Species Specificity of a Tyrosyl Transfer Ribonucleic Acid Synthetase from Calf Liver*

Ronald E. Pearlman† and Konrad Bloch

ABSTRACT: The species specificity of tyrosyl-tRNA formation catalyzed by an enzyme from calf liver has been investigated. Crude calf liver extracts and ammonium sulfate precipitated fractions catalyze the aminoacylation of yeast ribonucleic acid (RNA). However, on purification the liver enzyme loses the ability to catalyze the amino acylation of yeast RNA. A factor which apparently "modified" yeast RNA and restored its acceptor activity was separated from tyrosyl-tRNA synthetase by DEAE-cellulose chromatography.

The recognition of a specific RNA by an aminoacyl-tRNA synthetase is an aspect of protein biosynthesis that has received much attention (Yu and Zamecnik, 1963; Gottschling and Zachau, 1965; Baldwin and Berg, 1966; Calendar and Berg, 1966; Hayashi and Miura, 1966; Hayashi, 1966). In the course of purifying a tyrosyl-tRNA synthetase from calf liver, we have noted an interesting species specificity of this enzyme which may be useful in studying the recognition process. Purified liver tyrosyl-tRNA synthetase, in contrast to cruder enzyme, no longer catalyzes aminoacylation of yeast RNA. However, a yeast RNA acquires this acceptor function on exposure to certain fractions which separate from the liver tyrosyl-tRNA synthetase on DEAE-cellulose chromatography. Apparently yeast RNA must be modified in some way before it can accept tyrosine in the reaction catalyzed by partially purified liver tyrosyl-tRNA synthetase. This effect resembles the "modification" reaction described by Makman and Cantoni (1966) for the aminoacylation of *Eschericia coli* RNA by a purified seryl-tRNA synthetase from yeast.

Materials and Methods

Disodium-ATP[1] (Sigma Chemical Co. or Schwarz BioResearch, Inc.) was neutralized (pH 7) by the addition of $KHCO_3$. L-[^{14}C]Tyrosine (sp act. 360 μc/μmole) was obtained from Schwarz BioResearch, Inc. [^{14}C]ATP (25 μc/μmole) was a gift of Dr. M. Konrad. RNA from rat liver and yeast was purchased from General Biochemicals and tyrosine-specific yeast RNA was a gift of Dr. R. W. Holley.

Assay mixtures for measuring the formation of [^{14}C]tyrosyl-tRNA contained: Tris-chloride buffer (pH 7.4), 50 μmoles; $MgCl_2$, 4 μmoles; KCl, 3 μmoles; ATP, 5 μmoles; 2-mercaptoethanol, 5 μmoles; L-[^{14}C]tyrosine, specific activity 360 μc/μmoles, about 20,000 cpm; RNA, 0.1–1.0 mg; and enzyme and water in a total volume of 0.5 ml. After incubation at 37°, RNA was isolated from the reaction mixture by phenol extraction (Gierer and Schramm, 1956), precipitated with cold TCA, and collected on a Millipore filter. The material was washed and counted in a liquid scintillation counter. Zero-time controls were always quite high (~200 cpm) but the observed incorporations were significantly above this level.

Incubation mixtures for measuring the incorporation of [^{14}C]ATP into RNA contained Tris-chloride buffer (pH 7.4), 50 μmoles; $MgCl_2$, 4 μmoles; KCl, 6 μmoles; 2-mercaptoethanol, 5 μmoles; commercial yeast or rat liver RNA, 0.2 mg; [^{14}C]ATP, 0.5 μc, 25 μc/μmole; enzyme ($(NH_4)_2SO_4$ precipitate) 0.16 mg; and water in a total volume of 0.5 ml. After incubation at 37° RNA was isolated and counted as described above.

Enzyme from crude calf liver was prepared in the medium of Matthaei and Nirenberg (1961) as previously described (Pearlman and Bloch, 1963) and fractionated by $(NH_4)_2SO_4$ precipitation followed by chromatography on DEAE-cellulose. Fractions were eluted from DEAE-cellulose with a linear KCl gradient from 0.05 to 0.1 M in 0.01 M Tris-chloride buffer (pH 7.6), containing 0.001 M 2-mercaptoethanol. Figure 1 shows the activity profile obtained on DEAE-cellulose chromatography.

Results

Crude enzyme preparations and fractions precipitated by $(NH_4)_2SO_4$ aminoacylate yeast RNA and rat liver RNA with tyrosine at essentially the same rate and to the same extent. This is true for both commercial yeast RNA (unfractionated RNA) and partially purified tyrosine tRNA from yeast (Pearlman, 1966). The calf liver enzyme, therefore, "recognizes" a tyrosine-specific

* From the James Bryant Conant Laboratory of the Department of Chemistry, Harvard University, Cambridge, Massachusetts. *Received February 21, 1967.* This work was supported by grants-in-aid from the National Institutes of Health, the National Science Foundation, and the Eugene P. Higgins Trust Fund of Harvard University.

† Present address: Biological Institute, Carlsberg Foundation, Copenhagen, Denmark.

[1] Abbreviations used: ATP, adenosine triphosphate; TCA, trichloroacetic acid.

species of yeast RNA. However, after chromatography on DEAE-cellulose, the enzyme no longer catalyzes the aminoacylation of either commercial yeast RNA or of yeast RNA that is enriched for tyrosine acceptor activity. Rat liver RNA is aminoacylated with tyrosine by both the DEAE-enzyme and the $(NH_4)_2SO_4$-precipitated enzymes (Table I).

TABLE I: Species Specificity for RNA with Tyrosyl-tRNA Synthetase.[a]

RNA Species	Enzyme Fraction	Protein (mg)	TCA Ppt (cpm)
Rat liver	$(NH_4)_2SO_4$	0.16	2,070
Rat liver	DEAE-A	0.03	515
Yeast	$(NH_4)_2SO_4$	0.16	13,215
Yeast	DEAE-A	0.03	90

[a] The assays were performed as described in Methods. L-[^{14}C]Tyrosine (30,000 dpm, 360 μc/μmole) and 0.25 mg of RNA was used in all experiments; liver RNA was a commercial product. Yeast RNA enriched for tyrosine acceptor activity was a gift of Dr. R. W. Holley. The $(NH_4)_2SO_4$-precipitated enzyme was used after storage at $-20°$ for 10 weeks and served as the starting material for DEAE-cellulose chromatography (Figure 1). DEAE-A enzyme refers to eluent fractions 8–11 as described in Figure 1. Samples were incubated for 10 min and all values are the average of duplicates. A blank value of 200 cpm representing incorporation at zero time has been subtracted from all values.

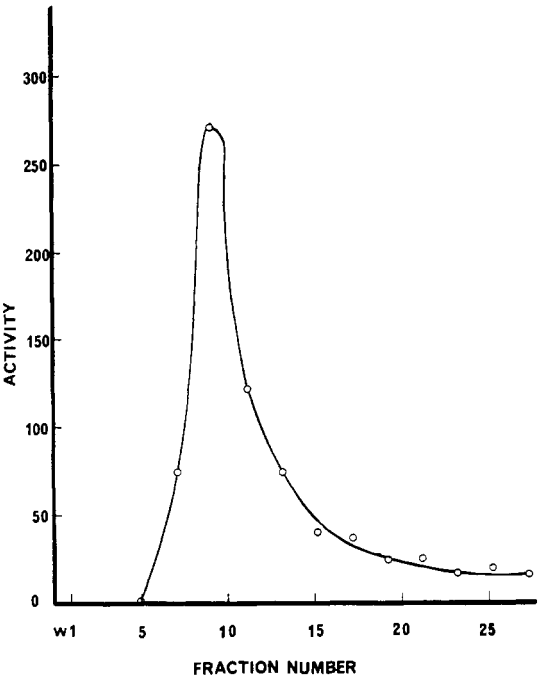

FIGURE 1: DEAE-cellulose chromatography of $(NH_4)_2SO_4$-precipitated enzyme. Approximately 25 mg of protein was applied to a DEAE-cellulose column having a column volume of 6 ml. W represents the "wash" eluted with 0.01 M Tris-chloride buffer (pH 7.6) containing 0.001 M 2-mercaptoethanol. Elution was then begun with 60 ml of a linear gradient of 0.05–0.1 M KCl in the above buffer. Fractions of approximately 2 ml were collected. Tyrosine-promoted $^{32}PP_i$–ATP-exchange activity (O———O) was assayed by the method of Norris and Berg (1964). Assay tubes contained in micromoles: Tris-chloride buffer (pH 8.7), 100; $MgCl_2$, 4; KF, 60; ATP, 5; L-tyrosine, 0.2; $^{32}PP_i$, 4 (containing from 20,000 to 50,000 cpm/μmole of PP_i); and enzyme and water in a final volume of 1 ml. Tubes were incubated at 37° and the reaction was stopped by placing the tubes in an ice bath and adding 0.25 ml of a solution of 14% perchloric acid. Nucleotide material was separated from nonnucleotide material by the method of Crane and Lipmann (1953). A blank of 5 mμmoles of PP_i exchanged into ATP in the absence of added amino acid has been subtracted. Activity is expressed as mμmoles of PP_i exchanged into ATP. One enzyme unit is defined as the amount of enzyme which will catalyze the exchange of 1 mμmole of PP_i into charcoal-adsorbable material in 15 min at 37°. Activity was eluted at a KCl concentration of approximately 0.06 M. Tyrosyl-tRNA formation with yeast RNA is not catalyzed by any of the individual fractions. The fractions were pooled as follows (with fractions given first followed by their designations): 8–11, A; 1–4, B; 5–7, C; 12–15, D; 16–19, E; 20–23, F; 24–27, G; and, 28 and 29, H.

Tables II and III show that tyrosine incorporation into yeast RNA by DEAE enzyme can be restored by combining DEAE fraction A with either fractions E, F, G, or H (Figure 1) before assay or by incubating the RNA with DEAE fractions E, F, G, or H, reisolating the RNA by phenol extraction and alcohol precipitation, and then assaying this material with fraction A.

One explanation for these results is that the rat liver RNA as used contains an intact pCpCpA-terminal sequence but that the terminal sequence in yeast RNA is pCpC. The function of fractions E, F, G, and H would then be to reconstitute the intact terminal sequence of yeast RNA by reactions which are well known (Canellakis and Herbert, 1960; Preiss *et al.*, 1961; Daniel and Littauer, 1963). R. W. Holley (personal communication) suggests that a large proportion of the yeast RNA as isolated in his laboratory lacks the terminal AMP moiety. The $(NH_4)_2SO_4$-precipitated enzyme, however, does not catalyze any significant incorporation of AMP from [^{14}C]ATP into either commercial yeast or rat liver RNA (less than 0.01 mμmole of AMP/mg of RNA.) This amount of AMP incorporated into RNA cannot account for the observed tyrosyl-tRNA

TABLE II: Recombination of DEAE Fractions.[a]

Enzyme Fractions	Total Protein (mg)[b]	TCA Ppt (cpm)
A	0.06	10
A + W	0.06	20
A + B	0.05	0
A + C	0.09	45
A + D	0.05	40
A + E	0.05	275
A + F	0.04	280
A + G	0.04	210
A + H	0.05	185
E	0.04	30
F	0.02	45
G	0.02	40

[a] The assays were performed as described in Methods with L-[^{14}C]tyrosine (30,000 dpm, 360 μc/μmole) and 0.2 mg of commercial yeast RNA in a total volume of 0.65 ml. The enzyme fractions are defined in Figure 1. In all the combination assays 0.03 mg of fraction A was present. Samples were incubated for 10 min and zero-time values of 200 cpm were subtracted in all cases. [b] The weight of total protein in the assay mixture of either single fractions when these were tested alone or of the sum of the two fractions in the combination assays.

TABLE III: Preincubation of Yeast RNA.[a]

Enzyme Fraction	Total Protein (mg)[b]	RNA Preincubn with Fraction	TCA Ppt (cpm)
A	0.06	E	250
A	0.06	F	390
A	0.06	G	225
A	0.06	H	245
A + E	0.05	E	190
A + F	0.04	F	295
A + G	0.04	G	195
A + H	0.05	H	200

[a] The assays were performed as described in Methods with 30,000 dpm of L-[^{14}C]tyrosine (360 μc/μmole) in a total volume of 0.65 ml. Enzyme fractions are as defined in Figure 1; 0.03 mg of fraction A was present in the combination assays. Commercial yeast RNA (0.25 mg) was incubated at 37° for 25 min with the appropriate enzyme fraction in the standard mixture for assay of aminoacyl-tRNA synthetase activity, omitting the amino acid. RNA was isolated by phenol extraction and alcohol precipitation. The preincubated RNA was assayed by incubation for 10 min. All values are the average of duplicates. A value of 230 cpm obtained with DEAE-A enzyme and yeast RNA which had not been preincubated was subtracted in all cases. [b] See footnote *b* in Table II.

formation (Table II) unless AMP is incorporated specifically into a $tRNA^{Tyr}$.

Results similar to those described here have recently been reported by Makman and Cantoni (1966) for a purified seryl-tRNA synthetase from yeast. The purified enzyme will not aminoacylate *E. coli* RNA with serine to the maximum extent (as determined with an *E. coli* synthetase) unless a heat-labile, nondialyzable factor from *E. coli* or yeast ("enhancing factor") is added. "Enhancing factor" does not contain seryl-tRNA synthetase or RNA pyrophosphorylase. The same authors have shown that RNA species of *E. coli* aminoacylated by *E. coli* seryl-tRNA synthetase are aminoacylated by yeast enzyme plus enhancing factor.

It has not as yet been established that the factor reported here is a protein and that an enzymic modification of RNA occurs. However, experiments with the crude enzyme preparations which catalyzes the aminoacylation of yeast RNA showed that yeast RNA was the substrate in the reaction and that no endogenous liver RNA was aminoacylated. This suggests that the factor present in DEAE-cellulose fractions E–H is not liver RNA.

The physiological significance of our factor and of Makman and Cantoni's enhancing factor is not clear, since its only apparent function is to modify heterologous RNA. The purified tyrosyl- and seryl-tRNA synthetases from calf liver and yeast need not be supplemented to catalyze aminoacylation of their homologous RNAs. Conceivably the modification reaction brings about a secondary structural alteration of the polynucleotide chain, *e.g.*, by methylation, which is necessary for reaction with the aminoacyl-tRNA synthetase.

Since the nucleotide sequence of a tyrosine tRNA from yeast is now known (Madison *et al.*, 1966), the exact nature of the "modification" process could be elucidated. Two different RNA species, one with and the other without tyrosine acceptor activity with heterologous enzyme, could be compared directly. Such an approach might be valuable in studying the mechanisms of recognition of RNA by aminoacyl-tRNA synthetase.

References

Baldwin, A. N., and Berg, P. (1966), *J. Biol. Chem. 241*, 831, 839.

Calendar, R., and Berg, P. (1966), *Biochemistry 5*, 1681.

Canellakis, E. S., and Herbert, E. (1960), *Biochim. Biophys. Acta 45*, 133.

Crane, R. K., and Lipmann, F. (1953), *J. Biol. Chem. 201*, 235.

Daniel, V., and Littauer, U. Z. (1963), *J. Biol. Chem. 238*, 2102.

Gierer, A., and Schramm, G. (1956), *Nature 177*, 702.
Gottschling, H., and Zachau, H. G. (1965), *Biochim. Biophys. Acta 103*, 418.
Hayashi, H. (1966), *J. Mol. Biol. 19*, 161.
Hayashi, H., and Miura, K. I. (1966), *Nature 209*, 376.
Madison, J. T., Everett, G. A., and Kung, H. (1966), *Science 153*, 531.
Makman, M. H., and Cantoni, G. L. (1966), *Biochemistry 5*, 2246.
Matthaei, J. H., and Nirenberg, M. W. (1961), *Proc. Natl. Acad. Sci. U. S. 47*, 1580.
Norris, A. T., and Berg, P. (1964), *Proc. Natl. Acad. Sci. U. S. 53*, 539.
Pearlman, R. (1966), Ph.D. Thesis, Harvard University, Boston, Mass.
Pearlman, R., and Bloch, K. (1963), *Proc. Natl. Acad. Sci. U. S. 50*, 533.
Preiss, J., Dieckmann, M., and Berg, P. (1961), *J. Biol. Chem. 236*, 1748.
Yu, C. T., and Zamecnik, P. C. (1963), *Biochim. Biophys. Acta 76*, 209.

Mitochondrial Glutamate–Aspartate Transaminase. I. Structural Comparison with the Supernatant Isozyme*

M. Martinez-Carrion and D. Tiemeier

ABSTRACT: Mitochondrial pig heart glutamate–aspartate transaminase (L-aspartate:2-oxoglutaratea minotransferase, EC 2.6.1.1) can be isolated in a state of purity suitable for detailed structural work. The absorption spectrum of this transaminase at pH 5 is identical with that of its supernatant isozyme but at pH 8.2 it is slightly different. This small discrepancy in spectra can be best explained in terms of some pyridoxal phosphate binding by the mitochondrial isozyme at sites other than the active center. The A_{280}:A_{355} ratio is 8.7 at pH 8.2. The molecular absorbancy coefficient due to the bound pyridoxal phosphate is 8350 ± 700 at 355 mμ (at pH 5) and 8050 ± 300 at 435 mμ (at pH 8.2). Pyridoxal phosphate is probably bound in an azomethine link to the protein and this bond can be reduced with sodium borohydride. This reduction shifts the absorption maxima to 330 mμ and renders the enzyme inactive. The purified enzyme yields two cationic protein bands that possess transaminase activity in starch gel electrophoresis at pH 8.6. The N-terminal amino acid is serine and the amino acid composition of this enzyme is distinct from that of the supernatant isozyme. Tryptic digests of both isozymes were fingerprinted and their patterns also indicate extreme dissimilarity. By comparing the N-terminal serine and pyridoxal phosphate contents as well as the number of ninhydrin-positive tryptophan and histidine spots of the fingerprints to its assigned molecular weight, it is concluded that the mitochondrial aspartate transaminase is a dimer made up of two very similar monomers. The significance of these findings in relation to the structure of the supernatant isozyme and their possible genetic implications is discussed.

After Fleischer *et al.* (1960) reported the separation of two isozymes of glutamate aspartate transaminase (L-aspartate:2-oxoglutarate aminotransferase EC 2.6.1.1.) from dog heart, the electrophoretically faster migrating enzyme was localized in the supernatant fraction while the slow one was associated with the mitochondrial fraction (Boyd, 1961). Later experiments led to the purification to varying degrees of the two enzymes from a variety of mammalian tissues including rat liver (Boyd, 1966), beef heart and liver, and pig heart (Wada and Morino, 1964). It is well established that these transaminases exist in two distinct forms or isozymes that are easily distinguishable immunologically (Wada and Morino, 1964) by starch gel electrophoresis (Martinez-Carrion *et al.*, 1965) and by differences in their substrate K_m's and rate dependence on pH (Fleischer *et al.*, 1960). In fact, differences in the properties of the two enzymes may result from distinct structural compositions dictated by variations in the genetic code of the particular isozyme. In addition, it is known (Martinez-Carrion *et al.*, 1965) that the S-GAT[1] can exist and be isolated in at least four forms that have similar primary structure and catalytic properties. Therefore, it would be of interest to determine to what extent we can differentiate between

* From the Department of Chemistry, University of Notre Dame, Notre Dame, Indiana. *Received January 4, 1967.* A preliminary report was presented at the Indiana Academy of Sciences Meeting, Fort Wayne, Ind., Oct 1966.

[1] Abbreviations used: supernatant glutamate–aspartate transaminase, S-GAT; mitochondrial glutamate–aspartate transaminase, M-GAT; *N*-bromosuccinimide, NBS; mercuribenzoate, MB.