecules may occur.
Tryptophan Synthetase of Proteus mirabilis

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REFERENCES
Purification and properties of the alpha2beta2 complex of tryptophan synthetase of Proteus mirabilis.
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