Tryptophanyl Transfer Ribonucleic Acid Synthetase from Bovine Pancreas

II. THE CHEMICALLY DIFFERENT SUBUNITS

ENRIQUE C. PREDDIE*

From the Institut de Biochimie, Faculté des Sciences de l'Université de Paris, Orsay, France

SUMMARY

Bovine pancreas tryptophanyl transfer RNA synthetase, a crystalline enzyme, is shown by filtration on a carefully calibrated Sephadex G-150 column and by equilibrium centrifugation to have a molecular weight of 110,000 ($s_{20,w} = 5.6$) in the presence of $10^{-4}$ M tryptophan. When the enzyme is treated with 4 M guanidine-hydrochloride for several days or with 8 M urea, values for the $s_{20,w}$ become 1.6 and 2.0, respectively. The enzyme dissociates slowly (to $s_{20,w} = 3.0$) on dialysis against Tris-glycine buffer, pH 8.5, or 0.1 M ammonium bicarbonate, pH 8.8. A very slow dissociation is caused by 0.2 M β-mercaptoethanol which is rapidly speeded up on the addition of 1% sodium dodecyl sulfate. Titration of native enzyme with 5,5'-dithiobis(2-nitrobenzoate) indicates the presence of four free —SH groups; titration in the presence of 8 M urea indicates six —SH groups; titration of sodium borohydride-reduced enzyme indicates 14 —SH groups per molecule of 110,000 molecular weight. The enzyme has been reduced with 0.2 M β-mercaptoethanol in 8 M urea and carboxymethylated with iodoacetic acid. The carboxymethylated protein is eluted as a single peak in a position corresponding to a particle with a molecular weight of 27,000 to 30,000 from a Sephadex G-150 column. Sedimentation equilibrium experiments show a single molecular weight species of 27,000 ($s_{20,w} = 1.9$). However, chromatography on carboxymethyl-Sephadex separates the carboxymethylated protein into two distinct radioactive protein peaks. The carboxymethylated proteins were acetylated, digested with trypsin, and subjected to peptide mapping. A total of seven 14C-cysteine peptides and 22 arginine-containing peptides was identified in both protein species, while the amino acid composition of the total unfractionated carboxymethylated protein shows 14 carboxymethylcysteine residues and 44 arginine residues per molecule of 110,000 molecular weight. The amino acid compositions of the two separated carboxymethylated proteins show significant differences, one carrying 6 half-cysteine residues, the other only 1. Catalytic hydrazinolysis of the individual carboxymethylated proteins gives 1 mole of glutamic acid and 1 mole of glycine, respectively, per 27,000 molecular weight. These experiments lead to the conclusion that a molecule of tryptophanyl-tRNA synthetase is made up of four subunits per molecular weight of 110,000. These subunits consist of two kinds of monomers with different primary structures. Tryptophanyl-tRNA synthetase activity is not restricted to the tetrameric protein but is also found in a smaller molecular species ($s_{20,w} = 3.0$ to 3.3). This molecule is found naturally in preparations of the enzyme and is also formed on dissociation of the tetrameric enzyme. These findings and those reported in the accompanying paper suggest that the catalytic form of the enzyme, the protomer, may be a dimer, and that the tetrameric form has no special functional significance and most likely is an artifact produced by the presence of tryptophan during the purification procedure.

EXPERIMENTAL PROCEDURE

Materials

Tryptophanyl-tRNA synthetase was prepared by a slight modification of the method described by Lemaire, Dorozi, and Labouesse (10). Guanidine-HCl was recrystallized from hot chloroform. Urea solutions were prepared immediately before use. Iodoacetic acid was recrystallized twice from heptane.
7% acetic acid. Stained with Amido black and destained electrophoretically in the same buffer made 0.2 M in β-mercaptoethanol. Protein was analyzed by the method of Davis (11). Gels were run in Tris-glycine buffer, pH 8.5, and in polyacrylamide gel electrophoresis at pH 8.5, as described by Gornall, Bardawill, and David (12).

Preparation of tRNA Synthetase

The enzyme was prepared by the following modifications of the procedure of Lemaire et al. (10). The precipitate obtained at 0.6 saturation with ammonium sulfate was dissolved in 10 to 15 ml of buffer (0.01 M potassium phosphate, pH 7.0, containing 0.1 M KCl, 10-4 M EDTA, 10-4 M L-tryptophan, and 10-4 M β-mercaptoethanol) and dialyzed against 2 liters of the same buffer. The nondiffusible material was subjected to gel filtration on a column (2.5 × 96 cm) of Sephadex G-150 (Pharmacia) equilibrated with the same buffer, at a flow rate of about 30 ml per hour. Ten-milliliter fractions were collected. The fractions showing enzymic activity were pooled and diluted with water to decrease the concentration of the buffer to about 0.05 M. Approximately 5 ml of moist DEAE-Sephadex A-50 (Pharmacia), previously equilibrated with buffer (0.05 M Tris-HCl, pH 7.5, 10-4 M tryptophan, 10-4 M EDTA, 10-4 M β-mercaptoethanol), were added to the pooled fractions. The slurry was stirred for about 30 min at 4°C and freed of solution by gentle suction on a Buchner funnel and the resin was washed with 20 to 20 ml of buffer. The moist resin was allowed to settle by gravity in the fluid on top of a column (2.5 × 30 cm) of DEAE-Sephadex A-50 equilibrated with the same buffer. The resin formed an even layer about 5 mm in height on top of the column, which was eluted with a 500-ml concentrated solution, about 5 M in Tris, is kept at -20°C. The nondiffusible material was frozen in a Dry Ice bath and lyophilized until about 90% of the water had been removed.

For storage the pooled fractions were dialyzed against a solution containing 0.01 M potassium phosphate, pH 7.0, 10-4 M tryptophan, 10-4 M EDTA, and 10-4 M β-mercaptoethanol. The nondiffusible material was frozen in a Dry Ice bath and lyophilized until about 90% of the water had been removed. For crystallization the pooled fractions were concentrated 10-fold at 30°C in a rotary evaporator, without prior dialysis. The concentrated solution, about 5 mM in Tris, is kept at -20°C. The solution does not freeze, and after about 1 week small crystals appear. These are unstable and tend to redissolve when the solution is brought to room temperature. They can be stabilized by the addition of either a few drops of absolute ethanol or of a saturated ammonium sulfate solution. The crystals were collected by centrifugation and stored in 70% saturated ammonium sulfate solution. The yield was 5 to 8% of the total enzyme protein.

Polyacrylamide Gel Electrophoresis

The homogeneity of the enzyme fraction was monitored by polyacrylamide gel electrophoresis at pH 8.6, as described by Davis (11). Gels were run in Tris-glycine buffer, pH 8.5, and in the same buffer made 0.2 mM in β-mercaptoethanol. Protein was stained with Amido black and destained electrophoretically in 7% acetic acid.

1 The abbreviations used are: DTNB, 5,5'-dithiobis(2-nitrobenzoate); SDS, sodium dodecyl sulfate; Cm, carboxymethyl.
protein in guanidine-HCl. Viscosities were measured with an Ubbelohde type viscometer mounted in a water bath at 25° ± 0.02°. The flow time of the viscometer was about 60 sec for water. Densities were obtained directly from the International Critical Tables. The protein was equilibrated by dialysis against the running buffer at 4°, except in some experiments in which urea was used as the denaturing agent. Titrations were made with an automatic titrator (Radiometer, model TTT1C). The solution was dialyzed in propriate amount of unlabeled "carrier" iodoacetic acid in 1.0 ml hours approximately 150 mg of 4 C-iodoacetic acid, plus an appropriate amount of sodium borohydride in 8 M urea and the -SH groups was reduced by sodium borohydride in 8 M urea immediately before titration was begun. The carboxymethylated protein was acetylated as described by Crestfield, Moore, and Stein (18). The carboxymethylated protein was acetylated essentially as described by Crestfield, Moore, and Stein (18). The protein was equilibrated by dialysis from Bovine Pancreas. II

Titration of Sulfhydryl Groups

Free — SH Groups—Tryptophanyl-tRNA synthetase was freed from β-mercaptoethanol by dialysis against several changes of 0.05 M potassium phosphate buffer, pH 7.5, containing 0.01 M EDTA. Titrations were carried out by the addition of molar equivalents of DTNB to a known amount of enzyme in Tris-HCl, pH 7.9, 0.01 M in EDTA. The increase in absorption at 412 μ it was noted that the solution in the dark and at pH 8.5 with an automatic titrator (Radiometer, model TTT1C). The solution was dialyzed for 16 hours against several changes of 0.5 M ammonium bicarbonate, pH 8.0, and lyophilized. The lyophilized material was reduced by sodium borohydride in 8 M urea and the -SH groups was reduced by sodium borohydride in 8 M urea immediately before titration was begun. The carboxymethylated protein was acetylated as described by Crestfield, Moore, and Stein (18). The carboxymethylated protein was acetylated essentially as described by Crestfield, Moore, and Stein (18). The protein was equilibrated by dialysis from Bovine Pancreas. II

Tryptophanyl-tRNA synthetase was carboxymethylated essentially as described by Crestfield, Moore, and Stein (18). The enzyme was dialyzed overnight against 0.1 M Tris-HCl, pH 8.5, containing 8 M urea and 0.01 M EDTA. The dialysed solution was flushed with nitrates for 30 min, made 0.2 M in β-mercaptoethanol, and allowed to stand at room temperature. After 4 hours, the solution was dialyzed against several changes of 0.1 M ammonium bicarbonate, pH 8.5, followed by dialysis against the same buffer adjusted to pH 10 with ammonium hydroxide. The concentration of ammonium bicarbonate was decreased to 0.05 M by the addition of water and the protein was lyophilized. For sedimentation equilibrium measurements, aliquots of the solution of carboxymethylated protein before lyophilization were used.

Acetylation

The carboxymethylated protein was acetylated as described by Oppenheimer, Labouesse, and Hess (19) with acetic anhydride.

Digestion with Trypsin and Peptide Mapping

Trypsin digests of carboxymethylated tryptophanyl-tRNA synthetase and of the acetylated and carboxymethylated enzyme were obtained in 0.1 M ammonium bicarbonate, pH 8.0, at 37°. Trypsin, in two aliquots of 2% (w/w protein) each, was added at the beginning and, after 4 hours of digestion, and digestion was allowed to continue for 16 hours. The digest was acidified with glacial acetic acid and lyophilized. Generally, no insoluble matter was observed after 16 hours digestion.

For peptide mapping, an aliquot of the digest in pyridinium acetate buffer, pH 6.4 (20), was spotted on Whatman No. 3MM paper. Electrophoresis in the same buffer at 2500 volts was for 1 hour and descending chromatography in 1-butanol-acetic acid-water (20) for 16 hours. The dried paper was sprayed with 2% ninhydrin in water-saturated butanol, and the color was developed at 100°.

14C-Labeled peptides were detected by cutting out areas corresponding to ninhydrin-positive spots from a duplicate peptide map. The cut-out areas were suspended in 5 ml of 95% ethanol and 12 ml of scintillation fluid were added. Radioactivity was counted in the Packard liquid scintillation counter. Arginine-containing peptides were detected by spraying a duplicate peptide map with a modified Sakaguchi reagent (21).

Amino Acid Analyses

The amino acid composition of carboxymethyltryptophanyl-tRNA synthetase was obtained with lyophilized or crystallized protein. Protein was dissolved (usually 1 mg in 5 ml) in 6 N HCl to which 1 crystal of phenol was added. The solution was sealed under vacuum in a Pyrex glass tube and hydrolysis was carried out at 110° for 24 to 72 hours. Hydrolysates were analyzed on a Technicon amino acid analyzer, essentially according to the method of Spackman, Stein, and Moore (22), or by the accelerated 6-hour procedure. Half-cystine was determined as carboxymethylcysteine. The tryptophan content was estimated by the method of Beaven and Holiday (23).

Analyses of Terminal Amino Acids

Carboxyl-terminal amino acids were determined by catalytic hydrazinolysis according to Bradbury (24). Each purified carboxymethyl-protein was analyzed separately. Carboxyl-terminal amino acids were identified on the Technicon amino acid analyzer following acid hydrolysis. Once the identity of the carboxyl-terminal residue was known, a correction factor, determined from the amount of destruction of an authentic sample of this amino acid, was used to obtain quantitative results.

Amino-terminal analyses were done by the phenylisothiocyanate method of Edman (25). The amino-terminal amino acid was identified on the Technicon amino acid analyzer after hydrolysis of the phenyl isothiocyanate derivative according to Frankel-Conrat, Harris, and Levy (26).

Electron Microscopy

Electron micrographs of tryptophanyl-tRNA synthetase were taken with a Siemens Elmiskop 1A electron microscope at 80 kv. Protein samples were prepared and negatively stained in 1 to 2% phosphotungstic acid in 0.1 M potassium phosphate buffer, pH 7.2, and in 0.5% uranyl acetate in ethanol buffered at pH 4.5, according to the methods of Brenner and Horne (27). Ilford N40 photographic plates were used; they were developed in Kodak D76 at 20°. Magnification calibration was performed with the aid of a carbon grating (Ladd Research Industries, Inc., Burlington, Vermont).
TABLE I

Purification of tryptophanyl-tRNA synthetase

The modified purification procedure is described in the text. The ammonium sulfate precipitate used was from about 5 kg of bovine pancreas. Specific activity is expressed as micromoles of hydroxamate formed per mg of protein per hour.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein (mg)</th>
<th>Total activity (units)</th>
<th>Specific activity (units/mg protein)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium sulfate</td>
<td>1,725</td>
<td>34,500</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>precipitate</td>
<td>470</td>
<td>33,870</td>
<td>72</td>
<td>97</td>
</tr>
<tr>
<td>DEAE-Sephadex</td>
<td>250</td>
<td>33,060</td>
<td>130</td>
<td>96</td>
</tr>
</tbody>
</table>

Preparation of Antibodies

The immunization procedure for tryptophanyl-tRNA synthetase was as follows. Each of two rabbits received three subcutaneous injections (each rabbit received 2 mg of enzyme1 per each injection) in 0.09% NaCl mixed with an equal volume of Freund's complete adjuvant (Difeo), followed by two intramuscular injections without adjuvant. All of the injections were made at 15-day intervals. The rabbits were bled 1 week after the final injection.

RESULTS

Purification of Tryptophanyl-tRNA Synthetase—The preparation and purification of bovine pancreas tryptophanyl-tRNA synthetase have been described in detail by Lemaire et al. (10). This method has been simplified so that starting from the ammonium sulfate precipitate the procedure involves only two steps. The results of the modified portion of the procedure are summarized in Table I. The final product was homogeneous as judged by polyacrylamide gel electrophoresis (Fig. 8) and by equilibrium centrifugation (Fig. 2). The final yield of active enzyme is a little less than 90% of the total activity in the ammonium sulfate precipitate.

Crystallization—Davie, Koningsberger, and Lipmann (28) reported the presence of crystals at a late stage during the preparation of bovine pancreas tryptophanyl-tRNA synthetase, but were unable to crystallize this enzyme in bulk. A typical crystal of tryptophanyl-tRNA synthetase is shown in Fig. 3. The method used to crystallize this protein is unusual, but it has not been possible to get comparative results with conventional methods, such as by salting out with ammonium sulfate. The crystals are enzymically active (160 units per mg of protein) and their chromatographic characteristics (in the presence of tryptophan) and amino acid composition (Table II) are similar to those of the native nontreastalline enzyme. They are very small and, so far, all attempts to grow them further have been unsuccessful.

Molecular Weight of Tryptophanyl-tRNA Synthetase—A standard molecular weight curve was calculated from the elution pattern obtained by the gel filtration of a mixture of markers (rabbit muscle aldolase, blue dextran, bovine serum albumin, chymotrypsinogen, and cytochrome c) by plotting the elution volume against the logarithm of the molecular weight of the markers.

The pattern obtained when tryptophanyl-tRNA synthetase was chromatographed with a mixture of these same markers is illustrated in Fig. 4A. Enzymic activity emerges a little after aldolase and before bovine serum albumin. A value of 110,000

![Figure 2](http://www.jbc.org/)

**Fig. 2.** Plot of log c against v² for equilibrium centrifugation of purified tryptophan-tRNA synthetase. The concentration, c, is in arbitrary units. v is the distance from the axis of rotation in centimeters. The data were obtained after 20 hours at 30,000 rpm. The concentration of protein was 1.5 mg per ml. The plots were used to calculate an Mₑ type average molecular weight.

![Figure 3](http://www.jbc.org/)

**Fig. 3.** Crystal of tryptophanyl-tRNA synthetase. Typical crystal (magnified 22,000 times) from concentrated Tris buffer, pH 7.5, stained with 1% phosphotungstic acid in phosphate buffer, pH 7.2 to 7.4. The photograph was taken with a Siemens Elmiskop 1A electron microscope. The negative was printed positively. Details of the crystallization procedure are given in the text.
The results for the titration of tryptophanyl-tRNA synthetase
reduced and carboxymethylated enzyme were taken to represent
residues of carboxymethylcysteine obtained from analyses of the
half-cystine content shows the most variation; however, the
ated tryptophanyl-tRNA synthetase is summarized in Table II.
(A) Determination of molecular weight by gel filtration on
was determined for the molecular weight of tryptophanyl-tRNA
Tryptophanyl-tRNA synthetase shows a single protein
Equilibrium sedimentation at 30,000 rpm (16 hours, 3-mm col-
ollected. Aliquots of fractions (10 gA) were dried on planchets, and
equilibrated with 0.1 M potassium phosphate, pH 7.5, containing
tryptophanogen, cytochrome c, and tryptophanyl-tRNA synthetase
-mersaptoethanol, 10^-4 M tryptophan, and 10^-4 M EDTA.
was filtered through a column of Sephadex G-150 (2.5 X 90 cm)
from 30-300 mg of protein was used for each analysis.
Sephadex G-150.
Tryptophanyl-tRNA synthetase was substituted for tryp-
B, the same mixture as in A except that reduced 14C-carboxymeth-
with DTNB are summarized in Table III. Results with par-
entially purified tryptophanyl-tRNA synthetase had shown that
molecule of 110,000 molecular weight
table of DTNB. After the end point was reached, 2 additional
eq of DTNB were added and the reaction was observed for 30
min more. A total of four titratable —SH groups was found
These results differ from those reported by DeLuca and McElroy (29); how-
other, their studies were done with an enzyme preparation which
zymes prepared by this method. However, a total of six —SH
groups reacted with DTNB when the titration was carried out in
in the presence of 8 M urea. The additional two titratable groups
must be masked or buried in the native enzyme. These results
may well not have been homogeneous, having been merely puri-
for gel filtration on Sephadex G-100.
Amino acid composition showed that tryptophanyl-tRNA
TABLE II
Amino acid composition of purified noncrystalline, native, of
crystalline, and of reduced and carboxymethylated
tryptophanyl-tRNA synthetase

Approximately 0.2 mg of protein was used for each analysis.
Unless otherwise indicated, the values listed are the average
values obtained from three 30-hour hydrolysates. The methods
of hydrolysis and determinations are described under "Experimental Procedures."

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Native enzyme</th>
<th>Crystalline enzyme</th>
<th>Reduced and carboxymethylated enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Residues per molecule of 110,000 molecular weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>62.2</td>
<td>60.3</td>
<td>62.0</td>
</tr>
<tr>
<td>Histidine</td>
<td>21.1</td>
<td>21.0</td>
<td>20.5</td>
</tr>
<tr>
<td>Arginine</td>
<td>44.0</td>
<td>45.4</td>
<td>44.5</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>97.1</td>
<td>90.4</td>
<td>97.0</td>
</tr>
<tr>
<td>Threonine</td>
<td>44.5</td>
<td>44.2</td>
<td>44.0</td>
</tr>
<tr>
<td>Serine</td>
<td>60.3</td>
<td>60.1</td>
<td>59.4</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>102.1</td>
<td>103.0</td>
<td>101.4</td>
</tr>
<tr>
<td>Proline</td>
<td>58.3</td>
<td>57.4</td>
<td>58.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>85.2</td>
<td>85.1</td>
<td>85.3</td>
</tr>
<tr>
<td>Alanine</td>
<td>78.0</td>
<td>78.2</td>
<td>78.2</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>13.2</td>
<td>12.0</td>
<td>14.1</td>
</tr>
<tr>
<td>Valine</td>
<td>48.1</td>
<td>47.5</td>
<td>48.0</td>
</tr>
<tr>
<td>Methionine</td>
<td>17.5</td>
<td>18.2</td>
<td>18.4</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>50.1</td>
<td>50.0</td>
<td>50.1</td>
</tr>
<tr>
<td>Leucine</td>
<td>70.2</td>
<td>70.5</td>
<td>70.2</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>33.3</td>
<td>33.1</td>
<td>33.1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>50.1</td>
<td>50.3</td>
<td>49.5</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>12.0</td>
<td>12.2</td>
<td>12.0</td>
</tr>
</tbody>
</table>

 States of titration.
* Extrapolated to zero time hydrolysis from 24-, 48-, and
72-hour hydrolysates.
* Average of spectrophotometric values from two analyses.
* This value for the carboxymethyllysine content was taken
to be a correct representative of the half-cystine content.

was seen in the linearity of this plot is shown in Fig. 2. The slight deviation from linearity of the
readings at the bottom of the cell is more likely caused by poly-
dispersity than by an impurity in the preparation.
Amino Acid Composition—The amino acid composition of
noncrystalline native, crystalline, and reduced-carboxymethyl-
ated tryptophanyl-tRNA synthetase is summarized in Table II.
The half-cystine content shows the most variation; however, the
residues of carboxymethyllysine obtained from analyses of the
reduced and carboxymethylated enzyme were taken to represent
the correct half-cystine content per 110,000 g of tryptophanyl-
tRNA synthetase.
Sulfhydryl Group Content of Tryptophanyl-tRNA Synthetase—
The results for the titration of tryptophanyl-tRNA synthetase
15 30 45 60
Counts/10^2 c.p.m.

A
Elution volume (ml)

B

Units/ml 10^-12

Fig. 4. Determination of molecular weight by gel filtration on
Sephadex G-150. A, a sample containing 1 drop of blue dextran
solution and 5 mg each of aldolase I, bovine serum albumin, chy-
motrypsinogen, cytochrome c, and tryptophanyl-tRNA synthetase
was filtered through a column of Sephadex G-150 (2.5 X 90 cm)
equilibrated with 0.1 M potassium phosphate, pH 7.5, containing
10^-3 M d-mercaptoethanol, 10^-4 M tryptophan, and 10^-4 M EDTA.
Fractions of 5 ml were collected and the protein-containing frac-
tions (0.1 ml) were assayed for tryptophanyl-tRNA synthetase
activity ( , protein concentration; --- , enzyme activity). B, the same mixture as in A except that reduced 14C-carboxymeth-
ylated tryptophanyl-tRNA synthetase was substituted for trypt-
ophanyl-tRNA synthetase. The column dimensions were 1.5 X
60 cm, and filtration was effected with 0.05 M ammonium bicarbo-
nate, pH 8.8, containing 0.1 M KCl. Fractions (3 ml) were collected.
Aliquots of fractions (10 ml) were dried on planchets, and
14C radioactivity was counted on a Nuclear-Chicago model CHS
low background automatic sample changer ( , protein concen-
tration; --- , 14C radioactivity). See text for additional de-
tails.
Fig. 5. Sedimentation patterns of tryptophanyl-tRNA synthetase under dissociating conditions. All Schlieren photographs were taken at a bar angle of 65° at about 40 min after the centrifuge had attained a speed of 67,770 rpm. a, tryptophanyl-tRNA synthetase in 0.05 M Tris HCl, pH 7.3, 10^{-2} M β-mercaptoethanol, 10^{-4} M L-tryptophan, 10^{-4} M EDTA. The protein concentration was about 6 mg per ml and was dialyzed against the buffer for 2 hours before the run. b, reduced and carboxymethylated tryptophanyl-tRNA synthetase. The protein (12 mg per ml) was dialyzed for several hours against 0.1 M ammonium bicarbonate, pH 9.5 to 10.0, and centrifuged in a synthetic boundary cell. c, tryptophanyl-tRNA synthetase (8 mg per ml) dialyzed for 7 days at 0° against 4 M guanidine hydrochloride in Tris-glycine, pH 8.5. d, tryptophanyl-tRNA synthetase (8 mg per ml) dialyzed for 2 hours against 8 M urea in Tris-glycine, pH 8.5. e, tryptophanyl-tRNA synthetase (3 mg per ml) dialyzed for 15 hours at 0° against Tris-glycine, pH 8.5, containing 0.2 M β-mercaptoethanol and 8 M urea. f, tryptophanyl-tRNA synthetase (8 mg per ml) dialyzed for 48 hours against 0.1 M ammonium bicarbonate, pH 8.8. g, tryptophanyl-tRNA synthetase (6 mg per ml) in 0.1 M Tris-glycine, pH 8.5, 0.4 M β-mercaptoethanol. h, solution from cell in Experiment g diluted with 1 volume of water to yield final concentrations of 0.05 M Tris, 0.2 M β-mercaptoethanol, and 3 mg per ml of enzyme. i, 0.5% SDS added to solution from cell in Experiment h. j, tryptophanyl-tRNA synthetase. The lower channel contained tryptophanyl-tRNA synthetase (3 mg per ml) in 0.05 M Tris HCl, pH 7.5, containing 10^{-4} M tryptophan, 10^{-4} M β-mercaptoethanol, and 10^{-4} M EDTA. The upper channel contained tryptophanyl-tRNA synthetase (3.5 mg per ml) in 0.05 M ammonium bicarbonate, pH 7.8. Urea to a final concentration of 8 M was added to each cell immediately before the run was started.

Synthetase contained 14 half-cystine residues measured as carboxymethylcysteine. Since only six —SH groups were found in the urea-denatured enzyme by DTNB titration, the other eight —SH groups are probably involved in the formation of four disulfide linkages in the native enzyme. To show this, the native enzyme was reduced in 8 M urea at 38° for 30 min with sodium borohydride and then titrated with DTNB. A total of 14 —SH groups were titrated per molecule of enzyme of molecular weight 110,000. This value did not change when the reduction with sodium borohydride was allowed to continue for 4 hours. These results confirm the half-cystine content determined by amino acid analysis.

Carboxymethylation—The carboxymethylation of tryptophanyl-tRNA synthetase was complete only if the enzyme was treated with urea for several hours prior to reduction with 0.2 M β-mercaptoethanol. When the enzyme, which had been reduced and carboxymethylated with 85C-iodoacetic acid, was subjected to gel filtration on a calibrated Sephadex G-150 column, the elution pattern shown in Fig. 4B was obtained. The radioactivity emerged after serum albumin, but a little before and al-

### Table III

**Titration of tryptophanyl-tRNA synthetase with 5,5-dithiobis-(2-nitrobenzoate)**

The titration was performed on 10 mmoles of enzyme at room temperature, in the presence of 1 mM EDTA. The details of the titration procedure are given in the text.

<table>
<thead>
<tr>
<th>Reaction with DTNB</th>
<th>Experimental conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titration to end point</td>
<td>0.1 M Tris-HCl, pH 7.9</td>
</tr>
<tr>
<td>Titration to end point</td>
<td>0.1 M Tris-HCl, pH 7.8, 8 M urea</td>
</tr>
<tr>
<td>Reduced with NaBH₄ in 8 M urea</td>
<td>30 min at room temperature in the dark</td>
</tr>
<tr>
<td>As in experiment with NaBH₄, except that no NaBH₄ was added</td>
<td>30 min at room temperature in the dark</td>
</tr>
</tbody>
</table>

---

| —SH groups per molecule of molecular weight 110,000 |
|----------------|----------------|----------------|
| 4             | 6              | 14             |
| 6              | 14             | 6              |

Downloaded from http://www.jbc.org by guest on August 28, 2017
most overlapping chymotrypsinogen. A value of 27,500 was obtained for the molecular weight of the carboxymethylated protein. No other radioactive protein peak was detected.

Carboxymethylated tryptophanyl-tRNA synthetase showed a single protein boundary ($s_{20,w} = 1.9$) by velocity sedimentation (Fig. 56). With equilibrium runs the attainment of equilibrium took about 20 hours at 30,000 rpm at a protein concentration 2.0 mg per ml. From a plot of $\ln c$ against $r^2$ (Fig. 6) an average molecular weight of 27,000 was estimated. Unlike the native enzyme, the plot shows no deviation whatsoever from linearity. This result indicates that tryptophanyl-tRNA synthetase, which in the native state has a molecular weight of 110,000, on reduction with $\beta$-mercaptoethanol, was broken into four fragments of equal molecular weight. However, a peptide map prepared from a trypsin digest of the reduced and carboxymethylated protein (Fig. 7a) showed about 52 ninhydrin-positive spots, approximately 50% of the total expected from the lysine and arginine content of the protein, as calculated for a molecular weight of 110,000 (Table II). These findings can be interpreted tentatively to indicate that tryptophanyl-tRNA synthetase is made up of at least 2 identical half-molecules and that each such subunit may be made up of two polypeptide chains (molecular weight approximately 27,000) joined together by disulfide bridges. To investigate this possibility the dissociation of the enzyme was examined.

**Dissociation of Tryptophanyl-tRNA Synthetase**—The $s_{20,w}$

---

**Fig. 6.** Plot of $\ln c$ against $r^2$ for equilibrium centrifugation of (a) reduced and carboxymethylated tryptophanyl-tRNA synthetase and (b) 4 M guanidine treated enzyme. The concentration, $c$, is in arbitrary units; $r$ is the distance from the axis of rotation in centimeters. The data were obtained after 30 hours at 30,000 rpm. The protein concentration was 2 mg per ml in a and 2.2 mg per ml in b. The plots were used to calculate $M_w$ type average molecular weights.

**Fig. 7.** Peptide maps of trypsin digests of (a) reduced and carboxymethylated tryptophanyl-tRNA synthetase, (b) acetyl Cm-protein-1, and (c) acetyl-Cm-protein-2. Approximately 2 to 2.5 mg of each digest were electrophoresed in one direction, followed by chromatography in the second. The hand-drawn maps corresponding to b and c are included to show the position of the $^{14}C$-containing peptides on the map. Further details are described in the text.
values for the dissociation products are listed in Table IV. Treatment of tryptophanyl-tRNA synthetase with 4 M guanidine hydrochloride in Tris-glycine buffer, pH 8.5, resulted in a single protein boundary (s_{20,w} = 1.6) on velocity centrifugation (Fig. 5c). An M_0 type plot (Fig. 6, Line b) from sedimentation equilibrium experiments at 30,000 rpm gave an average molecular weight of 27,600. When the experiment was done with 8 M urea in place of guanidine hydrochloride the result was a single protein boundary (s_{20,w} = 2.0) (Fig. 5d), and there was no change in the value of the sedimentation coefficient (s_{20,w} = 2.0) when the experiment was repeated in 8 M urea made 0.2 M in β-mercaptoethanol (Fig. 5e). In contrast to these results, when the enzyme is treated with 0.1 M ammonium bicarbonate, pH 8.8, a single protein boundary (s_{20,w} = 3.0) was obtained (Fig. 5f).

The treatment of tryptophanyl-tRNA synthetase with 0.2 M β-mercaptoethanol in Tris-glycine, pH 8.5, for 2 days resulted in the polyacrylamide gel electrophoresis pattern shown in Fig. 8. The dissociation of the enzyme by 0.4 M β-mercaptoethanol was observed over a period of time in the ultracentrifuge (Fig. 5, g and h). Approximately 45 min after the centrifuge had attained maximum velocity two protein boundaries were observed. The faster moving boundary contained approximately 40% of the sedimentation material (s_{20,w} = 3.3). The slower sedimenting material, comprising 60% of the total material, had an s_{20,w} = 1.8. When the concentration of buffer was decreased 2-fold by dilution with water in the equilibrium cell, the faster sedimenting boundary contained approximately 60% of the s_{20,w} = 3.3 material and 33% of the material was in the slower sedimenting boundary (s_{20,w} = 1.8). When 1% SDS was added to the equilibrium cell, only one protein boundary (s_{20,w} = 1.8), which contained 100% of the sedimenting material, was observed (Fig. 5i). The data from the dissociation experiments lead to the following conclusions: (a) there are no interchain disulfide bridges between the polypeptide chains in tryptophanyl-tRNA synthetase; (b) each polypeptide chain has a molecular weight equal to 27,000, and represents the monomeric unit, while the native enzyme is a tetramer; (c) the reduction in molecular weight observed following treatment of the native enzyme with 0.2 M β-mercaptoethanol in 8 M urea is mainly a result of dissociation by urea and not of reduction; (d) the disulfide bridges must be intra-chain in the monomeric units; and (e) some of the monomeric polypeptide chains must have a different primary structure.

Isolation of 14C-Carbomethyl Subunits—To show that the monomers of tryptophanyl-tRNA synthetase did indeed consist of chemically different polypeptide chains, attempts were made to isolate them in pure form. The separation of the 14C-carbamethylated subunits is illustrated in Fig. 9. The radioactive protein peaks were collected and acetylated to protect the ε-amino groups of lysyl residues from attack by trypsin. The acetylated proteins were freed of salt by dialysis. During dialysis one protein fraction precipitated and was collected by centrifugation. This fraction is referred to as acetyl-Cm-protein-1 and represents the chromatographic peak eluted first from the column depicted in Fig. 9. The other fraction, acetyl-Cm-protein-2, was lyophilized.

Amino Acid Composition and Peptide Mapping of Subunits—
The amino acid compositions of acetylated and carbamethylated protein-1 (acetyl-Cm-protein-1) and protein-2 (acetyl-Cm-protein-2) are given in Table V. The most striking difference was observed in the carboxymethyllysine content of both proteins. Protein-1 contains 6 carboxymethyllysine residues per molecule of molecular weight 27,000, while protein-2 contains only one carboxymethyllysine per molecule of the same molecular weight.

The differences observed in the amino acid analyses of the carbamethylated proteins were confirmed in peptide maps made from tryptic digests of acetyl-Cm-protein-1 and acetyl-Cm-protein-2 (Fig. 7, b and c). A total of 15 ninhydrin-stained spots, of which six were positive for radioactivity and 12 for

### Table IV

<table>
<thead>
<tr>
<th>Dissociation conditions</th>
<th>s_{20,w} value of protein boundary or boundaries</th>
<th>M_0 from equilibrium centrifugation</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5.6</td>
<td>110,000</td>
</tr>
<tr>
<td>Ammonium bicarbonate, pH 8.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 M guanidine hydrochloride, Tris-glycine, pH 8.5</td>
<td>3.0</td>
<td>27,800</td>
</tr>
<tr>
<td>8 M urea, Tris-glycine, pH 8.5</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>Reduced with β-mercaptoethanol and carbamylated</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>0.2 M β-mercaptoethanol, 0.1 M</td>
<td>1.9</td>
<td>27,000</td>
</tr>
<tr>
<td>Tris-glycine, pH 8.5, 45 min</td>
<td>1.8; 3.3</td>
<td></td>
</tr>
<tr>
<td>0.2 M β-mercaptoethanol, 0.05 M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tris-glycine, 1% SDS</td>
<td>1.8</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 8. Polyacrylamide gel electrophoresis pattern of tryptophanyl-tRNA synthetase. About 5.0 mg of enzyme were dialyzed for 48 hours at 0⁰ against Tris-glycine buffer, pH 8.5, containing 0.2 M β-mercaptoethanol. a, a sample of 50 μg in 10 μl was electrophoresed in the same buffer. See text for additional details. b, illustration of what is seen in a. c, gel electrophoretic pattern of enzyme eluted with 0.5 M Tris-HCl (Fig. 1) from a column of DEAE-Sephadex A-50. d, illustration of what is seen in c. e, gel electrophoretic pattern of enzyme eluted with 1.5 M Tris-HCl (Fig. 1, inset) from a column of DEAE-Sephadex A-50. f, illustration of what is seen in e.
the objective lens in order to enhance the contour contrast of the graphs were taken at an estimated 2 to 5 μm underfocus setting of 0.05 m ammonium acetate, pH 7.8 to 8.0. The column was eluted under a concentration gradient (0.05 to 0.25 M) of the same buffer; each vessel contained 20 ml of buffer. Friction (2.5 ml) were collected and protein concentration (solid curve), was measured on 1 ml of each fraction by the absorbance at 280 μm; aliquots (10 μl) of each fraction were dried on planchets and counted for $^{14}C$ radioactivity (dashed curve) on a Nuclear-Chicago model C115 low background automatic sample changer. The straight dashed line represents the salt concentration of the eluate.

![Diagram](https://via.placeholder.com/150)

**Fig. 9.** Chromatography of carboxymethylated tryptophanyl-tRNA synthetase. About 17 mg of the non(diffusible material in 0.5 ml dialysis buffer (ammonium bicarbonate adjusted to pH 10 with ammonium hydroxide) were adsorbed on a column (1.5 × 10 cm) of carboxymethyl-Sepharose previously equilibrated with 0.05 m ammonium acetate, pH 7.8 to 8.0. The column was eluted under a concentration gradient (0.05 to 0.25 M) of the same buffer; each vessel contained 20 ml of buffer. Fractions (2.5 ml) were collected and protein concentration (solid curve), was measured on 1 ml of each fraction by the absorbance at 280 μm; aliquots (10 μl) of each fraction were dried on planchets and counted for $^{14}C$ radioactivity (dashed curve) on a Nuclear-Chicago model C115 low background automatic sample changer. The straight dashed line represents the salt concentration of the eluate.

Arginine, were found on the peptide map of acetyl-Cm-protein-1. Only one radioactive spot was observed on the peptide map of acetyl-protein-Cm-2, while a total of 10 major ninhydrin-stained spots, nine of which were positive for arginine, was detected. It was concluded from these results and from the—SH group titrations of the entire enzyme, described above, that one subunit (Cm-protein-1) must contain two pairs of intrachain disulfide bridges and two free—SH groups, and the other subunit (Cm-protein-2) contains only one free—SH group, thus accounting for—SH group and disulfide bond contents of the native tetrameric structure (see above).

**Carboxyl-terminal and Amino-terminal Amino Acids of Subunits of Tryptophanyl-tRNA Synthetase—Hydrazinolysis of acetyl Cm-protein-1 in the presence of hydrazine sulfate as catalyst leads to the release of 1 eq of glutamic acid (after the experimental value was corrected for a recovery of 45%), together with small quantities of leucine, per 27,000 g. When Cm-protein-2 was similarly treated, 1 eq of glycine (after correction for a recovery of 60%) per 27,000 g was released. These findings indicate that the carboxyl-terminal amino acid for the monomeric units of tryptophanyl-tRNA synthetase are glutamic acid and glycine, respectively, and confirm that there is a single polypeptide chain in each subunit.

All attempts to obtain quantitative amino-terminal residue determinations with either of the nonacylated carboxymethylated subunits have so far been unsuccessful.

**Electron Microscopy—**Plates a to d (Fig. 10) show the electron micrographs of tryptophanyl-tRNA synthetase. These micrographs were taken at an estimated 2 to 5 μm underfocus setting of the objective lens in order to enhance the contour contrast of the molecules in the strain layer. A subunit structure for the enzyme is readily visible at low magnification (Plate a). The majority of the images probably represent different orientations of the same molecules or of dissociated subunits. At higher magnifications (Plate c), most of the molecules are visible as single molecules, many of which appear to have a tetrameric form with over-all dimensions 75 to 90 A × 60 to 80 A. Some of these tetrameric molecules appear to have a "hole" about 20 A in the diameter in the center. When the enzyme was treated with 4-methyltryptophan before staining, the majority of images (Plate b) appear uniform, a subunit structure is not readily visible, and the over-all dimensions of the images are 35 to 40 A × 30 to 50 A. These images probably represent a dimeric form of the tryptophanyl-tRNA synthetase molecule. Similarly, with enzyme dialyzed against 0.1 M ammonium bicarbonate before staining with uranyl acetate, uniform images, with no readily visible subunit structure and over-all dimensions 40 to 50 A × 40 to 50 A, are observed.

**Immunodiffusion—**The results of double diffusion tests in agar when antiserum was tested against tryptophanyl-tRNA synthetase are shown in Fig. 11. The native enzyme, specific activity 120 units per mg, forms a precipitin line in the presence of tryptophan + ATP, but not when tryptophan alone is present. The native enzyme treated with 0.5 or 1.0% SDS forms a precipitin line in the presence or absence of β-mercaptoethanol; however, enzyme which was reduced with β-mercaptoethanol in the

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Residues per molecule of 27,000 molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetyl-Cm-protein-1</td>
</tr>
<tr>
<td>Lysine</td>
<td>18.1</td>
</tr>
<tr>
<td>Histidine</td>
<td>6.7</td>
</tr>
<tr>
<td>Arginine</td>
<td>12.1</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>24.3</td>
</tr>
<tr>
<td>Threonine</td>
<td>5.0</td>
</tr>
<tr>
<td>Serine</td>
<td>13.6</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>27.1</td>
</tr>
<tr>
<td>Proline</td>
<td>19.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>13.2</td>
</tr>
<tr>
<td>Alanine</td>
<td>12.1</td>
</tr>
<tr>
<td>Valine</td>
<td>6.3</td>
</tr>
<tr>
<td>Leucine</td>
<td>11.0</td>
</tr>
<tr>
<td>Methionine</td>
<td>4.7</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>12.2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>15.2</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* Extrapolated to zero from 24- and 48-hour hydrolysates.
* Value from single 72-hour hydrolysates.
* Average of two spectrophotometric determinations.
* Not determined.

The amino acid compositions of purified acetylated and carboxymethylated proteins, acetyl-Cm-protein-1 and acetyl-Cm-protein-2

Approximately 0.2 mg of protein was used in each analysis. Unless otherwise indicated, the values listed are the average values obtained from three 24-hour hydrolysates. The method of hydrolysis and analysis is described in the text.
FIG. 10. Areas cut out of electron micrographs of tryptophanyl-tRNA synthetase. a, native tryptophanyl-tRNA synthetase in 0.1 M Tris-HCl containing 10^{-2} M β-mercaptoethanol, 10^{-4} M tryptophan, and 10^{-4} M EDTA, negatively stained with phosphotungstic acid. The projections are magnified 100,000 times. b, native tryptophanyl-tRNA synthetase—same conditions as a with the addition of 2 \times 10^{-2} M 4-methyl tryptophan. The projections are magnified 400,000 times. c, projections from a magnified 800,000 times. Several apparently tetrameric molecules can be seen. d, tryptophanyl-tRNA synthetase in 0.1 M ammonium bicarbonate, pH 8.8, negatively stained with uranyl acetate. The projections are magnified 450,000 times.

The absence of SDS does not form a precipitin line. These results seem to suggest that tryptophan and tryptophanyl-AMP are not bound to tryptophanyl-tRNA synthetase in the same manner. The SDS-dissociated enzyme forms a precipitin line with the antiserum because the antigenic site is probably on the monomeric subunit. No precipitin line is formed with enzyme which was reduced with β-mercaptoethanol in the presence of tryptophan, probably since reduction of the disulfide bridges does not lead to dissociation of the tetrameric enzyme or to a release of bound tryptophan.

FIG. 11. Immunodiffusion of tryptophanyl-tRNA synthetase. Well 1, 0.624 mg of enzyme and 10^{-4} M tryptophan in 0.1 M Tris-HCl, pH 7.3. SDS (0.5%) was added immediately before the solution was added to the well. Well 2, the same as Well 1 except that 0.2 M β-mercaptoethanol replaced the SDS. Well 3, the same as Well 1 plus 0.2 M β-mercaptoethanol. Well 4, the same as Well 1 except that tryptophan was excluded. Well 5, same as Well 1 but concentration of β-mercaptoethanol was 0.4 M. Well 6, same as Well 3 except that the concentration of SDS was 1%. Well 7, same as Well 1 except that SDS was excluded. Well 8, same as Well 1 except SDS was excluded and ATP was added. Well 9, same as Well 1 except tryptophan was removed by prolonged dialysis.

DISCUSSION

The titration of sulfhydryl groups before and after reduction with sodium borohydride, the amino acid compositions of the native enzyme and of the purified carboxymethylated subunits, the peptide maps of tryptic digests of the acetylated and carboxymethylated proteins, and the results from sedimentation velocity and sedimentation equilibrium experiments on the native, carboxymethylated, and dissociated enzyme all indicate that bovine pancreas tryptophanyl-tRNA synthetase (mol wt 110,000) is a tetramer in the native form; the tetramer contains monomers of molecular weight approximately 27,000 and the monomers include two kinds of polypeptide chains with different primary structures.

In the context of the allosteric theory of Monod, Wyman, and Changeux (30) this enzyme may be considered a “dimer” in which each “protomer” is made up of a pair of structurally different polypeptide chains. Some observations appear to support this “dimeric protomer” concept. Thus, (a) in the absence of added tryptophan the crystalline enzyme is eluted from a Seph dex G-150 column almost overlapping bovine serum albumin, corresponding to a particle of molecular weight 55,000 to 65,000; (b) Lemaire has shown that in some enzyme preparations, depending on the method used for purification, a second molecular

It is assumed that the aminocyl adenylate is formed when tryptophan and AMP are added to tryptophanyl-tRNA synthetase in solution.

G. Lemaire, personal communication.
species of tryptophanyl-tRNA synthetase equally active as the main enzyme fraction was present. The apparent molecular weight of this species, from filtration on a Sephadex G-100 column, was 70,000 to 80,000. This smaller molecular species has been purified by the modified method (see Fig. 1), and sedimentation velocity experiments show a single boundary (s20, w = 3.2), which corresponds to a molecule about half of the size of the native enzyme. Peptide maps of this fraction prepared from tryptic digest following reduction with β-mercaptoethanol and carboxymethylation show no qualitative differences as compared to similar maps made with the native enzyme; (c) enzyme dissociated in 0.1 M ammonium bicarbonate, pH 8.8, retained full activity although the s20, w value was reduced from 5.6 to 3.0; (d) the molecular weight of tryptophanyl-tRNA synthetase isolated from E. coli has an apparent molecular weight of 67,000, as estimated by filtration on a Sephadex G-200 column; and (e) when native enzyme (s20, w = 5.6) is mixed with transfer RNA in an ultracentrifuge cell, only one boundary (s20, w = 3.6) is observed in sedimentation velocity runs, indicating that it is a dimeric molecule which binds the tryptophan-specific transfer RNA. Therefore, it appears likely that the tetrameric form of tryptophanyl-tRNA synthetase isolated from E. coli is not a prerequisite for enzyme activity. The dimeric form, presumably made up of one of each variety monomeric unit, can do as well.

The observations that a lack of tryptophan in the buffers used for the preparation of tryptophanyl-tRNA synthetase led to a much higher proportion of the lower molecular weight enzyme, that the stability of the enzyme in solution is greatly enhanced by the presence of tryptophan, and that the native enzyme dialyzed against ammonium bicarbonate and lyophilized (s20, w = 3.0 material) is very stable suggest that added tryptophan might lead to the formation of tetrameric molecules by encouraging the aggregation of two protomers. This “dimerization” might change the conformation of the enzyme to the extent that important surface regions on the protomer are protected from non-specific interactions in solution. It follows then that, for enzyme activity to take place, tryptophan must either be easily displaced from its binding site on the enzyme by substrate (the aminocyl adenylate) which is more hydrophobic, or must bind at a different site from the substrate on the native enzyme. There is evidence which appears to support both possibilities. Thus, it has been found that in the presence of tryptophan the native enzyme is dissociated only very slowly by 8 M urea (Fig. 5g) while dissociation by 0.5% sodium dodecyl sulfate is rapid and complete. Moreover, treatment of the native enzyme in the presence of tryptophan with an excess of 4-methyltryptophan, a more hydrophobic substance than tryptophan, changes the sedimentation properties of the enzyme from (s20, w = 5.6) to (s20, w = 3.3) (9) and also changes the over-all dimensions of images seen in electron micrographs to about half their size. Finally, rabbit antisera prepared against the native enzyme freed of tryptophan does not react with the native enzyme in the presence of tryptophan, while both of the enzymes in the presence of the amino acyl-adenylate and the smaller molecules obtained on dissociation by sodium dodecyl sulfate and ammonium bicarbonate reacted with the antisera.

Because of the bifunctional nature of this enzyme, it is tempting to speculate that a different monomer may be responsible for each of the two enzymic activities. It has been possible, so far, to isolate and study chemically only the denatured derivatives of these monomers which are not biologically active. All attempts to purify the monomers in their native forms have been unsuccessful. Nevertheless, it should be pointed out that the subunits of methionyl-tRNA synthetase from E. coli appear to be equal and similar by amino-terminal analysis and by the behavior of the oxidized enzyme on polyacrylamide gel electrophoresis (8). Although this is certainly not definitive evidence, it does serve to put a brake on such speculations and, at least for the present, encourages the conservative view that the two different types of monomers of tryptophanyl-tRNA synthetase from bovine pancreas might be unique to this tRNA synthetase and not directly related to its bifunctional nature.

**REFERENCES**

Tryptophanyl Transfer Ribonucleic Acid Synthetase from Bovine Pancreas: II. THE CHEMICALLY DIFFERENT SUBUNITS
Enrique C. Preddie

J. Biol. Chem. 1969, 244:3958-3968.

Access the most updated version of this article at http://www.jbc.org/content/244/14/3958

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/244/14/3958.full.html#ref-list-1