ANTICODON STRUCTURE OF GAA-SPECIFIC GLUTAMIC ACID tRNA FROM YEAST

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Summary. A yeast glutamic acid tRNA, which specifically recognizes GAA codon but not GAG codon, was digested with RNase T_1 and the nucleotide sequences of the fragments were determined. A minor nucleoside, 2-thiouridine derivative (S), was found in the presumed anticodon, SpUpCp. The possible function of this minor nucleoside for the specific recognition of GAA by this tRNA was discussed.

Many purified tRNA's[1-4] have been reported to recognize more than two synonym codons, which are different by one base usually at third position, and these phenomena were explained by the "Wobble Hypothesis" proposed by Crick[5]. Recently, Sekiya et al[6] of our laboratory discovered a unique species of glutamic acid tRNA from yeast ($tRNA^{Glu}_{III}$) which recognized only GAA but not GAG as codon for glutamic acid. Since this specific codon recognition by $tRNA^{Glu}_{III}$ could not be explained by Crick's "Wobble Hypothesis", an unusual structure of anticodon region in the $tRNA^{Glu}_{III}$ was proposed[6].

This paper describes the identification of a 2-thiouridine derivative (S) in the first letter of the presumed anticodon in $tRNA^{Glu}_{III}$ and discussion with respect to the possible role of this minor nucleoside (S) in GAA specific recognition by this tRNA.

Materials and Methods

$tRNA^{Glu}_{III}$ was purified from unfractionated tRNA of baker's yeast by column chromatography on DEAE-Sephadex A-50 as described previously[7] and subsequently on benzoylated DEAE-cellulose according to Gillam et al[8]. The purity of the final preparation of $tRNA^{Glu}_{III}$ was estimated to be at least 85 % from the observed acceptance of 1400 pmoles of glutamic acid by 1 A_{260} unit of the tRNA

The purified tRNA was confirmed to be specific to GAA by the ribosomal binding experiments as described previously[9]. The $tRNA^{Glu}_{III}$ (100 A_{260} units) was digested with RNase T_1 (200 units) at pH 7.5 for 15 hours and the fragments were separated by DEAE-Sephadex A-25 column chromatography in the presence of 7 M urea. The nucleotide sequence of the fragment was determined by digestion with various enzymes having different base specificity and mode of action and subsequent identification of the products. The details of these procedures will be reported elsewhere[10].

Results and Discussion

The nucleotide sequences of all fragments obtained by RNase T_1 digestion of $tRNA^{Glu}_{III}$ and the degradation experiments used for the sequence determination of fragment X are summarized in Table I and Fig. 1, respectively. A minor nucleotide, Sp, isolated from fragment X showed unique ultraviolet absorption spectra (Fig. 2a). The similarities of this absorption spectra to

Table I. Nucleotide sequence of fragments obtained by RNase T_1 digest of $tRNA^{Glu}_{III}$

Fragment No	Nucleotide sequence	Molar ratio
I	CpC	1.00
II	Gp	6.85
III	UpGp	2.20
IV	ApGp	3.28
V	TpΨpCpGp	1.21
VI	ApCp5MeCpGp	1.16
VII-I	ΨpApApCpGp	1.08
VII-II	UpApUpCpGp	1.06
VIII-I	ApUpApUpApGp	1.10
VIII-II	pUpCpCpGp	1.06
IX	ApCpUpCpCpCpCpGp	1.08
X	CpUpSpUpCpApCpCpGp	0.95
XI	CpdiHUpApUpCpApCpApΨpCpApCpGp	0.90

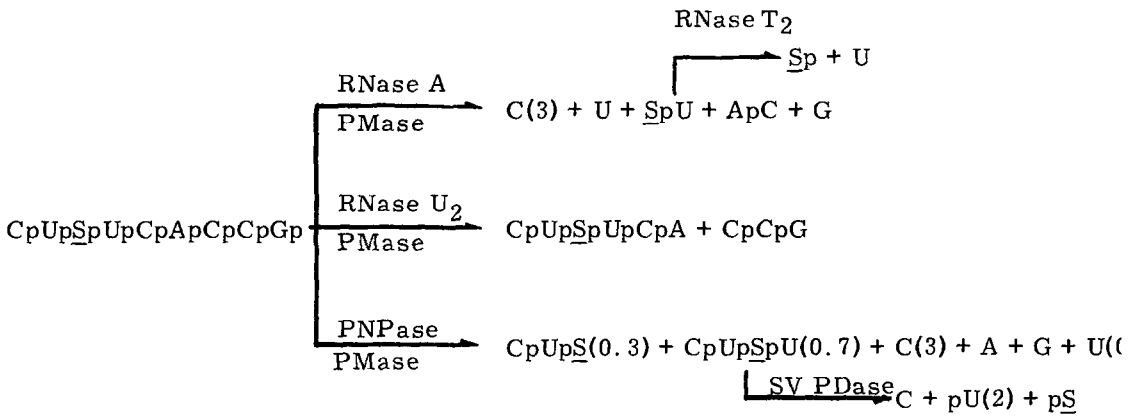

Fig. 1. Scheme of sequential analysis of fragment X. PMase, E. coli alkaline phosphomonoesterase; SV PDase, snake venom phosphodiesterase; PNPase, polynucleotide phosphorylase from M. lysodeikticus. The numerals parenthesis indicate the molar ratio of the products.

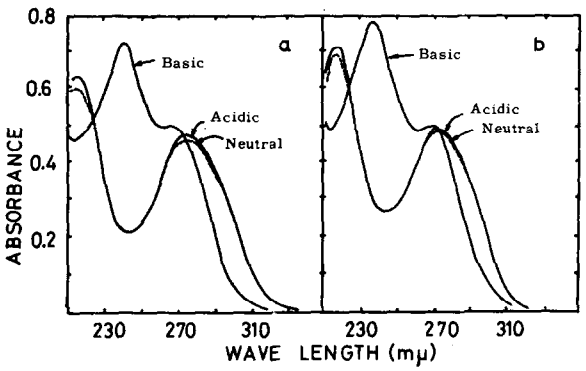

Fig. 2. Ultraviolet absorption spectra of a minor nucleotide, Sp, isolated from fragment X (a) and of 2-thiouridine (b).

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that of 2-thiouridine (2tU) (Fig. 2b) indicated that Sp was a derivative of 2-thiouridine. The nucleoside, S, obtained by phosphatase treatment of Sp, however, was different from 2tU or 5-methyl-2tU in the cellulose thin layer chromatography as shown in Table II. Although the nucleoside, S, was electrophoretically neutral at pH 6.8, a product formed by 0.2 M NaOH-treatment of S at 100° for 40 minutes showed negative charge at pH 6.8. These results indicated that this nucleoside, S, is probably identical with 2-thio-5(6)-uridine

Table II. Relative Rf values of a minor nucleoside, S, in cellulose thin layer chromatography

Compounds	Relative mobilities to uridine	
	in Solvent A*	in Solvent B*
S	1.75	1.30
2-Thiouridine	1.30	1.01
5-methyl-2-thiouridine	1.54	1.07

* Solvent A: n-butanol-acetic acid-water (5:3:2) and Solvent B: 2-propanol-conc, ammonia-water (7:1:2).

acetic acid methyl ester found in the total mixture of yeast tRNA by Baczynsky, et al[11].

Since $tRNA^{Glu}_{III}$ can recognize only GAA codon, the corresponding anticodon can be UpUpCp or ?pUpCp (?p represents an unusual nucleotide). The data in Table I show that no UpUpCp sequence is present in $tRNA^{Glu}_{III}$ and the only possible anticodon sequence is SpUpCp in fragment X. On the other hand, the primary structures of tRNA's hitherto reported indicate that the anticodon sequence is generally preceded by PypUp sequence at the 5'-side and followed by a modified nucleotide or unmodified adenylic acid at the 3'-side. From these respects, it is most probable that the sequence SpUpCp in the fragment X, CpUpSpUpCpApCpCpGp, is the anticodon of $tRNA^{Glu}_{III}$.

According to Crick's "Wobble Hypothesis"[5], C-2 oxygen and N-3 hydrogen of uracil perticipate in U-G pairing instead of C-4 oxygen and N-3 hydrogen in the standard U-A pair (Fig. 3a and b). Since S in the presumed anticodon, SpUpCp, contains sulfur at the C-2 position, it will not be able to form a stable S-G pair as shown in Fig. 3c, because S···H hydrogen bonding, if present, is known to be very weak compared with O···H hydrogen bonds[12]. On the other hand, S is able to pair with A providing C-4 oxygen and N-3 hydrogen to the hydrogen bonding (Fig. 3a). These considerations can reasonably explain the role of the presumed anticodon SpUpCp in the mechanism of the

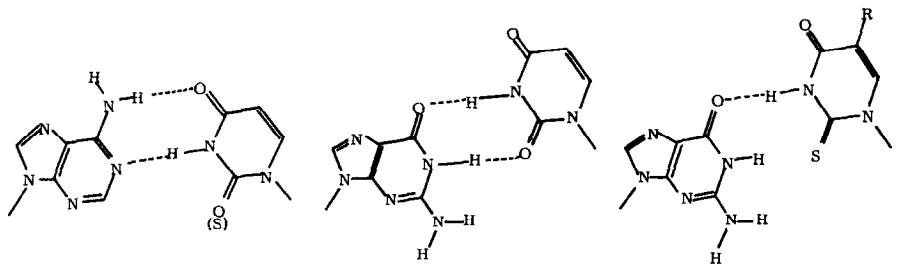

Fig. 3. Possible base pairing in the specific recognition of A by a 2-thiouridine derivative, S.

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specific recognition of GAA codon by $tRNA^{Glu}_{III}$. The presence of thiated base in the anticodon of glutamic acid tRNA which was found this time and its possible function in the specific recognition of GAA by this tRNA are very interesting in connection with the observations that some species of glutamic acid tRNA from E. coli and rat liver also contain a 2-thiouridine derivative (S. Nishimura, personal communication).

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