RECOVERY OF TYROSINE ACCEPTOR ACTIVITY BY COMBINING 3'-HALF MOLECULE WITH STEPWISE DEGRADATION PRODUCTS OF 5'-HALF MOLECULE OBTAINED FROM TYROSINE tRNA

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Two half molecules were obtained from purified Torulopsis utilis tyrosine tRNA by scission with RNase T_1 of the G-Ψ bond of the presumed anticodon GΨA. When these halves were mixed, full tyrosine acceptor activity was recovered, although each of the halves was inactive. After splitting the 3'-terminal residues of the 5'-half and mixing the products with the 3'-half, the activity was fully recovered as compared with native tyrosine tRNA, clearly indicating that the first letter of the anticodon is not regarded as one of the tyrosine recognition sites.

There have been many reports suggesting that codon recognition site in tRNA might also function as amino acid recognition site (e.g., 1 - 3). More recently some data however have suggested that the anticodon might not be the site for determining amino acid acceptor specificity (4 - 7).

Yeast $tRNA^{Val}$ was split by Bayev et al. (8) into two half molecules by digestion of the I-A bond of the presumed anticodon with guanyloribonuclease (RNase T_1-like enzyme). A mixture of the halves accepted valine to the same extent as did the intact molecule Nishimura et al. showed that digestion of E. coli $tRNA^{Val}$ with B. subtilis RNase (9), of E. coli $tRNA^{Met}$ and $tRNA^{Tyr}$ with RNase T_1 (10, 11) produced respectively a set of two large fragments whose mixture accepted respective amino acids. These fragments were formed by splitting somewhere in the anticodon and dihydrouridine loops and S-region, respectively. Scission of yeast $tRNA^{Phe}$ at the base next to the anticodon was achieved by Zachau et al. with

a chemical treatment (12). They showed that the mixture of the resulted two halves had 50 - 60 % of phenylalanine acceptor activity as compared with unmodified $tRNA^{Phe}$.

We previously reported the primary structure of T. utilis $tRNA^{Tyr}$ (13). During the determination of the structure, we obtained half molecules of this RNA by splitting the G-ψ bond of the presumed anticodon GψA by limited RNase T_1 digestion. Then studies have been planned to determine whether the anticodon is related to amino acid recognition site or not, by measuring the tyrosine acceptor activity of the mixture of stepwise degradation products of the half molecules. This paper reports that the tyrosine acceptor activity is recovered when the 3'-half molecule is combined with the 5'-half molecule of which 3'-terminal Gp is removed (first letter of the anticodon).

MATERIALS AND METHODS

$tRNA^{Tyr}$ was isolated from T. utilis tRNA mixture as described previously (13). Tyrosyl-tRNA synthetase was extracted from baker's yeast and partially purified by chromatography on a DEAE-cellulose column. Method of assay for amino acid acceptor activity described previously (14) was used with a minor modification. tRNA fragments in 5 mM Tris-HCl (pH 7.5) containing 10 mM $MgCl_2$ were preincubated at 37°C for 1 hour, cooled immediately at room temperature (20 - 25°C), and then assayed.

A typical method of preparation of the half molecules was as follows: Purified $tRNA^{Tyr}$ (13.1 mg) in 3 ml of 0.05 M Tris buffer (pH 7.5) and 0.1 M $MgCl_2$ was digested with 100 units of RNase T_1 at 0°C for 30 min. Human serum albumin was then added and the enzyme was extracted with phenol saturated with 0.05 M Tris buffer (pH 7.5). The aqueous layer was washed with ether and then kept overnight at pH 1 and 0°C to cleave terminal cyclic phosphate.

The digest was chromatographed on a column of DEAE-cellulose (0.5 x 150 cm), eluting with a linear gradient from 0.2 to 0.5 M NaCl in 7 M urea - 0.005 M Tris-HCl, pH 7.5 (300 ml each). The main peak which contained half molecules and intact tRNA was rechromatographed on a column of DEAE-cellulose (0.4 x 120 cm), eluting with a linear gradient from 0.15 to 0.4 M NaCl in 7 M urea - 0.1 M HCOOH (300 ml each). Fractions were pooled and desalted with dialysis four times against 50 - 100 volumes of 1 mM $MgCl_2$ in 1 mM Tris-HCl (pH 7.0). The inner fluid was dried with repeated evaporation and dissolved in 5 mM Tris-HCl (pH 7.5) containing 10 mM $MgCl_2$ to make about 10 OD_{260} units per ml solution.

Phosphate of both ends of the 5'-half molecule was removed by E. coli alkaline phosphatase treatment. Phosphatase (10 µg in 20 µl) was mixed with 7 OD_{260} units of the 5'-half in 1 ml of 20 mM Tris-HCl (pH 8.3). After two hour incubation at room temperature, the enzyme was removed by phenol extraction.

3'-Terminal guanosine moiety of the 5'-half molecule was removed according to Khym and Uziel (15).

RESULTS

Fig. 1 shows the profiles of fractionation of half molecules obtained by limited digestion of $tRNA^{Tyr}$ with RNase T_1. The main peak in Fig. 1a consisting of the 3'- and 5'-halves and a small amount of intact $tRNA^{Tyr}$ was rechromatographed as shown in Fig. 1b. Fraction IV in Fig. 1b was treated with phosphatase (fraction IV-1) followed by treatment with $NaIO_4$ - lysine buffer (fraction IV-2). Analysis of complete RNase T_1 digests of the fractions indicated that fraction I was composed of about 90 % of the 3'-half molecule and 10 % of the 5'-half molecule, and fraction IV-2 was about 75 % of the 5'-half and 25 % of the 3'-half as shown in Table I. Fraction VIII was intact $tRNA^{Tyr}$. The 5'-half in fraction IV-2 was

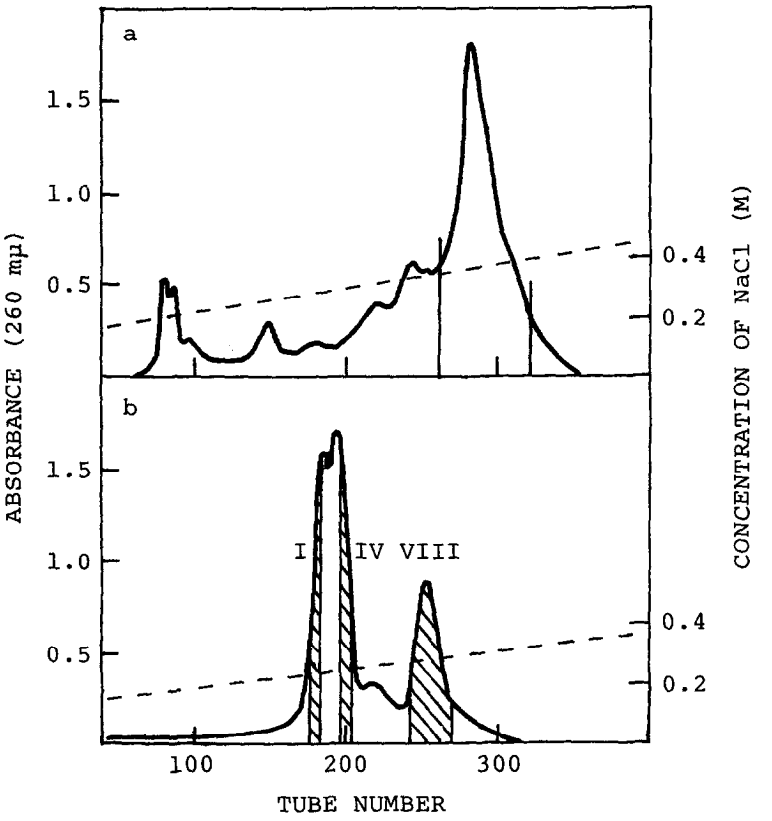

Fig. 1. Separation of half molecules produced by limited digestion of tyrosine tRNA with RNase T_1. a: Chromatography on a DEAE-cellulose column at pH 7.5. b: Rechromatography of the main peak fraction of Fig. 1a on a DEAE-cellulose column at pH 3.5.

almost free from Gp of the 3'-end but phosphate was incompletely removed from the 5'-end.

Tyrosine acceptor activity was measured for each fraction and for the mixture of fraction I with fractions IV, IV-1 and IV-2. As shown in Table II, fractions I and IV alone accepted a small amount of tyrosine, as either half molecule contained a small amount of its counterpart. By mixing fraction I with fraction IV or with IV-1 in nearly equimolar ratios with respect to the 5'- and 3'-halves, the acceptor activity per mole of the 3'-half molecule of the mixture reached to its maximum. When fractions I and IV-2 were mixed, the activity per mole of the 3'-half was fully recovered as

compared with native $tRNA^{Tyr}$.

Table I

Analysis of RNase T_1 Digests of Fractions I and IV in Fig. 1b*

	Fragment	Molar ratio based on established sequence of $tRNA^{Tyr}$ (13)	Analysis	
			Fr. I moles per mole of A-C-C-Aoh	Fr. IV-2** moles per mole of U-m^1G-m^2Gp
5'-Half	C-U-C-U-C-Gp	-	-	0.4 } 0.9
	pC-U-C-U-C-Gp	1	<0.1	0.5 }
	U-m^1G-m^2Gp	1	<0.1	1.0
	C-C-A-A-Gp	1	<0.1	1.1
	hU-hU-G^m-Gp	1	<0.1	0.9
	hU-hU-hU-A-A-Gp	1	<0.1	1.0
	C-m^2_2G-Ψ-C-A-Gp	1	<0.1	1.1
	Gp	2		4.0
	A-C-U-Gp	1	<0.1	-
	A-C-Up	-	-	1.0
	Guanine***	-	-	1.0
3'-Half	Gp	2	1.6	
	Ψ-A-iA-A-Ψ-C-U-Gp	1	0.8	0.2-0.3
	A-A-C-A-hU-m^5C-Gp	1	1.0	0.2-0.3
	C-Gp	1	0.9	0.2-0.3
	T-Ψ-C-Gp	1	0.8	0.2-0.3
	m^1A-A-U-C-Gp	1	0.6	0.2-0.3
	C-C-C-C-C-Gp	1	0.7	0.2-0.3
	A-Gp	2	1.6	0.6
	A-C-C-Aoh	1	1.0	-
	A-C-Cp	-	-	0.3
	Adenine***	-	-	0.4

* The abbreviations were used according to the new IUPAC-ICB nomenclature rules regarding nucleic acids.

** Fraction IV was analyzed after the stepwise removal of the 3'-terminal nucleotide.

*** When the 3'-terminal residue was released from the dephosphorylated product of fraction IV, guanine and adenine were formed which were identified by chromatography on Sephadex G-10. Adenine was derived from the 3'-end of the 3'-half contained in fraction IV.

Table II

Recovery of Tyrosine Acceptor Activity by Combining 3'-Half with Derivatives of 5'-Half of $tRNA^{Tyr}$ Molecule

Fraction	Composition of fraction* μμmoles					[^{14}C]Tyrosine** accepted	
	3'	5'	5'(-p)	5'(-Gp)	3'(-A)	cpm per reaction mixture	cpm per mμmole of 3'-half
I	172	20				160	930
IV	43	142				212	4930
I+IV	95	148				608	6420
	129	152				906	7020
	387	182				1921	4960
I+IV-1	227	20	183			1747	7700
IV-2				158	48	22	—
I+IV-2	172	20		158	48	902	5420
VIII	intact $tRNA^{Tyr}$, 110 μμmoles					625	5700
Purified native $tRNA^{Tyr}$, 68 μμmoles						355	5250
				136 μμmoles		711	5260

* The abbreviations used were: 3', 3'-half molecule; 5', 5'-half molecule; 5'(-p), 5'-half molecule treated with E. coli alkaline phosphatase; 5'(-Gp), 5'-half molecule deprived of its 3'-end Gp; 3'(-A), 3'-half molecule deprived of its 3'-end adenosine. Composition of each fraction was estimated from Table I.

** Specific activity, 5 μc/μmole.

DISCUSSION

The anticodon sequence of E. coli tyrosine tRNA is GUA and the suppressor gene (Su^{+}_{III}) product is tyrosine tRNA of which anticodon is altered to CUA (4). This change of G to C enables the tRNA to recognize the amber codon UAG instead of the tyrosine codons UAU and UAC, but does not influence the tyrosine recognition specificity. Yoshida et al. (5) modified alanine tRNA with acrylonitrile and showed that the product was fully active in the alanine acceptance, in spite of that the first letter (Ip) of its anticodon

and ψp in the anticodon loop were cyanoethylated. These results suggest that the first letter of anticodon may not be one of the amino acid recognition sites. The present paper proves this clearly on the structural basis.

In Bacillus megaterium there are found two tRNAs which both accept glutamic acid. One of them recognizes glutamine codons CAA and CAG, and the other does glutamic acid codons GAA and GAG (6). Hence the third letter of the anticodon seems to be not concerned in glutamic acid accepting specificity. Considering these together, it is possible that all anticodon bases may not function as the amino acid recognition sites.

When the 3'-half was mixed with the 5'-half and its derivative free from end-phosphorus in nearly equimolar ratios, the reconstituted molecule appeared to accept tyrosine more than intact tyrosine tRNA. Besides only less than 60 % of the intact RNA molecules accepted tyrosine under the conditions used and also even with much higher concentration of enzyme and prolonged reaction time. The reason why the combined fragments are so active is not clear. Further studies are in progress using shorter fragments formed from either half molecule with stepwise degradation.

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