Thyroid Ribonucleic Acid-Iodopeptides. Comparison of Tyrosyl-Complex II and Tyrosyl-tRNA†

Paul A. Fisher,‡ Judd G. Goodman,§ and Fredrick J. Kull*

ABSTRACT: It has previously been shown that mammalian RNA-peptidyl complexes are found in close association with tRNA, but can be separated from the bulk of the tRNA by benzoylated diethylaminoethylcellulose chromatography (Kull, F. J., and Soodak, M. (1971), *Biochim. Biophys. Acta 246,* 1; Gadski, R. A., and Kull, F. J. (1973), *Biochemistry 12,* 1907). These studies also showed that under aminoacylation conditions the complex fractions were able to act as acceptors for certain amino acids and that the formation of porcine thyroid tyrosyl-complex II was particularly high. Because of this high acceptor function, and because of the importance of tyrosine to thyroid metabolism, further studies were conducted comparing some of the properties of porcine thyroid tyrosyl-complex II with those of porcine thyroid tyrosyl-tRNA. Porcine thyroid tyrosyl-tRNA synthetase was purified in excess of 200-fold and characterized. It was found that maximal aminoacylation was achieved at pH 8.1 in the presence of 150 mM KCl. The K_m for tyrosine was determined to be 3.0×10^{-6} M. The purified thyroid tyrosyl-tRNA synthetase was used under aminoacylation conditions to prepare radioactively labeled porcine thyroid tyrosyl-tRNA and tyrosyl-complex II. Comparisons made using reversed-phase column chromatography (RPC-5) showed distinct differences between the two aminoacylated species and revealed, in addition, a number of isoaccepting forms of tyrosine tRNA. Tyrosyl-complex II was also found to differ from tyrosyl-tRNA in that it is more stable to deacylation at pH 7.0 and at pH 4.4 and to degradation by ribonuclease A. In addition, tyrosyl-complex II, unlike tyrosyl-tRNA, is degraded by trypsin. Ribosomal binding studies showed that tyrosyl-complex II did not respond to the codons for tyrosine, UpApU and UpApC, whereas tyrosyl-tRNA responded to both. It is suggested that thyroid tyrosine complex II is representative of a group of related complexes that constitute the complex II fraction and that, although the complexes resemble tRNA in many respects, they have distinctly different characteristics than conventional tRNA.

Oligonucleotidylpeptidyl complexes (complex II) are present in the nonparticulate RNA fraction of porcine liver (Kelly and Kull, 1974)[1] and in the thyroid glands of several mammalian species (Kull and Soodak, 1971); the porcine thyroid complex II has been shown to contain thyroxine (Gadski and Kull, 1973). Two functions have been described for the complex II fraction that could have biological importance. At low concentrations relative to tRNA, the complex II fraction affects or modifies the aminoacylation of several amino acids to tRNA while at higher concentrations the fraction appears to act as an acceptor for several amino acids in a manner analogous to tRNA (Gadski and Kull, 1973). The purpose of this investigation was to examine the latter function: the apparent aminoacylation of complex II.

The questions we addressed were: is the apparent aminoacylation of the complex II fraction the result of contamination of the fraction by tRNA or does the complex II fraction contain species of acceptor molecules different from tRNA? To answer these questions we set out to partially purify and characterize porcine thyroid tyrosyl-tRNA synthetase and then to use the purified enzyme to prepare the porcine thyroid tyrosyl-tRNA and porcine thyroid tyrosyl-complex II used for comparisons. The tyrosyl enzyme was chosen because initial studies had demonstrated that the complex II fraction was a good acceptor of tyrosine and because of the obvious importance of tyrosine to thyroid function.

The results of our study indicate that tyrosyl-complex II is different from tyrosyl-tRNA with respect to chromatography on reversed-phase columns, nonenzymatic deacylation, susceptibility to ribonuclease A and trypsin, and in response to the codons for tyrosine in a ribosomal binding experiment using *Escherichia coli* ribosomes.

Materials and Methods

Materials. Porcine thyroid glands, purchased from Arbogast and Bastian Meat Packing Co. (Allentown, Pa.), were frozen on dry ice immediately after removal from the animals and were stored at −70 °C. The DEAE-cellulose used was Whatman DE-23, and BD-cellulose[2] was obtained from Gallard-Schlesinger Chemical Mfg. Co. (Carle Place, N.Y.). L-[^{14}C]- and L-[^{3}H]tyrosine of high specific activity were obtained from Amersham/Searle Co. (Arlington Heights, Ill.). Adogen 464 was obtained from the Ashland Chemical Co. (Columbus, Ohio) and Plaskon CTFE 2300 powder from the Allied Chemical Corp. (Morristown, N.J.). Uridylyl(3′-5′)adenosine was purchased from Sigma Chemical Corp. (St. Louis, Mo.) and polynucleotide phosphorylase and alkaline phosphatase were obtained from P-L Biochemicals (Milwaukee, Wis.) and Worthington Biochemical Corp. (Freehold, N.J.), respectively. All other chemicals were of the highest grade commercially available and were used without further purification.

Tyrosyl-tRNA Synthetase. (All work was done at 4 °C

† From the Department of Biological Sciences, State University of New York, Binghamton, New York 13901. *Received December 16, 1975.* This research was sponsored by the State University of New York and supported by United States Public Health Service Research Grant Number AM 15292 from the National Institute of Arthritis, Metabolism and Digestive Diseases.

‡ Present address: Department of Pathology, Stanford University School of Medicine, Stanford, California 94305.

§ Present address: Baylor College of Medicine, Texas Medical Center, Houston, Texas 77027.

[1] E. P. Kelly and F. J. Kull, 1976, submitted for publication.

[2] Abbreviations used: BD-cellulose, benzoylated diethylaminoethylcellulose; CTFE, polychlorotrifluorethylene resin; Tris, tris(hydroxymethyl)aminomethane; DEAE, diethylaminoethyl.

TABLE I: Optimal Conditions for Aminoacylation of Porcine Thyroid tRNA and the K_m for Tyrosine of Porcine Thyroid Tyrosyl-tRNA Synthetase.[a]

K_m (tyrosine, mM)	3×10^{-3}
Mg^{2+} (mM)	5.0
ATP (mM)	3.3
pH	8.1
KCl (mM)	150.0
Ionic strength (mM)	200.0

[a] Aminoacylation assays were performed in a final volume of 0.15 ml as described in Materials and Methods and Results.

unless otherwise indicated.) Frozen pig thyroid glands were ground in a commercial meat grinder and then homogenized by means of a Waring Blendor in 2 volumes of a buffer composed of 50 mM Tris-HCl (pH 7.5), 10 mM 2-mercaptoethanol, and 10% glycerol (v/v) (buffer A). The homogenate was filtered through buffer-soaked cheesecloth to remove lipid and unhomogenized tissue and centrifuged at 12 000*g* for 20 min. The resulting supernatant was decanted through buffer-soaked cheesecloth and applied immediately to a DEAE-cellulose column (2.5 × 70 cm) that had been previously equilibrated in buffer A. The column was washed with buffer A until the absorbance of the effluent at 280 nm (A_{280}) was less than 0.6 in a 1-cm light path. At this point, the column was eluted by a 2-l. linear gradient (0.05–0.30 M KCl in buffer A) and 15-ml fractions were collected. Fractions of peak activity were pooled, dialyzed twice against 4 l. of a buffer composed of 10 mM Tris-HCl (pH 7.5), 10 mM 2-mercaptoethanol, and 10% glycerol (v/v) (buffer B), and added to a second DEAE-cellulose column (1.2 × 40 cm) which had been preequilibrated in buffer B. Following the addition of the enzyme fraction, the column was washed with buffer B until the A_{280} of the effluent was negligible and then eluted as before except that the gradient was 1 l. and was in buffer B. Peak fractions were pooled and dialyzed into 10 mM Tris-HCl (pH 7.5), 10 mM 2-mercaptoethanol, and 50% glycerol (v/v). Upon completion of dialysis, the enzyme was stored at −20 °C. The purified enzyme, appropriately diluted prior to use, was used for all the experiments described in this study.

RNA–Iodoproteinaceous Complex II and tRNA. The preparation of porcine thyroid tRNA and total complex II was carried out according to the procedure of Gadski and Kull (1973) except that the 2-propanol fractional precipitation step was omitted. Following the second gradient on BD-cellulose, the total complex II fraction was eluted batchwise from the chromatographic medium with a solvent consisting of 1 M LiCl, 10% benzyl alcohol (v/v), and 40% ethyl alcohol (v/v). After concentration and dialysis into 10 mM Tris-HCl (pH 7.5) and 1 mM $MgCl_2$, tRNA and complex II were stored at −20 °C. In order to ensure that purified species were available for certain experiments, tyrosine complex II and tRNA were separated from each other and further purified by RPC-5 chromatography as described below.

Crude tRNA, unfractionated from complex II, was prepared by the same procedure as above through the deacylation step but was not applied to BD-cellulose; after concentration and dialysis, it was stored as above.

The Aminoacylation Reaction. The aminoacylation of either tRNA or complex II with tyrosine was carried out under conditions found to be optimal for the aminoacylation of tRNA (see Results). Prior to optimization, final incubation concentrations were as follows: 50 mM Tris-HCl (pH 7.5), 2.5 mM ATP, 10 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 20 μM tyrosine, and greater than 10 A_{260} units of tRNA per ml. Incubation volumes varied according to the nature of the specific experiment with typical quantities being 75 and 150 μl for analytical work and 1.5 to 3.0 ml for preparative scale operations. Initial velocities were used for the measurement of activity during the optimization procedures and for determination of the specific activities of tyrosyl-tRNA synthetase during purification. Initial velocities were estimated by extrapolation of 5 and 10 min time points to zero time. The filter paper disk procedure of Bollum (1959) was used to measure the amount of radioactive tyrosyl-tRNA formed.

Reversed-Phase Chromatography (RPC-5). RPC-5 packing was prepared according to Pearson et al., method C (1971). The packing was degassed in the equilibration buffer: 10 mM sodium acetate (pH 4.4), 10 mM $MgCl_2$, 1 mM 2-mercaptoethanol, and from 0.35 to 0.45 M NaCl (the exact salt concentration was dependent on the nature of the particular experiment). After degassing, columns were poured as rapidly as possible, packed at maximum flow rate (Milton-Roy Minipump, Model G), and maintained at 37 °C in the equilibrating buffer.

After aminoacylation, samples of labeled tyrosyl-tRNA and tyrosyl-complex II were separated from the other components of the incubation mixture on a 1.5-cm^3 DEAE-cellulose column or were stored at −20 °C for future use. Typical chromatographic trials used linear NaCl gradients of 1 or 2 l. over a total salt concentration range of 0.4 to 0.9 M that were maintained at a constant flow rate of 0.7 ml/min. In all cases, 10-ml fractions were collected at 2 °C and maintained as such until assayed. Assays were done following the method of Ortwerth et al. (1973), and the dried Millipore filters were counted in a Packard Tri-Carb liquid scintillation counter. In the case of cochromatographic experiments, where two different labels were used, samples were counted on attenuated channels and raw counts were adjusted according to the discriminator ratio method of Okita et al. (1957) (method C).

RPC-5 chromatography was also utilized to further purify tyrosyl-complex II and tyrosyl-tRNA. This was accomplished by the stepwise elution of the labeled, aminoacylated species, as defined by their respective positions of elution, 0.43 M NaCl and 0.53 M NaCl, from small (1.2 × 18 cm) columns. After elution from the RPC-5 columns the labeled species were diluted and then condensed by means of small DEAE-cellulose columns as described above (Yang and Novelli, 1968).

Stability and Hydrolytic Studies on Tyrosyl-tRNA and Tyrosyl-Complex II. The stabilities of tyrosyl-tRNA and tyrosyl-complex II at various pHs were determined using L-[^{3}H]tyrosine as a marker. Aminoacylated samples were obtained as when prepared for RPC-5. Chromatographic profiles from RPC-5 revealed that the complex II sample used for this study was composed of 74% material that eluted at 0.43 M NaCl and 26% material that eluted at 0.53 M NaCl. The pHs were adjusted with sodium acetate (pH 4.4) and Tris-HCl (pH 7.0 and 8.85) all at final concentrations of 10 mM, and incubations were at 37 °C.

The effects of trypsin and ribonuclease A on partially purified tyrosyl-tRNA and tyrosyl-complex II labeled with L-[^{3}H]tyrosine were studied at room temperature. The rates of hydrolysis of the aminoacylated species separated from each other by RPC-5 as described above were determined by measuring the amount of cold-acid-precipitable counts remaining after various times of incubation.

Ribosomal Binding Assay. The codons for tyrosine, UpApU and UpApC, were synthesized enzymatically, purified, and characterized according to Leder (1968a). Ribosomes were

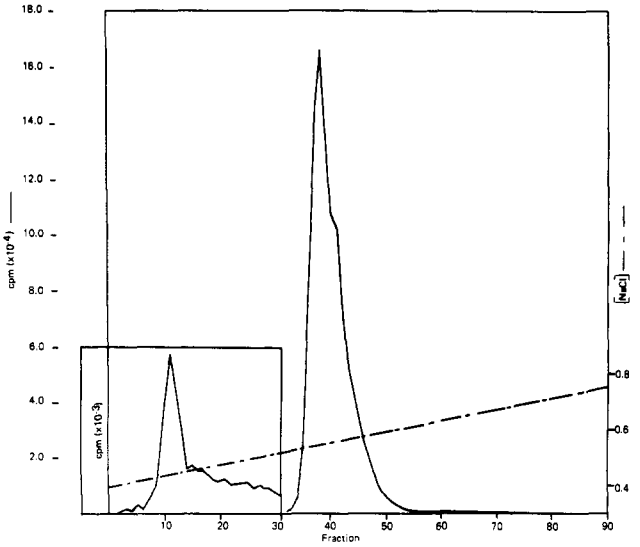

FIGURE 1: Chromatography of unfractionated tyrosyl-tRNA on RPC-5. Crude tRNA, unfractionated from complex II, was prepared as described in Materials and Methods. Approximately 33 A_{260} units was incubated under aminoacylation conditions with L-[^{3}H]tyrosine, isolated in the aminoacyl form as described in Materials and Methods, and added to a RPC-5 column (1.2 × 18 cm) which had been preequilibrated at 37 °C in 10 mM sodium acetate (pH 4.4), 10 mM $MgCl_2$, 1 mM 2-mercaptoethanol, and 0.35 M NaCl. The column was eluted with a 1-l. linear gradient (0.4 to 0.8 M NaCl in buffer); 10-ml fractions were collected and kept at 2 °C until assayed as described in Materials and Methods.

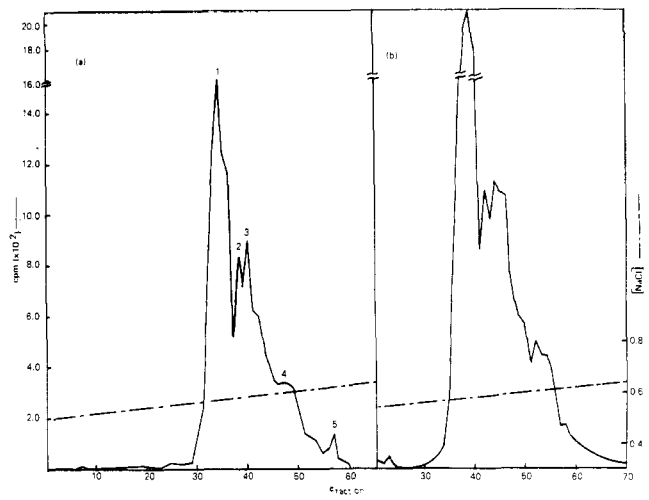

FIGURE 2: Chromatography of tyrosyl-tRNA on RPC-5. The tRNA fraction that was separated from the complex II fraction by BD-cellulose was aminoacylated as described in Materials and Methods. The RPC-5 column (1.2 × 40 cm) was preequilibrated at 37 °C in 10 mM sodium acetate (pH 4.4), 10 mM $MgCl_2$, 1 mM 2-mercaptoethanol, and 0.45 M NaCl. In a, 5.85 A_{260} units of L-[^{14}C]tyrosyl-tRNA was added to the RPC-5 column and eluted with a 2-l. linear gradient (0.50 to 0.90 M NaCl in buffer); 10-ml fractions were collected and assayed as described in Materials and Methods. b is a repetition of a, except that L-[^{3}H]tyrosyl-tRNA was chromatographed.

prepared from *E. coli B* according to Kaji (1968) (deoxyribonuclease step omitted) and further purified according to Stanley and Wahba (1968). Ribosomes obtained by these methods were dispersed in "standard buffer" (Leder, 1968b), centrifuged to remove debris, and used immediately. Complex II and tRNA were aminoacylated with L-[^{3}H]tyrosine as previously described and further purified by stepwise elution from a RPC-5 column, also as described above. The binding assay was carried out according to Leder (1968b).

Results

Tyrosyl-tRNA Synthetase. The enzyme fraction which eluted from the second DEAE-cellulose column (see Materials and Methods) was optimized and characterized with the results summarized in Table I. Optimization was accomplished as follows. First, a preliminary optimum of 10 mM $MgCl_2$ was found. Various ATP levels were then tested and a concentration of 2.5 mM was found to yield maximal aminoacylation. Under these conditions, a pH optimum of 8.1 was indicated using both Tris-HCl and potassium phosphate buffers. The use of the potassium phosphate buffer led to the observation that potassium ion stimulated the reaction. Using both KCl and potassium phosphate under otherwise identical conditions ruled out the possibility of stimulation by phosphate and showed that the optimum potassium ion concentration was 150 mM. The $MgCl_2$ and ATP concentrations were then reexamined under the new reaction conditions: 50 mM Tris-HCl (pH 8.1), 150 mM KCl, 5 mM 2-mercaptoethanol, 2×10^{-2} mM tyrosine, and greater than 10 A_{260} units per ml of tRNA. The optimum $MgCl_2$ concentration was unaltered, but a new ATP optimum of 3.3 mM was found. Using the optimized system, the K_m for tyrosine was determined to be 3.0×10^{-6} M.

A 208-fold purification of the tyrosyl-tRNA synthetase was achieved as measured using the optimized system. Porcine thyroid tyrosyl-tRNA synthetase is remarkably stable for a mammalian synthetase; no measurable loss of activity was observed after storage for 6 months at −20 °C in 50% glycerol. Furthermore, no significant inactivation was encountered at any of the preparative stages and the enzyme is capable of tolerating a variety of common technical manipulations.

Reversed-Phase Chromatography. Total crude tRNA, unfractionated from complex II, was prepared as described in Materials and Methods and aminoacylated with L-[^{3}H]tyrosine. Figure 1 shows the result of chromatographing this crude fraction on a 1.2 × 18 cm RPC-5 column. Two prominent peaks were obtained that are well separated from each other. The smaller of these eluted at 0.43 M NaCl, contained approximately 3% of the total recovered radioactivity, and was associated with several minor peaks. The bulk of the radioactivity eluted from the column at a salt concentration of 0.53 M as an asymmetrical peak with prominent trailing shoulder.

To determine which if either of the two peaks shown in Figure 1 represented the tRNA and/or complex II fractions obtained by fractionation of crude tRNA on BD-cellulose columns, the tRNA fraction obtained from BD-cellulose was aminoacylated with L-[^{14}C]tyrosine and was chromatographed on a larger RPC-5 column (1.2 × 40 cm). Under the conditions described in Figure 2, it can be seen that several peaks were resolved by chromatography on the longer column; the trailing shoulder on the major peak seen in Figure 1 appears to contain at least two and possibly as many as four discrete peaks (numbered 2 through 5 in Figure 2a). Two of these (peaks 2 and 3) eluted at virtually identical salt concentrations and were separated by only a single fraction. Figure 2b illustrates a repetition of this experiment that reproduces Figure 2a in that peaks 2 and 3 are resolved and thus supports the conclusion that, despite the small degree of separation achieved by the RPC-5 column, peaks 2 and 3 most likely represent different isoaccepting forms of tyrosine tRNA.

In order to conserve complex II and to facilitate the chromatographic comparison of tyrosyl-tRNA and tyrosyl-complex II, we used the shorter RPC-5 column (1.2 × 18 cm) for further analyses. The results shown in Figure 3a are in accord with

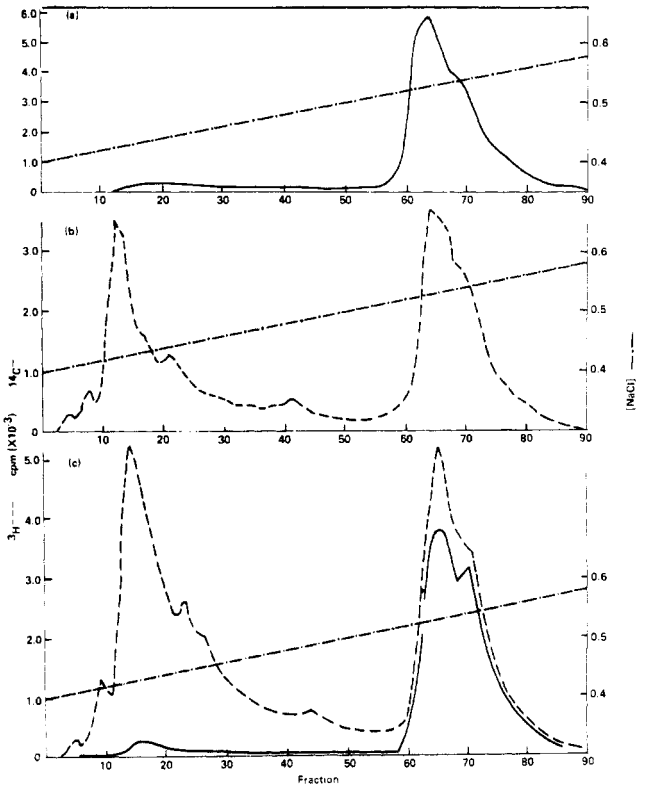

FIGURE 3: A comparison of tyrosyl-tRNA and tyrosyl-complex II by RPC-5. The tRNA and complex II fractions were prepared as described in Materials and Methods. The RPC-5 column (1.2 × 18 cm) was pre-equilibrated at 37 °C in 10 mM sodium acetate (pH 4.4), 10 mM $MgCl_2$, 1 mM 2-mercaptoethanol, and 0.35 M NaCl. Samples of tRNA and complex II were aminoacylated with tyrosine and isolated as described in Materials and Methods. In a, 10.4 A_{260} units of L-[^{14}C]tyrosyl-tRNA was added to the RPC-5 column and eluted with a 1-l. linear gradient (0.4 to 0.6 M NaCl in buffer); 10-ml fractions were collected and assayed as described in Materials and Methods. In b, 9.0 A_{260} units of L-[^{14}C]tyrosyl-complex II was chromatographed as in a. In c, 18.0 A_{260} units of L-[^{3}H]tyrosyl-complex II and 10.4 A_{260} units of L-[^{14}C]tyrosyl-tRNA were cochromatographed as in a and b.

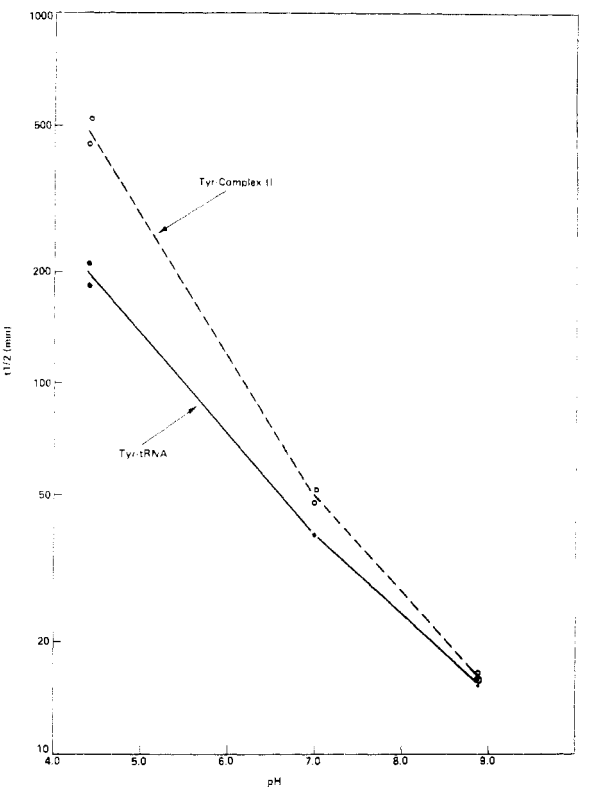

FIGURE 4: Sensitivity of tyrosyl-tRNA and tyrosyl-complex II to pH. L-[^{3}H]tyrosyl-tRNA and L-[^{3}H]tyrosyl-complex II, prepared as described in Materials and Methods, were incubated in duplicate at 37 °C in a final concentration of 110 mM NaCl, 0.1 mM $MgCl_2$, and 10 mM buffer (sodium acetate, pH 4.4; Tris-HCl, pH 7.0 and 8.85) in an initial volume of 0.9 ml. At 0, 6, 12, 18, 30, 60, 90, and 120 min, 0.1 ml was removed from each incubation and applied to a filter paper disc. Discs were washed as was described for the aminoacylation assay (Materials and Methods) and half-lives were calculated at each pH from the amount of labeled material still precipitable by cold acid at the various times.

the data presented in Figures 1 and 2; it can be seen that a single major peak of radioactivity eluted at 0.53 M NaCl, again with a significant trailing shoulder. Note also that a very small amount of radioactivity eluted rather indistinctly at the front of the profile.

When tyrosyl-complex II was chromatographed on the same RPC-5 column (1.2 × 18 cm), profiles typified by Figure 3b were obtained. Two major peaks of approximately equal magnitude were resolved. The first of the peaks eluted at 0.43 M NaCl in association with several, additional, small, but quite distinct peaks, whereas the second major peak was virtually identical, in position of elution and shape, with the profile obtained for tyrosyl-tRNA.

A double labeling experiment is illustrated in Figure 3c. In this experiment, complex II was labeled with L-[^{3}H]tyrosine and tRNA with L-[^{14}C]tyrosine. When cochromatographed, the samples eluted at salt concentrations identical with those required to effect their elution when chromatographed separately. Reversal of the labels caused no change in the resulting profiles.

During the course of the RPC-5 experiments, complex II fractions were usually found to contain tRNA, although the relative amounts varied from virtually none to an amount equal to, or even in excess of, complex II. We found that the amount of contaminating tRNA present in fresh complex II preparations could be reduced, but not completely eliminated, by extensive washing of the BD-cellulose column prior to elution of complex II.[1] Reduction of the amount of contaminating tRNA was accomplished by following the first (0.3 to 1.0 M NaCl) and second (1.0 to 1.2 M NaCl and 0 to 20% ethyl alcohol) gradients by exhaustive washings with 1.0 M NaCl and 1.2 M NaCl, 20% ethyl alcohol, respectively.

Stability and Hydrolytic Studies. Figure 4 shows the stability, at various pHs, of tyrosyl-complex II relative to tyrosyl-tRNA. The most striking difference between the two was seen at pH 4.4 where tyrosyl-complex II was more than twice as stable as tyrosyl-tRNA; the average half-life of tyrosyl-complex II was 488 min while that for tyrosyl-tRNA was 198 min. At pH 7.0, tyrosyl-complex II was again more stable, but the half-lives differed much less than at pH 4.4 (52 min for tyrosyl-complex II vs. 40 min for tyrosyl-tRNA). At pH 8.85, the stabilities of the two species were very similar; the average half-life of tyrosyl-complex II was only 0.5 min longer than that for tyrosyl-tRNA.

Incubation of tyrosyl-complex II and tyrosyl-tRNA with ribonuclease A revealed two significant facts (Table II). Firstly, tyrosyl-complex II was degraded to cold-acid-soluble fragments. Secondly, tyrosyl-complex II was much less sensitive to nucleolytic attack by ribonuclease A than was tyrosyl-tRNA. Half-lives of the former were on the order of ten times greater than those of the latter.

The results with ribonuclease A were opposite to those obtained with trypsin. In the presence of trypsin, tyrosyl-tRNA,

as expected, showed no detectable degradation over nonenzymatic hydrolysis. However, tyrosyl-complex II was degraded with a half-life of approximately 50 min.

Ribosomal Binding Assays. The results described above show several distinct differences between tyrosyl-tRNA and tyrosyl-complex II, but at the same time clearly indicate that complex II is capable of acting as an acceptor of tyrosine in a manner quite analogous to tRNA. In fact, the tyrosine acceptor activity could be rationalized if complex II could be shown to represent a class of minor isoaccepting forms of tyrosine tRNA (disregarding the evidence for a peptidyl component presented here and previously). To test this possibility, we examined the binding of partially purified tyrosyl-tRNA and tyrosyl-complex II to the two tyrosine codons in the presence of *E. coli* ribosomes.

Both codons for tyrosine, UpApU and UpApC, were tested in quadruplicate in the ribosomal binding system. In each of the eight trials, the binding of tyrosyl-tRNA was stimulated over the duplicate control experiments done in the absence of exogenous codon. Specific binding of the added tyrosyl-tRNA represented 6.6% for UpApC and 5.1% for UpApU. Nonspecific binding in the absence of either codon accounted for 66% of the added tyrosyl-tRNA.

Because of the high nonspecific binding of labeled tyrosyl-tRNA and tyrosyl-complex II, the data were evaluated by *t* test. The response of tyrosyl-tRNA to the UpApU and UpApC codons showed significance to the >95% and >99% levels of confidence, respectively.

An identical experiment was done except that tyrosyl-complex II was used in place of tyrosyl-tRNA. Unlike the results obtained with tyrosyl-tRNA, none of the eight samples incubated with codons showed any stimulation of binding. Furthermore, the nonspecific binding of tyrosyl-complex II was only 31%, appreciably less than that seen for tyrosyl-tRNA. Of possible additional interest was the observation that the nonspecific binding of tyrosyl-complex II to the ribosomes was very significantly diminished (p values $\ll 0.01$ for each codon) in the presence of the tyrosine codons.

TABLE II: Stability of Tyrosyl-tRNA and Tyrosyl-Complex II to Incubation with Ribonuclease A and Trypsin.[a]

Hydrolytic Enzyme	(μg/ml)	Half-Life (min)	
		Tyrosyl-tRNA	Tyrosyl-Complex II
Ribonuclease A	0	>290.00	>490.00
	0.4	18.50	>270.00
	1.2	8.50	87.00
	140.0	<0.25	<0.25
Trypsin	167.0	>290.00	49.50

[a] Incubations in 10 mM sodium acetate (pH 5.5) were at room temperature in 0.275 ml for ribonuclease A and in 0.350 ml for trypsin. In both cases the final concentration of L-[^{3}H]tyrosyl-complex II or L-[^{3}H]tyrosyl-tRNA was 0.552 A_{260} unit per ml. Samples (50 μl) of each incubation were removed for assay at 0.5, 1, 2, 5, and 10 min for ribonuclease A and 0.5, 1, 2, 5, 10, and 30 min for trypsin. The filter paper disc technique (Materials and Methods) was used to measure the amount of cold-acid-precipitable counts that remained at the various times.

Discussion

The 208-fold purification of porcine thyroid tyrosyl-tRNA synthetase we achieved by two successive gradient elutions from DEAE-cellulose columns represents the first reported purification of tyrosyl-tRNA synthetase from thyroid glands, and, to our knowledge is the highest purification reported for a mammalian tyrosyl-tRNA synthetase. The enzyme is remarkably stable when compared with several other mammalian aminoacyl-tRNA synthetases we have purified in our laboratory. For example, porcine thyroid isoleucyl-tRNA synthetase is denatured by freezing and thawing and has a half-life of 9 min at 42 °C (Dibner et al., 1974). Also the leucyl-tRNA synthetase from porcine liver and the aspartyl-tRNA synthetase from porcine thyroid are both extremely sensitive to temperature and environment (Curatolo, 1973).[3]

Optimization and characterization of the thyroid tyrosyl-tRNA synthetase did not reveal any unusual results with the possible exception of the pronounced stimulation seen in the presence of potassium ions. Several authors have reported stimulation of aminoacyl-tRNA synthetases by various cations and anions (Yu, 1966; Rubin et al., 1967; Curatolo, 1973). However, the mechanism for such stimulations is largely unknown.

The magnesium ion and ATP concentrations and pH optimum are all well within ranges reported for numerous other aminoacyl-tRNA synthetases as is the K_m of 3×10^{-6} M that we determined for tyrosine (Novelli, 1967).

Previous studies of the complex II fraction from porcine thyroid revealed two types of in vitro activity that could possibly be related to the in vivo function of the complex: an ability to alter the rate and the extent of the aminoacylation of several amino acids to tRNA and the capacity to act as an acceptor of several amino acids in a manner seemingly analogous to the aminoacylation of tRNA (Gadski and Kull, 1973). Although many questions remain to be answered regarding both the nature and mechanism of these phenomena, the results presented here are basic to an understanding of the latter activity, the apparent aminoacylation of complex II with tyrosine.

Of several different hypotheses that can be advanced to account for the apparent aminoacylation of tyrosine to the complex II fraction, two seem most reasonable. The aminoacylation could be the result of the contamination of the complex II fraction by tRNA or the aminoacylation could be to a molecule in the complex II fraction that is similar to tRNA but one that differs sufficiently from tRNA such that it can be separated from tRNA. Our experiments were designed to compare tyrosyl-tRNA and tyrosyl-complex II in hopes of distinguishing between these hypotheses. We reasoned that if the incorporation of tyrosine was due to the presence of conventional tRNA in the complex II fraction, no differences in response to the various tests we devised would be observed between the aminoacylated tRNA and complex II fractions. Conversely, any differences we would observe would serve to strengthen the second hypothesis, that there exists a distinct class of acceptor molecules represented by tyrosine complex II.

The reversed-phase chromatography experiments using tyrosine-labeled, crude, unfractionated tRNA, prepared under aminoacylation conditions, revealed that no less than three and perhaps as many as ten elution peaks that contain radioactive tyrosine could be partially or completely resolved by RPC-5. It should be pointed out that this experiment also serves as a control that rules out the possibility that the first elution peak from RPC-5 is due to an artifact that arose during chromatography and/or elution from BD-cellulose. Additional RPC-5

[3] H. J. Brandwein and F. J. Kull, 1976, submitted for publication.

chromatography using tRNA and complex II, fractionated by BD-cellulose, showed that the major peak obtained with tyrosine-labeled, crude, unfractionated tRNA, corresponded to the fractionated tRNA and that the minor peak corresponded to complex II. Thus, these experiments established a second major chromatographic difference between tRNA and complex II (the first being manifested on BD-cellulose).

Although the appearance of a "tRNA" peak in our RPC-5 profile for tyrosyl-complex II caused some initial concern about the selectivity of BD-cellulose, it was later found that a considerable portion of the tRNA that was present in the complex II fraction could be eliminated if additional precautions were taken in eluting the tRNA and complex II fractions from BD-cellulose.[1] However, while establishing this, it became clear that the complex II fraction underwent some sort of gradual transition such that it could no longer act as an acceptor of tyrosine. It was observed that with time, and/or freezing and thawing, there is a decrease in the ability of complex II to accept tyrosine. However, any tRNA present remains essentially unaltered and, therefore, the tRNA to complex II ratio as measured by tyrosine acceptance, increases. These observations point out another basic difference between the complex II fraction and tRNA.

In contrast to the presence of varying amounts of tRNA in the complex II fraction, the tRNA fraction contains little complex II. Thus, it is clear that if the fraction that elutes early from RPC-5 represents a conventional, minor, isoaccepting form of tyrosyl-tRNA, it must differ from tRNA such that it is bound very tightly by BD-cellulose. We have shown previously (Gadski and Kull, 1973) that a second deacylation of the complex II fraction has no effect on its rechromatography on BD-cellulose (which rules out absorption to BD-cellulose because of the incomplete deacylation of aromatic aminoacyl-tRNA). Nor was complex II found to contain either "Y" base or any other derivative that was eliminated by incubation at pH 2.9 according to the procedure described by Thiebe and Zachau (1968) which could also have accounted for retardation of the complex on BD-cellulose (Fink et al., 1971). Therefore, after consideration of the RPC-5 experiments, we are left with the possibilities that the complex II fraction either represents minor isoaccepting forms of tRNA that are tightly bound to BD-cellulose because of some unique aromatic constituent or that the fraction represents a class of acceptor molecules radically different from conventional tRNA.

In addition to the chromatographic differences seen between the tRNA and complex II fractions using RPC-5 chromatography, our other data support the argument that the complex II fraction is distinct from tRNA. The greater stability of tyrosyl-complex II to incubation at acidic and neutral pH can only be interpreted to mean that, if an aminoacyl linkage is present in tyrosyl-complex II, it is in a different environment than that in tyrosyl-tRNA. The possibility that complex II and tyrosyl-tRNA have widely different structural organizations is also suggested by the pronounced resistance of tyrosyl-complex II to ribonuclease A and by its gradual degradation by trypsin. These data, presented in Table II, strongly support our earlier results (Kull and Soodak, 1971; Gadski and Kull, 1973), that indicated that complex II has both oligonucleotidyl and peptidyl portions and further demonstrate that disruption of the complex can be brought about by either nucleolytic or proteolytic enzymes. These results demonstrate striking differences between tyrosyl-tRNA and tyrosyl-complex II and cannot be easily explained if one considers the tyrosyl-complex II to be a minor isoaccepting form of conventional tRNA.

Finally, the results of the ribosomal binding studies are good preliminary evidence that tyrosyl-complex II either does not have an anticodon complementary to the codons for tyrosine or that an anticodon is unavailable for interaction with the codons; thus, we assume that complex II could not act as a transfer molecule in conventional protein biosynthesis.

The data presented here and previously (Kull and Soodak, 1971; Gadski and Kull, 1973) are consistent with the speculation that the complex II fraction represents either a highly modified and unusual group of minor isoaccepting forms of tRNA or a hitherto unrecognized group of acceptor molecules, present in small amounts, in what is generally considered to be the tRNA fraction of the cellular RNA.

References

Bollum, F. J. (1959), *J. Biol. Chem. 234,* 2733.

Curatolo, W. J. (1973), M.A. Thesis, State University of New York at Binghamton.

Dibner, J. J., Brandwein, H. J., and Kull, F. J. (1974), 168th National Meeting of the American Chemical Society, Atlantic City, New Jersey, Sept 1974.

Fink, L. M., Lanks, K. W., Goto, T., and Weinstein, I. B. (1971), *Biochemistry 10,* 1873.

Gadski, R. A., and Kull, F. J. (1973), *Biochemistry 12,* 1907.

Holmes, W. M., Hurd, R. E., Reid, B. R., Rimerman, R. A., and Hatfield, G. W. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 1068.

Kaji, A. (1968), *Methods Enzymol. 12B,* 692.

Kelly, E. P., and Kull, F. J. (1974), 168th National Meeting of the American Chemical Society, Atlantic City, N.J., Sept 1974.

Kull, F. J., and Soodak, M. (1971), *Biochim. Biophys. Acta 246,* 1.

Leder, P. (1968a), *Methods Enzymol. 12B,* 837.

Leder, P. (1968b), *Methods Enzymol. 12B,* 725.

Novelli, G. D. (1967), *Annu. Rev. Biochem. 36,* 449.

Okita, G. T., Kabara, J. J., Richardson, F., and LeRoy, G. V. (1957), *Nucleonics 15,* No. 6, 111.

Ortwerth, B. J., Yonuschot, G. R., and Carlson, J. V. (1973), *Biochemistry 12,* 3985.

Pearson, R. L., Weiss, J. F., and Kelmers, A. D. (1971), *Biochim. Biophys. Acta 228,* 770.

Rubin, I. B., Kelmers, A. D., and Goldstein, G. (1967), *Anal. Biochem. 20,* 533.

Stanley, W. M., and Wahba, A. J. (1968), *Methods Enzymol. 12A,* 524.

Thiebe, R., and Zachau, H. G. (1968), *Eur. J. Biochem. 5,* 546.

Yang, W.-K., and Novelli, G. D. (1968), *Biochem. Biophys. Res. Commun. 31,* 534.

Yu, C.-T. (1966), *Cold Spring Harbor Symp. Quant. Biol., 31,* 565.