The Subunit Structure of Tryptophan Synthase from Neurospora crassa*

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SUMMARY

Tryptophan synthase of Neurospora crassa was purified to electrophoretic homogeneity from the wild type strain 74A which had been derepressed by the presence of 0.5 mM indoleacrylic acid in the growth medium. The isolated material migrated as a single symmetrical peak in the ultracentrifuge with a sedimentation constant of 6.0 S. Gel filtration on Sephadex G-200 and conventional sedimentation equilibrium yielded molecular weight estimates of 151,000 ± 10,000 and 149,000 ± 10,000, respectively. Treatment of the enzyme with sodium dodecyl sulfate followed by polyacrylamide gel electrophoresis gave a single band with a relative mobility suggesting a molecular weight of 76,000 ± 2000. Aspartic acid was the only detectable N-terminal amino acid and experiments with carboxypeptidases A and B revealed that the three amino acids, isoleucine, leucine, and phenylalanine, were released rapidly and in the order mentioned. These results are interpreted as indicating that the Neurospora enzyme is a homodimer.

The tryptophan synthase system in Neurospora crassa provided an important early model for the study of gene-enzyme relationships. The genetics (1, 2), enzymology (3), and physiology (4) of this system have received much experimental attention during the past 25 years. Despite these extensive investigations, questions concerning the size and subunit structure of the wild type enzyme have remained. Such information would have significance for the question concerning the evolutionary relationships which might exist between the N. crassa enzyme and the enzyme of Escherichia coli. Speculation about these relationships derives from a consideration of the catalytic similarities of each enzyme as compared with the apparent structural dissimilarities between them (5).

The enzymes from both N. crassa and E. coli catalyze the following reactions (6):

\[
\text{indole} + \text{L-serine pyridoxal phosphate} \rightarrow \text{L-tryptophan (1)}
\]

\[
\text{indole} + \text{glyceraldehyde 3-phosphate} \rightarrow \text{indoleglycerol phosphate (2)}
\]

The enzyme from E. coli consists of two easily dissociable subunits, α (MW 29,000) and β (MW 45,000), in the form of a tetramer (α2β2). The two subunits are coded for by distinct but adjacent genes (6) and their individual catalytic activities have been clearly elucidated (7–9). In contrast, the Neurospora system appears to consist of a single gene coding for a single protein (5).

Previous efforts (10–13) directed at purification of the N. crassa enzyme have been compromised primarily by the relatively low specific enzyme activity of crude extracts of representative wild type strains. Mohler and Suskind (10) developed a purification scheme which has been followed by subsequent workers. Ensign et al. (11) obtained a homogeneous preparation of altered tryptophan synthase from a mutant strain (ld 141) which produces approximately 10 times the wild type level of enzyme. This preparation exhibited a molecular weight of ~110,000. No information on subunit structure was reported. Carsiotis et al. (12) reported the purification of tryptophan synthase from mutant strain C-84 of N. crassa. This strain, like strain (ld 141), exhibits high levels of the enzyme. On the basis of physical and chemical methods of analysis they reported that their material had a molecular weight of about 135,000, and that upon treatment with 5 M guanidine hydrochloride it dissociated into a homogeneous material having a molecular weight of ~35,000. Their conclusion concerning the size and number of subunits was further supported by an analysis of tryptic peptides and by the results of a partial digestion of the material with carboxypeptidases A and B. Phenylalanine and leucine were released at approximately equal rates and in quantities sufficient to account for two distinct COOH-terminal amino acid residues. These results led them to suggest that tryptophan synthase consisted of four polypeptide chains of approximately equal size, two of which had COOH-terminal phenylalanine and two of which had leucine residues in the COOH-terminal position.

Subsequent work (13, 14) has indicated the presence of extremely active proteases in extracts of certain strains of Neurospora and, moreover, that these proteases co-purify with tryptophan synthase under certain circumstances. The uncertainties which the presence of proteases generate, coupled with the diffi-
ulty of interpreting the postulated heterotetrameric structure in the context of the genetic and enzymological data on the tryptophan synthase system prompted us to seek a means of preparing homogeneous tryptophan synthase from a wild type strain under conditions which would minimize the presence of contaminating proteases. Toward this end we have taken advantage of the fact that when mycelia are caused to accumulate indoleglycerol phosphate, the penultimate intermediate in the tryptophan pathway, they exhibit a 20-fold increase in the differential rate at which they form tryptophan synthase (15-17). Crude extracts of mycelia grown in this way have considerably higher levels of the enzyme (17) and were used as the source of tryptophan synthase. Using methods (13) already published, we readily obtained preparations of the enzyme which appeared to be free of contaminating proteases (14) even at relatively early steps in the purification. In this report we detail the methods for growing the mycelia from which the enzyme was obtained. From the results of physical and chemical studies we conclude that tryptophan synthase of wild type strain 74A is a protein of molecular weight of ~150,000 which apparently consists of two identical monomers with a molecular weight of ~75,000.

METHODS AND MATERIALS

Organisms—Stock cultures of strain 74A (FGSC No. A262) of Neurospora crassa were grown on slants of agar containing Vogel's minimal medium (18) supplemented with 2% sucrose. For growth of vegetative mycelia, a loop of conidia was placed on the surface of a 50-ml agar butt in a 250-ml conical flask. The culture was incubated 72 hours at 30° and 45 hours at 25°, by which time the culture was well conidiated. The conidia were suspended in sterile water and filtered through cheesecloth into a liter of liquid minimal medium. The final concentration in the 1-liter culture was ~10^6 conidia per ml. This culture was incubated at 30° with vigorous gyratory shaking for 16 hours. The contents of this culture then were delivered into 15 liters of minimal medium which was 0.5 mx in indoleacrylic acid. The 16-liter culture, in a 20-liter carboy, was sparged vigorously and maintained at 30° in a water bath thermostated at 33°. The incubation was continued for 24 hours after which the mycelia were harvested by filtering the culture through several layers of cheesecloth. The mycelia were washed quickly with distilled water, blotted to remove excess moisture with paper toweling, and quickly frozen. Mycelia were routinely stored at -20° for several weeks prior to lyophilizing. According to Suskind, such storage has an important effect in re-establishing the level of enzymatic activities. Preparation of Extracts—Extracts were prepared and fractionated according to the procedure of Meyer et al. (13) with only minor modifications. Tryptophanases were prepared by the method of Dutcher and DeMoss (19). Rabbit liver dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase were purchased from Calbiochem. Protein standards for molecular weight determinations were purchased from Pharmacia.

Reagents and Substrates—Indoleglycerol phosphate was prepared as described previously (20). Indoleacrylic acid was purchased from the Aldrich Chemical Company. Other reagents and chemicals were obtained from previously mentioned sources (17) or from standard sources of supply.

Assay of Enzymic Activities—The activities of tryptophan synthase were measured as described previously (3, 17). The L-serine deaminating activity was measured by the method of Morino and Snell (21). One unit of enzymic activity is that amount of activity required to catalyze the appearance of 1 nmol of product in 1 min at 37°. Efforts to determine the presence of protease in extracts at various stages of purification were similar to the experiments described by Yu et al. (14).

Gel Electrophoresis—Electrophoresis through native analytical polyacrylamide gels was performed according to the procedure of Davis (22) with modifications as described by Gaertner and DeMoss (23). Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was carried out according to the procedure of Weber et al. (24) except that proteins were routinely treated at 100° for 15 min (14) in the standard procedure. Performic acid oxidation and denaturation by guanidine hydrochloride were carried out precisely as described (24). The gels were stained for protein with Coomassie blue by the method of Fairbanks et al. (25).

Ultracentrifugation—Sedimentation velocities were measured in a Beckman model E analytical ultracentrifuge with electronic speed control by the method of Schachman (26). Molecular weights were estimated from the results of conventional sedimentation equilibrium centrifugation (27) in a Beckman model E analytical ultracentrifuge equipped with electronic speed and temperature controls and a photoelectric scanning system.

Amino Acid Analyses—Protein samples were hydrolyzed and analyzed according to the method of Spackman et al. (28). The apparent partial specific volume of tryptophan synthase was calculated from its amino acid composition by the method of Schachman (26). Methionine and cysteine were determined as methionine sulfone and cysteic acid on protein samples which had been oxidized with performic acid (29). Tryptophan and tyrosine were determined by the method of Goodwin and Morton (30).

RESULTS

Growth of wild type mycelia of Neurospora crassa in medium containing 0.5 mx indoleacrylic acid causes the cells to accumulate indoleglycerol phosphate (17). This accumulation apparently causes a 20-fold increase in the differential rate at which the cells synthesize tryptophan synthase. Crude extracts of this strain grown in the absence of indoleacrylic acid usually exhibit a specific activity of the order of 0.0017 unit of Reaction 2 per mg of protein (1). Crude extracts used in this study exhibited a specific activity of 0.031 unit of Reaction 2 per mg of protein. This derepression of the enzyme coupled with the general applicability of previously described methods of purification (10, 11, 13) has permitted the preparation of considerable quantities of tryptophan synthase which was homogeneous as judged by several criteria to be described below. With an 85-fold purification, we achieved a preparation with a specific activity of 2.05 units of Reaction 2 per mg of protein. The overall recovery of activity was 33%.

Fig. 1 shows the elution profile obtained when a sample of the protein which precipitated during the second ammonium sulfate step (13) was applied to a column (2.5 x 100 cm) packed with Sephadex G-200 and fitted with backflow adaptors. A flow rate of approximately 7 ml per hour was maintained with a peristaltic pump and fractions of ~2.3 ml were collected. Protein emerged from the column in three peaks located at Fractions 21 to 22 (the void volume of the column), at Fractions 34 to 35, and at Fractions 57 to 58. Reaction 1 (closed circles) and Reaction 2 (open squares) of tryptophan synthase eluted as symmetrical peaks, each centered on the third protein peak. The serine-deaminating activity (open triangles), which routinely contaminated the second ammonium sulfate step (13, 17), was cleanly and unambiguously resolved from the tryptophan synthase activities. Inset 1 of the figure shows analytical acrylamide gels prepared from: the material applied to the column (A); material in Fraction 22 (B); material in Fraction 36 (C); and material in Fraction 58 (D).

Gel A shows the presence of five major components in the material which was applied to the column. Of these bands, 1, 2, and 4 emerged from the column with the void volume. Band 8 and a trace of Band 4 emerged with Peak 2. We inferred that Band 4

1 S. R. Suskind, personal communication.
was tryptophan synthase since it was the only one of the five bands which could be identified in the third peak to emerge from the column. Inset 2 shows analytical gels prepared from material obtained from pooling the fractions of highest specific activity.

Fig. 1. Preparative gel filtration of tryptophan synthase on Sephadex G-200. Approximately 87 mg of protein (second ammonium sulfate step (33)) in 3.0 ml were applied to a column (2.5 x 91 cm) of Sephadex G-200. The column was eluted with Solution M of Ref. 13. A flow rate of 7 ml per hour was maintained with a peristaltic pump. Fractions of 2.3 ml were collected. Inset 1 shows the appearance of analytical polyacrylamide gels prepared from: A, 8 µg of the protein applied to the column; B, 14 µg of the protein which emerged from the column with the void volume; C, 9 µg of protein from Fraction 36; D, 5 µg of protein from Fraction 58. Inset 2 shows the appearance of: A, 3 µg; B, 6 µg; C, 12 µg; D, 18 µg of the pooled protein from the fractions of highest specific enzyme activity with respect to Reaction 2 of tryptophan synthase.

Fig. 2 (left). Determination of molecular weight of native tryptophan synthase by the method of gel filtration on Sephadex G-200. \( K_{av} \) was calculated from the expression \( K_{av} = \frac{V_e - V_d}{V_d} \) where \( V_e \) = elution volume for the protein, \( V_d \) = the void volume = the elution volume of blue dextran 2000, \( V_t \) = the total bed volume (34). Samples of standard proteins (20 mg in 2.0 ml of Solution M) were individually applied to the column of Fig. 1. For other experimental details, see legend of Fig. 1 and text. The results are plotted according to the method of Andrews (35). The arrow on the abscissa indicates the average of three separate determinations of \( K_{av} \) for tryptophan synthase.

Fig. 3 (right). Sedimentation pattern of tryptophan synthase. Bar angle was 65°, rotor speed was 56,000 rpm. Temperature was 20°. Photograph was taken at 24 min after the rotor had reached speed. This pattern was obtained from a solution containing 8.0 mg per ml of tryptophan synthase. Other concentrations (in milligrams per ml) examined were 2.0, 3.8, 6.0, and 7.0. Extrapolation to zero protein concentration (36) provided a value for \( s_{20,w} \) of 6.0. The average of these values, 0.282, is indicated by the position of the arrow on the abscissa and corresponds to a molecular weight of 151,000 ± 10,000.

Fig. 4. Sedimentation equilibrium plots of pure tryptophan synthase. In each panel the logarithm of the absorbance at 280 nm of protein is plotted as a function of the square of the radial distance. Panels A and B, rotor speed 8000 rpm, temperature = 22.3°; Panels C and D, rotor speed 10,000 rpm, temperature = 21.0°. Other details are given in Table II and in the text. Rotor speeds and duration of runs were exactly as described in Ref. 36.

Increasing the quantities of protein applied to the gels, from 3 to 18 µg per gel, failed to reveal the presence of other protein bands. We regard these results as evidence of homogeneity of the enzyme preparation even though we were not successful in attempts to recover enzyme activity from gels like those in Inset 2.

In the molecular weight determination presented in Fig. 2, \( K_{av} \) values for three standard proteins are plotted as a function of their molecular weights. Three separate determinations of \( K_{av} \) for tryptophan synthase gave values of 0.268, 0.263, and 0.234. The average of these values, 0.282, is indicated by the position of the arrow on the abscissa and corresponds to a molecular weight of 151,000 ± 10,000.

Fig. 3 shows a schlieren sedimentation pattern which is typical of those obtained in experiments carried out to measure the sedimentation velocity of the material isolated from 74A. Values for \( s_{20,w} \) ranged from 5.4 to 5.9 S, depending upon the solvent and the protein concentration. Extrapolation to zero protein concentration (36) provided a value for \( s_{20,w} \) of 6.0. The pattern shown in Fig. 3 was obtained from a solution (8.0 mg per ml) of material which had been treated with hydroxylamine to remove pyridoxal phosphate. The photograph was made 24 min after the rotor had reached a speed of 56,000 rpm. The symmetry of the pattern provides further indication of the homogeneity of the preparation.

Sedimentation equilibrium studies provided independent confirmation of the molecular weight determinations carried out by gel filtration through columns of Sephadex G-200. Plots of the logarithm of the absorbance at 280 nm against the square of the radial distance (Fig. 4) were prepared from data obtained following the procedure of Schachman (37) and with the exact speed and duration recommended in Ref. 36. The slope of such plots is proportional to the molecular weight of the protein according to the expression

\[
M = \frac{2RT}{(1 - \epsilon P) \omega^2} \frac{2.303 (\text{log } A)_{280}}{d^2}
\]

The partial specific volume of the material was calculated (26).
TABLE I
Amino acid ratio composition of tryptophan synthase and calculation of partial specific volume

Partial specific volume was calculated by the method of Schachman (26). Partial specific volume ($\bar{V}$) = $73.0 / 8.0 = 0.74$.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Molar ratio to aspartic acid</th>
<th>Residues per 100,000 daltons of protein</th>
<th>Residue weight</th>
<th>Per cent by weight of residue ($W_i$)</th>
<th>$\bar{V}$ of amino acid ($\bar{V}_i$)</th>
<th>$\bar{V} W_i$</th>
</tr>
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<tbody>
<tr>
<td>Asp</td>
<td>1.0</td>
<td>114</td>
<td>13,100</td>
<td>8.8</td>
<td>0.60</td>
<td>5.3</td>
</tr>
<tr>
<td>Thr</td>
<td>0.62</td>
<td>70</td>
<td>7,070</td>
<td>4.7</td>
<td>0.70</td>
<td>3.3</td>
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<tr>
<td>Ser</td>
<td>0.57</td>
<td>64</td>
<td>5,570</td>
<td>3.7</td>
<td>0.63</td>
<td>2.3</td>
</tr>
<tr>
<td>Glu</td>
<td>1.3</td>
<td>148</td>
<td>18,100</td>
<td>13.0</td>
<td>0.66</td>
<td>3.4</td>
</tr>
<tr>
<td>Pro</td>
<td>0.76</td>
<td>88</td>
<td>8,540</td>
<td>5.7</td>
<td>0.76</td>
<td>4.3</td>
</tr>
<tr>
<td>Gly</td>
<td>1.1</td>
<td>126</td>
<td>9,500</td>
<td>4.0</td>
<td>0.74</td>
<td>3.4</td>
</tr>
<tr>
<td>Ala</td>
<td>1.2</td>
<td>136</td>
<td>9,510</td>
<td>6.4</td>
<td>0.74</td>
<td>4.7</td>
</tr>
<tr>
<td>Cys</td>
<td>0.14</td>
<td>15</td>
<td>3,260</td>
<td>2.2</td>
<td>0.61</td>
<td>1.3</td>
</tr>
<tr>
<td>Val</td>
<td>0.76</td>
<td>86</td>
<td>8,510</td>
<td>5.7</td>
<td>0.86</td>
<td>4.9</td>
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<tr>
<td>Met</td>
<td>0.31</td>
<td>56</td>
<td>6,430</td>
<td>4.2</td>
<td>0.90</td>
<td>3.8</td>
</tr>
<tr>
<td>Ile</td>
<td>0.50</td>
<td>56</td>
<td>6,330</td>
<td>4.2</td>
<td>0.90</td>
<td>3.8</td>
</tr>
<tr>
<td>Leu</td>
<td>1.0</td>
<td>114</td>
<td>13,100</td>
<td>8.8</td>
<td>0.90</td>
<td>7.9</td>
</tr>
<tr>
<td>Nor</td>
<td>0.19</td>
<td>22</td>
<td>2,260</td>
<td>1.5</td>
<td>0.90</td>
<td>1.4</td>
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<tr>
<td>Tyr</td>
<td>0.30</td>
<td>34</td>
<td>5,540</td>
<td>3.7</td>
<td>0.71</td>
<td>2.5</td>
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<tr>
<td>Phe</td>
<td>0.37</td>
<td>42</td>
<td>6,170</td>
<td>4.1</td>
<td>0.77</td>
<td>3.2</td>
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<tr>
<td>Lys</td>
<td>0.63</td>
<td>72</td>
<td>9,220</td>
<td>6.2</td>
<td>0.82</td>
<td>5.1</td>
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<tr>
<td>His</td>
<td>0.28</td>
<td>32</td>
<td>4,380</td>
<td>2.9</td>
<td>0.67</td>
<td>1.9</td>
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<tr>
<td>NH$_3$</td>
<td>0.59</td>
<td>68</td>
<td>10,600</td>
<td>7.1</td>
<td>0.70</td>
<td>5.0</td>
</tr>
<tr>
<td>Arg</td>
<td>0.11</td>
<td>12</td>
<td>2,230</td>
<td>1.5</td>
<td>0.74</td>
<td>1.1</td>
</tr>
<tr>
<td>Trp</td>
<td>0.11</td>
<td>12</td>
<td>2,230</td>
<td>1.5</td>
<td>0.74</td>
<td>1.1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1,336</td>
<td>146,000</td>
<td>98.0</td>
<td>$\sum \bar{V} W_i$</td>
<td>73.0</td>
</tr>
</tbody>
</table>

* Determined by the method of Goodwin and Morton (30).

TABLE II
Molecular weight of tryptophan synthase as determined by sedimentation equilibrium studies

Molecular weights were calculated from the slopes of the curves depicted in Fig. 4. Partial specific volume was calculated from amino acid composition shown in Table I. For other details see text and Ref. 36. Mean = 145,000; standard deviation = 10,000.

<table>
<thead>
<tr>
<th>Protein concentration (mg/ml)</th>
<th>Rotor speed (rpm)</th>
<th>Molecular weight</th>
<th>Reference panel in Fig. 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.33</td>
<td>8,000</td>
<td>158,400</td>
<td>A</td>
</tr>
<tr>
<td>0.44</td>
<td>8,000</td>
<td>144,600</td>
<td>B</td>
</tr>
<tr>
<td>0.33</td>
<td>10,000</td>
<td>145,000</td>
<td>C</td>
</tr>
<tr>
<td>0.44</td>
<td>10,000</td>
<td>134,000</td>
<td>D</td>
</tr>
</tbody>
</table>

Fig. 5. Subunit molecular weight determination by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Standard curve plotting the logarithm of the molecular weight of several standards as a function of the relative mobility was prepared following the procedure of Weber et al. (24). Standards employed: A, transferrin, 95,000; B, bovine serum albumin, 68,000; C, catalase, 60,000; D, ovalbumin, 45,000; E, ribonuclease, 13,700. Inset 1 shows appearance of typical set of standard gels; 4 µg of protein applied to each gel. Inset 2, Appearance of gels prepared from tryptophan synthase. A and B, 4 and 8 µg of protein from Fraction 55 of the profile shown in Fig. 1. B and C, 4 and 8 µg of protein from Fraction 58 of that profile. Arrow on the abscissa indicates the average relative mobility obtained from 10 determinations of that parameter of tryptophan synthase.

The value is indicated by the arrow on the abscissa of the graph. A total of 10 separate determinations of this value gave an average molecular weight of 76,000 with a standard deviation of 2000. The facts that the gels showed a single band coupled with a molecular weight almost exactly one-half that observed for the native molecule suggested to us that the native molecule was a dimer consisting of monomers of equal size. To assure that the native material was completely denatured, samples either were oxidized with performic acid (24) or were treated with 6 M guanidine hydrochloride (24) before running on sodium dodecyl sulfate gels. Neither treatment caused the appearance of additional bands on gels like those in Inset 2 of Fig. 5.

The question concerning identity or nonidentity of the monomers was approached as follows. Tryptic digestion should yield either about 70 peptides for identical monomers or about 140 peptides for nonidentical monomers. Incomplete separation of the tryptic peptides in our preparations prevented accurate determination of their number. However, Carstairs et al. (20) have reported the occurrence of 48 to 53 tryptic peptides in digests of
Fig. 6. Partial digestion and release of amino acids from tryptophan synthase by carboxypeptidase A. Equivalents of amino acid per equivalent of protein are plotted against time. Procedure followed was that described by Ambler (31).

The C-84 enzyme. To determine the NH$_2$-terminal residue(s), the protein was dansylated* and hydrolyzed by the procedure of Gray (33) and the hydrolysate was analyzed by a modification of the polyamide chromatographic method of Woods and Wang (32). Aspartic acid was the only dansylated amino acid detected which could be readily interpreted as indicating its NH$_2$-terminal location in the polypeptide chain. A third development in Solvent System 2 was required to render the dansylated aspartate patent on the chromatogram. In earlier stages its presence was obscured by the large spot of dansylated tyrosine which is to be expected in the hydrolysate of a protein of molecular weight ~150,000. This could account for the report by Carsiotis et al. (12) of their failure to detect any NH$_2$-terminal amino acid. The results of partial digestions of the enzyme with carboxypeptidases A and B (31) are presented in Fig. 6. The three amino acids, isoleucine, leucine, and phenylalanine, were released rapidly by carboxypeptidase A in that order. Isoleucine was released most rapidly and in amounts consistent with its COOH-terminal position. Carboxypeptidase B failed to reveal the presence of proline, arginine, or lysine at or near the COOH-terminal. These results suggest that both subunits have aspartic acid residues at the NH$_2$-terminus and isoleucine residues at the COOH terminus.

**DISCUSSION**

Other workers (11–13) have reported previously the purification to homogeneity of tryptophan synthase from various mutant strains of *N. crassa*. Precise interpretation of structural studies on these preparations has been compromised by the apparent presence at early stages of extremely active proteases, some of which may co-purify with the enzyme through the final steps in the purification procedure. Recognition of the presence of these contaminating proteases has only recently occurred (14).

We have taken advantage of our previous physiological and enzymological studies (17) on the wild type tryptophan synthase system of strain 74A to obtain homogeneous preparations of the enzyme which are apparently free of the protease contaminants which occur in the preparations of others. Our preparations are quite stable in experiments of the sort (14) which have detected the contaminating activities in other preparations. Our preparations are homogeneous as judged in terms of: (a) polyacrylamide disc gel electrophoresis; (b) sedimentation velocity studies on solutions of high protein concentration; and (c) sedimentation equilibrium studies on solutions of various low concentrations of protein and at various rotor speeds. Failure to detect contaminating protease activities suggests that the native conformation of the enzyme is stable through the final stages of the purification procedure and also during the various analytical procedures we have employed to study its structure.

The preparation we have described here is a protein of molecular weight of 149,000 ± 10,000 with a maximum specific enzyme activity of 2.65 i.u. per mg. The molecule, thus, exhibits a catalytic constant of the order of 396 mol of product per mol of enzyme per min with respect to Reaction 2. This molecular weight is the average of three separate determinations utilizing the technique of gel filtration on columns of Sephadex G-200 and four separate determinations based on sedimentation equilibrium studies.

The preparation we have described appears to consist of two subunits, each of equal size. The average molecular weight obtained from 10 separate determinations was 76,000 with a standard deviation of 2000. It is unlikely that these subunits are themselves composed of smaller subunits since treatment of the material with either guanidine hydrochloride or performic acid failed to reveal the presence of more rapidly migrating material. The observation of aspartic acid at the NH$_2$-terminal position and of isoleucine at the COOH-terminal position is consistent with the assertion that both subunits are identical. In experiments with carboxypeptidases A and B Carsiotis et al. (12) reported the occurrence of leucine and phenylalanine in essentially equimolar quantities and at approximately the same rates of release. On this basis they concluded that the C-84 enzyme possessed two COOH-terminal amino acids and, therefore, two nonidentical subunits. The occurrence of proteases in their preparations could conceivably account for the appearance of more than one COOH-terminal amino acid.

The catalytic properties of the *N. crassa* enzyme are quite similar to those of the holoenzyme of *E. coli*. When the similarity of these properties is considered in the context of the large body of genetic evidence in the *Neurospora* system, it is apparent that there are regions within the *Neurospora* enzyme which are functional analogs of the α subunit and the β subunit of the *Escherichia coli* system. The results reported here provide evidence for the existence of monomers in the *Neurospora* native enzyme which are of the size (~74,000 daltons) which would be expected of a polypeptide which was the evolutionary fusion product of two distinct polypeptides like the α (~29,000 daltons) and β (~45,000 daltons) polypeptides of *E. coli*. We interpret these results as lending support to D. M. Bonner's (5) theory to account for the evolution of complex proteins.

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* The abbreviation used is: dansyl, 5-dimethylaminonaphthalene-1-sulfonyl.
The subunit structure of tryptophan synthase from Neurospora crassa.
W H Matchett and J A DeMoss


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