Human Placental Tryptophanyl Transfer Ribonucleic Acid Synthetase. Purification and Subunit Structure†

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ABSTRACT: Tryptophanyl-tRNA synthetase was purified to homogeneity from human placenta with standard techniques. A neutral detergent, polyoxyethylene cetyl ether (Brij 58), stabilized the enzyme throughout the purification. The enzyme appeared to be homogeneous by standard disc gel polyacrylamide electrophoresis, but a minor contaminant was defined with multiple gels of differing porosity. Inclusion of Brij 58 in polyacrylamide gels allowed us to recover 100% of the enzyme activity and to demonstrate that the activity was associated with the major protein band. The mol wt of the enzyme is 100,000 by sucrose density gradient centrifugation, 125,000 by gel filtration chromatography, and 118,000 by electrophoretic mobility on polyacrylamide gels of differing porosity. Sodium dodecyl sulfate–polyacrylamide electrophoresis demonstrated a single band of mol wt 58,000. Polyacrylamide electrophoresis in the presence of 8 M urea revealed a single major band. Therefore, the enzyme appears to be a dimer composed of subunits of identical mass and charge. We have modified the technique of isoelectric focusing in two ways with the result that up to 100% of our enzyme activity is recoverable after focusing. The isoelectric point of human tryptophanyl-tRNA synthetase is 5.2.

Most of the aminoacyl-tRNA synthetases from *Escherichia coli* have been purified and studied (Muench, 1971). Several mammalian synthetases have also been highly purified and characterized (Loftfield, 1972). Although Tchou *et al.* (1971) partially purified the tryptophanyl-tRNA synthetase from human leukemic lymphocytes, none of the human aminoacyl-tRNA synthetases heretofore have been purified to homogeneity. A rich and plentiful human source of enzyme has been lacking.

We have found that human placenta is a rich source of tryptophanyl-tRNA synthetase; the yield from this source is sufficient to allow chemical studies on the purified enzyme. In addition, human tryptophanyl-tRNA synthetase charges brewers' yeast tRNA, an easily obtained substrate in contrast to human tRNA.

In this paper we present the purification and initial characterization of human placental tryptophanyl-tRNA synthetase.

Experimental Procedure

Materials. Brewers' yeast tRNA (24–42 pmol of $tRNA^{Trp}$/A_{260} unit) was purchased from Boehringer Mannheim Corporation or from Schwarz Bio-Research. One A_{260} unit of tRNA has an A_{260} of 1.0 in a 1.0-cm optical path when dissolved in 1.0 ml of 5 mM KH_2PO_4–5 mM K_2HPO_4 (pH 6.9). [^{32}P]H_3PO_4 and L-[3-^{14}C]tryptophan were from New England Nuclear Corporation. L-[3-^{14}C]Tryptophan was purified as previously described (Muench and Safille, 1968) with spectrophotometric determination of its concentration (Greenstein and Winitz, 1961). [^{32}P]PP_i was prepared as by Berg (1958). Bicine[1] was obtained from Calbiochem. Brij 58[1] was from ICI America, Inc. Poly(ethylene glycol) 6000 was from Dow Chemical Co. Hydroxylapatite was prepared as the "CPA" material of Main *et al.* (1959). DE52 cellulose and GF/C glass fiber filters were from Reeve Angel. Amberlite CG-50 (100–400 mesh)–poly(methacrylic acid) was obtained from Mallinckrodt Chemical Works and prepared as described by Hirs *et al.* (1953). Sephadex G-150 was from Pharmacia Fine Chemicals. Sucrose (RNase free) was from Schwarz Bio-Research. Sodium dodecyl sulfate was purchased from Mallinckrodt and recrystallized from boiling ethanol. The materials for disc gel electrophoresis were purchased from Canalco. ATP, AMP, glutathione, L-tryptophan, and crystalline bovine serum albumin were from Sigma. Ampholyte solutions were obtained from LKB. Ammonium sulfate, urea (Ultra Pure), beef pancreas chymotrypsinogen A, horse heart cytochrome *c*, and apoferritin were from Mann Research Laboratories. Trypsin, rabbit muscle aldolase, and soybean trypsin inhibitor were from Worthington. Homogeneous human placental alkaline phosphatase was a gift of Dr. D. Harkness (Harkness, 1968). Rabbit muscle phosphorylase was a gift from Dr. A. Yunis. *E. coli* isoleucyl-tRNA synthetase was in a preparation of aminoacyl-tRNA synthetases (Muench, 1971). *E. coli* tryptophanyl-tRNA synthetase was a pure preparation obtained with the procedure of Joseph and Muench (1971a). *E. coli* valyl-tRNA synthetase was a nearly homogeneous preparation obtained by DE52 cellulose and hydroxylapatite batch steps followed by hydroxylapatite chromatography (Muench, 1971). All other materials used were the highest grades available commercially.

Methods. The activity of tryptophanyl-tRNA synthetase was assayed by measuring either the incorporation of L-[3-^{14}C]tryptophan into tRNA as previously described (Joseph and Muench, 1971a) or by measuring L-tryptophan-dependent ATP–[^{32}P]PP_i exchange. For the tRNA charging reaction, the 0.5-ml reaction mixture was 100 mM potassium bicine buffer

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‡ Leukemia Society of America Scholar.

[1] Abbreviations used are: Brij 58, polyoxyethylene cetyl ether with 5 ppm of citric acid and 10 ppm of butylated hydroxyanisole; bicine, *N,N*-bis(2-hydroxyethyl)glycine.

(pH 8.0), 20 mM $MgCl_2$, 2 mM ATP, 20 A_{260} units of brewers' yeast tRNA, and 0.1 mM L-[3-^{14}C]tryptophan. Addition of 0.003–0.025 ml of enzyme (0.06–0.50 unit) previously diluted in 20 mM 2-mercaptoethanol, 10% glycerol, and 0.01 M potassium phosphate buffer (pH 6.9) initiated the reaction which was continued for 10 min at 30°. Under these conditions, the amount of Trp-tRNA formed is proportional to time and to the amount of enzyme added. One unit of enzyme forms 1 nmol of Trp-tRNA in 10 min under the stated conditions. The concentration of $tRNA^{Trp}$ was measured by the extent of Trp-tRNA formation under the same conditions but with limiting amounts of $tRNA^{Trp}$ and excess enzyme.

For the exchange reaction, the 1.0-ml reaction mixture was 100 mM potassium bicine buffer (pH 8.0), 2 mM ATP, 5 mM $MgCl_2$, 2 mM [^{32}P]PP_i, and 0–7.5 mM L-tryptophan. Addition of 1–5 units of enzyme as defined above initiated the reaction, which was continued for 10 min at 30°. The reaction was stopped with the addition of 0.5 ml of 250 mM $NaPP_i$ containing 10% perchloric acid; 30 mg of acid-washed Norit was added, and the Norit with adsorbed [^{32}P]ATP was collected on a GF/C disc, washed five times with 5 ml of H_2O, dried under an infrared lamp, and counted in a toluene-based scintillation medium.

Polyacrylamide disc gel electrophoresis of 15-, 25-, and 40-μg aliquots of the Amberlite III fraction was done in a conventional manner as described under Figure 2. Brij 58 (0.1%) was not included in the gels; the electrophoresis was done at room temperature at 3 mA/gel.

Polyacrylamide disc gel electrophoresis of the Amberlite III fraction was also performed with 15 μg of enzyme in the system described by Hedrick and Smith (1968) in 5.0, 6.0, 7.5, 9.0, 10.0, and 11% polyacrylamide gels, running at pH 9.5. Tris-glycine buffer (pH 8.5) was at the anode and the cathode. The ratio of acrylamide to bisacrylamide was 140:1 in all gels. At room temperature, the gels, 6.0 × 0.5 cm, were subjected to 2 mA/gel in a Canalco Model 12 apparatus until the dye front had passed through the stacking gel, when the current was increased to 4 mA/gel. Electrophoresis was discontinued when the dye front had migrated to within 1 cm of the end of the gel. The gels were removed from the columns, cut horizontally through the dye front, and stained with 0.25% Coomassie Blue.

The molecular weight of human tryptophanyl-tRNA synthetase was determined by polyacrylamide disc gel electrophoresis. Soybean trypsin inhibitor, bovine serum albumin, *E. coli* tryptophanyl-tRNA synthetase, valyl-tRNA synthetase, human placental tryptophanyl-tRNA syhthetase, and rabbit muscle aldolase were subjected to disc gel electrophoresis as described above. Measurements and calculations were performed as described by Hedrick and Smith (1968).

A Sephadex G-150 column, 2.5 × 80 cm, was prepared and equilibrated with 100 mM potassium phosphate buffer (pH 6.9), 20 mM 2-mercaptoethanol, and 10% glycerol at 2° by upward flow at 4 ml/hr. The column was equilibrated with 2 mg of Blue Dextran 2000 (mol wt 2,000,000), 4 mg of rabbit muscle aldolase (mol wt 158,000), 4 mg of chymotrypsinogen A (mol wt 25,700), 3 mg of horse heart cytochrome *c* (mol wt 12,400), 5 mg of apoferritin (mol wt 480,000), 4 μg of *E. coli* tryptophanyl-tRNA synthetase (mol wt 74,000), and 150 nmol of D,L-[^{3}H]valine. In a typical experiment, 200 units of human placental tryptophanyl-tRNA synthetase and one or more of the standards were dissolved in 2 ml of the buffer and applied to the column at 4 ml/hr. The column was developed at 4 ml/hr. Fractions (2 ml) were assayed for tryptophanyl-tRNA synthetase as described above. *E. coli* tryptophanyl-tRNA synthetase was assayed as previously described (Joseph and Muench, 1971a). Chymotrypsinogen A, apoferritin, and rabbit muscle aldolase were measured by absorbance at 280 nm, the blue dextran by absorbance at 620 nm, and the horse heart cytochrome *c* by absorbance at 410 nm. D,L-[^{3}H]Valine was determined on aliquots placed on glass fiber filters and counted in scintillation fluid. The molecular weights of the standard proteins were plotted on a logarithmic scale against their respective elution volumes (milliliters) at the peak concentrations (Andrews, 1964).

TABLE I: Purification of Tryptophanyl-tRNA Synthetase.

Fraction	Protein (mg)	10^{-2} × Units	Sp Act. (U/mg)	Recovery (%)	Purification
Extract	21,000	3000	14	100	1.0
Ammonium sulfate	1,400	1800	130	60	9.3
DE52	95	1100	1,200	37	86
Hydroxylapatite	19	1100	5,700	37	420
Amberlite CG-50 I	8.4	860	10,000	29	710
Amberlite CG-50 II	3.0	630	21,000	21	1500
Amberlite CG-50 III	1.3	310	24,000	10	1700

Sucrose density gradient centrifugation was performed as described by Martin and Ames (1961) with *E. coli* isoleucyl-tRNA synthetase and human placental alkaline phosphatase as the standards. Sodium dodecyl sulfate gel electrophoresis was performed according to the method of Weber and Osborn (1969) on a 12-μg sample of the Amberlite III fraction with rabbit muscle phosphorylase (mol wt 94,000), bovine serum albumin (mol wt 68,000), rabbit muscle aldolase (mol wt 40,000), and trypsin (mol wt 24,000) as standards. Alkaline phosphatase was assayed according to Garen and Levinthal (1960). Protein was measured by the method of Lowry *et al.* (1951) with bovine serum albumin as the standard.

Results

Purification. All operations were done at 2° unless otherwise noted. All phosphate buffers were pH 6.9, composed of equimolar amounts of KH_2PO_4 and K_2HPO_4. The purification is summarized in Table I.

Cotyledons from a fresh human placenta obtained at normal full-term delivery were mixed with 10 mM phosphate buffer containing 20 mM 2-mercaptoethanol and 10% glycerol (1:2, w/v) and homogenized for 5 sec in a Polytron homogenizer (Kinematica, Lucern, Switzerland) at intermediate speeds. The homogenates were centrifuged for 1 hr at 15,000*g*.

Crystalline ammonium sulfate (39 g) was added for every 100 ml of supernatant (60% saturation), and after stirring for 2 hr the suspension was centrifuged for 30 min at 15,000*g*. The supernatant was discarded, and the pellet was successively suspended in and centrifuged from 50% (2.37 M), 45% (2.10 M), 40% (1.84 M), and 35% (1.59 M) saturated ammonium sulfate solutions containing 50 mM phosphate buffer, 20 mM 2-mercaptoethanol, and 10% glycerol. The supernatants in 45, 40, and 35% saturated ammonium sulfate were pooled and made to 60% saturation by the addition of crystalline ammonium sulfate. After 30 min the suspension was centrifuged and the pellet was dissolved in 20 mM 2-mercaptoethanol, 10% glycerol, and 0.1% Brij 58 (solution A) containing 10 mM potassium phosphate buffer (pH 6.9), and dialyzed against this solution for 24 hr (ammonium sulfate fraction).

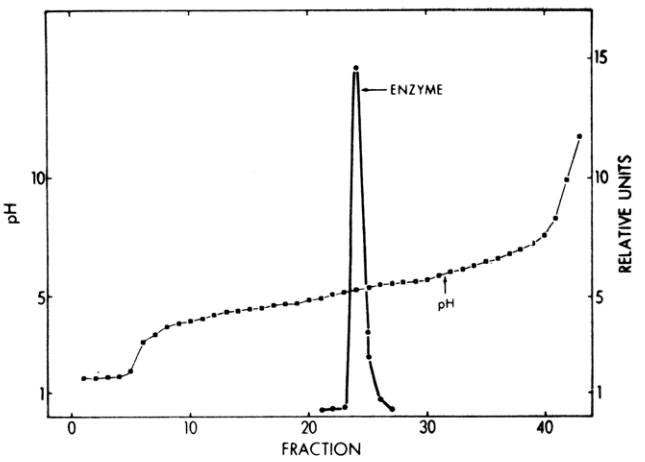

FIGURE 1: Isoelectric focusing. In a stepwise gradient from 0 to 70% glycerol containing 0.1% Brij 58, a pH gradient was established with 1% ampholyte, pH 3–6, at 0° by operation of the LKB Model 8101 apparatus at 300–1100 V for 36 hr. The higher glycerol concentrations were at the higher pH. Then 100 units (5 μg) of Amberlite II fraction in 0.25 ml of 10 mM potassium phosphate buffer (pH 6.9)–20 mM 2-mercaptoethanol–50% glycerol were placed at the 50% glycerol level (pH ~5.5) and focused for 10 hr at 0°, 700 V. The pH (■) was measured directly on 2-ml fractions at 0°. The activity (●) of tryptophanyl-tRNA synthetase was measured as described under Methods. Recovery was 90% of input activity.

The ammonium sulfate fraction was applied to a column of DEAE-cellulose (40 cm × 2.5 cm) equilibrated with 10 mM phosphate buffer in solution A. The column was washed with 1500 ml of equilibration buffer, and development was effected with a linear gradient from 10 to 250 mM phosphate buffer in 4000 ml of solution A. Fractions (40 ml) were collected, and those containing significant enzyme activity were pooled and dialyzed against 10 mM phosphate buffer–10% poly(ethylene glycol) 6000 in solution A (DE52 fraction).

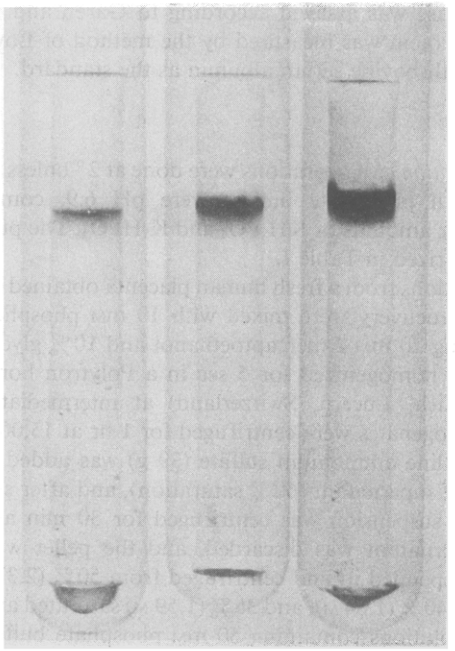

FIGURE 2: Polyacrylamide disc gel electrophoresis of the Amberlite II fraction. The enzyme, 10 (left), 25 (center), and 40 (right) μg, was subjected to disc gel electrophoresis in the system described by the Canalco Brochure (April, 1965) with the standard 7.0% polyacrylamide gel, running at pH 9.5. Tris-glycine buffer (pH 8.5) was at the anode and the cathode. The gel, 6.3 × 0.5 cm, was subjected to 3 mA/gel in a Canalco Model 12 apparatus until the dye front had reached the bottom of the gel. The gel was stained with 0.25% Coomassie Blue.

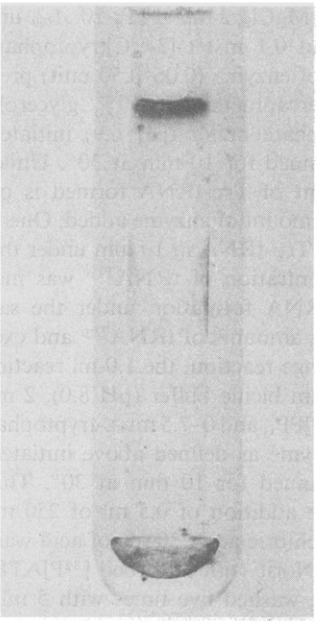

FIGURE 3: Disc gel electrophoresis in 8 M urea. The polyacrylamide gels were prepared and developed as described under Figure 2, except all gel solutions contained 8 M urea and one-half running gel buffer concentration, and the polyacrylamide gels were run for 2 hr before application of the enzyme. The Amberlite III fraction (15 μg) was treated for 2 hr at 37° in the presence of 8 M urea, 1% 2-mercaptoethanol, and 10 mM potassium phosphate buffer (pH 6.9) in a total volume of 0.100 ml. The solution was made 20% in glycerol, and the entire sample was applied to the top of the stacking gel and developed at 2.5 mA/tube for 1 hr. The gel was removed, stained, and destained as before.

The DE52 fraction was applied to a column of hydroxylapatite (1.5 cm × 30 cm) equilibrated with 50 mM phosphate buffer in solution A. The enzyme was eluted with a linear gradient from 50 to 300 mM phosphate buffer in 1200 ml of solution A. Fractions (20 ml) were collected, and those containing significant enzyme activity were pooled and dialyzed as before (hydroxylapatite fraction).

The hydroxylapatite fraction was applied to a 15 cm × 0.9 cm column of Amberlite CG-50 (200–400 mesh) equilibrated with 10 mM phosphate buffer in solution A. The enzyme was eluted with a linear gradient from 10 to 300 mM phosphate buffer in 260 ml of solution A. Fractions (4 ml) were collected, and those containing significant enzyme activity were pooled and dialyzed against 10 mM phosphate buffer–10% poly(ethylene glycol) 6000 in solution A (Amberlite I fraction).

The Amberlite I fraction was applied to a 10 cm × 0.9 cm column of Amberlite CG-50, equilibrated as described above. The enzyme was eluted, collected, and dialyzed as described above (Amberlite II fraction).

The Amberlite II fraction was applied to an 8 cm × 0.9 cm column of Amberlite CG-50 equilibrated as described above. The enzyme was eluted, collected, and concentrated as described above (Amberlite III fraction).

Isoelectric Focusing. Isoelectric focusing was done in a manner previously reported (Joseph and Muench, 1971a). Several isoelectric focusing experiments were done with the introduction of the enzyme sample prior to establishment of a pH gradient. Determinations of the isoelectric point of 5.2 were precise, but recoveries of active enzyme were poor, never

TABLE II: Quaternary Structures of Some Aminoacyl-tRNA Synthetases.

Amino Acid	Source	Mol Wt	Reference
		Single Polypeptide Chains	
Gln	*E. coli*	69,000	Folk, 1971
Ile	*E. coli*	112,000	Arndt and Berg, 1970; Berthelot and Yaniv, 1970
Leu	*E. coli*	105,000	Rouget and Chapeville, 1971; Hayashi *et al.*, 1970
Tyr	Yeast	40,000	Beikirch *et al.*, 1972
Val	*E. coli*	110,000	Yaniv and Gros, 1969
Val	Yeast	112,000	Lagerkvist and Waldenström, 1967
		Identical Subunits[a]	
His	*Salmonella typhimurium*	100,000 (dimer)	De Lorenzo and Ames, 1970
Leu	Yeast	120,000 (dimer)	Chirikjian *et al.*, 1973
Lys	*E. coli*	114,000 (dimer)	Rymo *et al.*, 1972
Lys	Yeast	120,000 (dimer)	Rymo *et al.*, 1972
Met	*E. coli*	190,000 (dimer)	Bruton and Hartley, 1968
Met	*E. coli*	190,000 (tetramer)	Lemoine *et al.*, 1968
Phe	*E. coli*	181,000 (tetramer)	Kosakowski and Bock, 1970
Ser	*E. coli*	100,000 (dimer)	Katze and Konigsberg, 1970
Ser	Yeast	120,000 (dimer)	Heider *et al.*, 1971
Tyr	*E. coli*	95,000 (dimer)	Calendar and Berg, 1966; Muench *et al.*, 1970
Trp	*E. coli*	74,000 (dimer)	Joseph and Muench, 1971b
Trp	*Bacillus stearothermophilus*	70,000 (dimer)	B. S. Hartley (personal communication)
Trp	Beef pancreas	108,000 (dimer)	Gros *et al.*, 1972
Trp	Human placenta	116,000 (dimer)	This work
Trp	Water buffalo brain	150,000 (trimer)	Liu *et al.*, 1973
		Differing Subunits	
Glu	*E. coli*	102,000 ($\alpha\beta$)	Lapointe and Söll, 1972
Gly	*E. coli*	230,000 ($\alpha_2\beta_2$)	Ostrem and Berg, 1970
Phe	Yeast	237,000 ($\alpha_2\beta_2$)	Fasiolo *et al.*, 1970; Schmidt *et al.*, 1971

[a] Presumed identical if of same molecular weight and with no contrary evidence.

exceeding 27%. Moreover, a large precipitation of protein always occurred in the electrophoresis chamber at the pH zone of the tryptophanyl-tRNA synthetase isoelectric point. These experiments required approximately 68 hr for completion.

In order to increase enzyme recovery, two modifications were made in the isoelectric focusing technique: (1) addition of Brij 58 to the glycerol density gradient (Friesen *et al.*, 1971) and (2) formation of the pH gradient prior to addition of the enzyme sample. A 0–70% glycerol density step gradient was formed in 0.1% Brij 58 and 1% LKB pH 3–6 ampholyte. High voltages were then applied to form rapidly the pH gradient in the electrophoresis chamber; when the change in current approached zero, the pH gradient was considered to be formed, the higher pH values being in the higher glycerol concentrations. The voltage was discontinued, and a length of Tygon tubing (i.d. $^1/_{16}$ in.) connected to a Sigma motor pump was inserted into the electrophoresis chamber to the level of 50% glycerol concentration and at a pH between 5 and 6. One milliliter of the gradient was removed, mixed with an aliquot of enzyme solution, and reinserted into the electrophoresis chamber at the original level. The tubing was removed, and the electrophoresis was resumed at lower voltages for 10 hr.

As shown in Figure 1, an isoelectric point of 5.2 was determined for tryptophanyl-tRNA synthetase. This value was identical with that for the tryptophanyl-tRNA synthetase of human leukemic lymphocytes (Tchou *et al.*, 1971) but differed from the value of 6.2 obtained for the *E. coli* tryptophanyl-tRNA synthetase by Joseph and Muench (1971a). With a preformed gradient and Brij 58 up to 100% recovery of enzyme activity was achieved and no precipitation occurred, even though up to 4 mg of enzyme was electrophoresed at one time. At 0°, Brij 58 partially precipitates in the electrophoresis cell, but this precipitation does not interfere with the pH gradient, the determination of the isoelectric point, or the recovery of active enzyme.

Polyacrylamide Disc Gel Electrophoresis. By disc gel electrophoresis, the Amberlite III fraction exhibited one major band. The band corresponded with the major band in the Amberlite I and II and hydroxylapatite fractions. No enzyme activity was recovered from 1-mm gel slices crushed into 10 mM phosphate buffer containing 20 mM 2-mercaptoethanol and 10% glycerol after electrophoresis of the Amberlite II fraction at 2° with 10% glycerol and 20 mM 2-mercaptoethanol in the gels. However, addition of 0.1% Brij 58 (final concentration, w/v) to the gels allowed full recovery of activity from the gel, and the activity migrated with the major band (Figure 2). Electrophoresis of the Amberlite III fraction in gels containing either 5, 6, 7.5, 8, 10, or 11% acrylamide with the technique described by Hedrick and Smith (1968) defined a minor contaminant visible at the leading edge of the enzyme band in the 10 and 11% gels. The increased sieving properties of the 10 and 11% gels reduce diffusion and allow visualization of the contaminating band.

Molecular Weight. Sucrose density gradient centrifugation with human placental alkaline phosphatase and *E. coli* isoleucyl-tRNA synthetase as standards revealed an apparent mol wt of 100,000 for the human placental tryptophanyl-

TABLE III: Comparison of *E. coli*, Bovine, and Human Tryptophanyl-tRNA Synthetases.

	E. coli[a]	Bovine[b]	Human[c]
Mol wt	74,000	108,000	116,000
Subunit mol wt	37,000	54,000	58,000
L-Trp binding sites	2	2	
ATP binding sites		2	
tRNA binding sites	2	1	
Trp-ATP binding sites	2		
Synthesizes Trp-ATP ester	Yes	Yes	Yes
Essential thiol for act.	Yes	Yes	Yes
Thiols per subunit	1	7	4
Free N-terminal AA	No	No	No
C-terminal sequence		-Leu-Phe-Gln	
K_m for L-Trp	1×10^{-5}	2.5×10^{-6}	2.8×10^{-6}
K_m for ATP	9×10^{-5}	1.5×10^{-4}	1.6×10^{-4}
K_m for $tRNA^{Trp}$ of *coli*	5×10^{-7}	*d*	*d*
K_m for $tRNA^{Trp}$ of yeast	*d*	3.8×10^{-7}	1.1×10^{-7}
K_m for $tRNA^{Trp}$ of beef		1.5×10^{-7}	
Turnover no.	1200	240	200
Isoelectric point	6.2		5.2

[a] Data in this column are from Joseph and Muench (1971a,b) and Penneys *et al.* (1973) (N terminal). [b] Data in this column are from Gros *et al.* (1972) and Dorizzi (1972) (kinetics) and from Weiss *et al.* (1959) (Trp-ATP ester). [c] Data in this column are from the present work, Penneys and Muench (1974), and Penneys *et al.* (1973). [d] Not a substrate.

tRNA synethestase. By the polyacrylamide disc gel electrophoretic technique of Hedrick and Smith (1968) the mol wt is 118,000. The mol wt is 125,000 by gel filtration on Sephadex G-150.

Subunit Structure. An aliquot of the Amberlite III fraction was subjected to sodium dodecyl sulfate–gel electrophoresis according to the method of Weber and Osborn (1969). One major band of mol wt 58,000 was seen on the gel along with two trace contaminants. Electrophoresis of a sample taken from the Amberlite III fraction in a polyacrylamide gel containing 8 M urea demonstrated one homogeneous band (Figure 3). Therefore, tryptophanyl-tRNA synthetase is a dimer composed of subunits with identical charge at pH 9.5 and of mol wt 58,000. The human enzyme is similar to the tryptophanyl-tRNA synthetases purified from *E. coli* (Joseph and Muench, 1971b) and from bovine pancreas (Gros *et al.*, 1972) in that all three are dimers with identical subunits.

Discussion

The quaternary structures of the aminoacyl-tRNA synthetases are diverse and can be categorized as shown in Table II. Some are single polypeptide chains, and these ordinarily have molecular weights near 100,000, but range as low as 40,000 for the tyrosyl-tRNA synthetase of yeast (Beikirch *et al.*, 1972). Others consist of probably identical subunits, the subunit molecular weights ranging from 35,000 to 95,000. The third variety consist of subunits of differing molecular weights. In cases so far examined those enzymes consisting of a single polypeptide chain or of identical subunits have one binding site for each substrate on each polypeptide chain. So far no rule relates amino acid specificity to quaternary structure. Thus, the valine enzymes of *E. coli* and yeast are both single polypeptide chains, and the lysine enzyme pair and serine enzyme pair of *E. coli* and yeast consist of identical subunits, but the leucine, tyrosine, and phenylalanine enzyme pairs from these species all differ in quaternary structure.

The tryptophanyl-tRNA synthetase of beef pancreas was the first to be purified (Davie *et al.*, 1956) to homogeneity and is now well characterized with respect to quaternary structure (Gros *et al.*, 1972). With the present work information on the tryptophan enzymes spans the evolutionary range from *E. coli* to man. Each of the tryptophan enzymes consists of identical subunits, as shown in Table II. A more extensive comparison of the tryptophan enzymes from *E. coli*, beef pancreas, and human placenta is given in Table III. All are inactivated by sulfhydryl reagents. All synthesize tryptophanyl ATP ester. In no case has a free N-terminal amino acid been detected, but the single C-terminal sequence of the beef pancreas enzyme has confirmed the identity of the subunits. The apparent Michaelis constants are generally typical of aminoacyl-tRNA synthetases, although the constants for tryptophan are particularly low in the cases of the mammalian enzymes. Whereas *E. coli* tRNA is not a substrate for either mammalian enzyme, yeast tRNA is a substrate for both mammalian enzymes and is equivalent to beef tRNA for the beef pancreas enzyme. Yeast tRNA is not a substrate for the *E. coli* enzyme.

We have previously purified 850-fold the tryptophanyl-tRNA synthetase from human skin (Penneys and Muench, 1971). By the criteria of chromatographic characteristics on all three media, molecular weight, isoelectric point, K_m values, and species specificity for tRNA, the enzyme from human skin is identical with the tryptophanyl-tRNA synthetase of human placenta.

The *E. coli* and human tryptophanyl-tRNA synthetases have similar but not identical catalytic mechanisms as determined by steady-state kinetic studies supported in part by dead-end inhibitor experiments (Penneys and Muench, 1974). Although both enzymes have a bi-uni–uni-bi Ping-Pong mechanism (Cleland, 1963), the placental enzyme has random release of products whereas the *E. coli* enzyme has ordered release, AMP first. The bovine pancreas enzyme has not been studied in this way.

Further studies are in progress with the *E. coli* and placental enzymes to define the structural similarities and dissimilarities and to further extend the comparisons with the bovine pancreas enzyme.

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