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ACCEPTOR ACTIVITY OF INCOMPLETE AGGREGATE MOLECULES OF VALINE tRNA 1 CONSISTING OF FOUR FRAGMENTS

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1. Introduction

The study of the functional topography of yeast valine tRNA by the "dissected molecules" method is being continued. The primary structure of yeast valine tRNA has been established earlier [1]. Bayev et al. [2] were the first to show that $tRNA_1^{Val}$ halves prepared by splitting one phosphodiester bond in the anticodon region form "dissected aggregate molecules" possessing acceptor but no transfer activity [3]. In some cases dissected incomplete $tRNA_1^{Val}$ molecules (lacking some oligonucleotides) have also been shown to possess acceptor activity [4–6]. Recently it has been shown that in mixtures of fragments of formylmethionine [7], phenylalanine [8], *E. coli* valine tRNA [9] and alanine tRNA 2 [10] the acceptor activity is restored.

In the present paper we describe the acceptor activity of incomplete $tRNA_1^{Val}$ molecules, lacking the dinucleotide C_{17}-Gp in the dehydrouridylic loop. The molecules were reconstituted from four fragments.

2. Methods

The fragments F_{19-35}, F_{36-57} and F_{58-77} were prepared by partial digestion of the 3′- and 5′-halves $tRNA_1^{Val}$ *Saccharomyces cerevisiae* molecules with guanylo-ribonuclease from *Actinomyces aureoverticillatus* (EC 2.7.7.26); the fragment F_{1-16} was prepared by partial digestion of whole $tRNA_1^{Val}$ molecules with pancreatic RNase (EC 2.7.7.16). Pure fragments were isolated by ion exchange chromatography on DEAE-cellulose in 7 M urea at pH 8.0 and subsequent rechromatography at pH 3.3 [6]. Fragment F_{1-16} was identified by two-dimensional thin-layer chromatography of its pyrimidylo-RNase digestion products and by the determination of the nucleotide composition of each oligonucleotide. The other fragments were identified as described previously [6].

The assay mixture for the estimation of the acceptor activity of $tRNA_1^{Val}$ fragments contained in a total volume of 0.5 ml (μmole): Tris-HCl buffer, pH 7.5, 50; $MgCl_2$, 10; KCl, 10; EDTA, 1; ATP, 5; ^{14}C-labelled valine (specific activity 105 μc/μmole) 1 μc; partially purified yeast aminoacyl-tRNA-synthetase 0.2 mg (protein) and specified amounts of $tRNA_1^{Val}$ fragments. The fragments were dissolved in water and after addition of all other components the mixture was kept at 37° for 5 min and slowly cooled (20 min) to 0°. After addition of the enzyme the mixture was incubated at 0° for 6 hr. Further steps of the procedure have been described elsewhere [6].

3. Results

The studied fragments (quarters) of $tRNA_1^{Val}$ have nearly equal length and consist of 16 to 23 nucleotides. They were prepared by splitting $tRNA_1^{Val}$ molecules at the hU-, T- and anti-codon loops.

Data concerning the acceptor activity of the fragment mixtures are summarized in table 1. Three fragments H_{36-77}, F_{1-16}, F_{19-35} form incomplete molecules with excised dinucleotide C_{17}–Gp in the hU-loop (fig. 1). The aggregate exhibits nearly the same acceptor activity as the dissected molecule formed from two halves (table 1, exp. 1,2). Incomplete molecules with three broken phosphodiester

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Table 1
Acceptor activity of $tRNA_I^{Val}$ fragments. Incubation mixture (see Methods) contained 3′- or 5′-halves 0.05 A_{260} unit and quarter-fragments 0.03 A_{260} unit each. Halves and quarter-fragments of $tRNA_I^{Val}$ are symbolized by H and F respectively. Indices of H and F represent the ordinal number of the corresponding nucleotide beginning from the 5′-end of the $tRNA_I^{Val}$ molecule.

Exp. No.	$tRNA_I^{Val}$ fragments Halves		Quarter	^{14}C-valine incorporation cpm
1	H_{1-35} +	H_{36-77}	–	18,200
2	–	H_{36-77} +	F_{1-16} + F_{19-35}	13,800
3	H_{1-35}	–	+ F_{36-57} + F_{58-77}	16.500
4	–	–	F_{1-16} + F_{19-35} + F_{36-57} + F_{58-77}	9,600
5	H_{1-35}	–	–	205
6	–	H_{36-77}	–	240
7	–	H_{36-77} +	F_{1-16}	60
8	–	H_{36-77} +	F_{19-35}	150
9	H_{1-35}	–	+ F_{36-57}	230
10	H_{1-35}		+ F_{58-77}	110
11	–	–	F_{1-16} + F_{58-77}	300

bonds (in the hU-, T- and anticodon loops) and consisting of the four quarter-fragments have nearly 50% activity (table 1, exp. 4).

Mixtures consisting of either the 3′-or the 5′-halves plus one quarter of the opposite half are fully inactive at 0° (table 1, exp. 7–10). Similarly, a system consisting of the F_{1-16} + F_{58-77} fragments, representing the acceptor part of tRNA molecule, are also inactive. Earlier, negative results have already been obtained for the aminoacylation at 15°C of the mixtures described in experiments 8–10 of table 1 [5].

4. Discussion

It has been shown earlier that the 3′- and 5′-halves of $tRNA_I^{Val}$ have no acceptor activity and do not inhibit enzymatic aminoacylation. However, the halves easily form "dissected aggregate molecules" upon mixing and thus the full acceptor activity is restored [2,12]. By analogy, we suppose that the restoration of acceptor activity of quarter fragments is also preceded by their self-assembly in aggregate molecules. Incomplete $tRNA_I^{Val}$ molecules devoid of the $pI_{35}p$ or ψ_{33}-C-Ip sequence in the anticodon loop [4,5] or of the ubiquitous tetranucleotide sequence T_{54}-ψ-C-Gp in the T-loop [6] also exhibit acceptor activity. Incomplete molecules lacking the C_{17}-Gp dinucleotide in the hU-loop also have full acceptor activity. Thus, we conclude that the above-mentioned nucleotide sequences in the anticodon, dihydrouridylic and the thymidylic loops and perhaps all these loops in toto are not necessary for the interaction between tRNA and aminoacyl-tRNA-synthetase. However, the removal of any of the possible four quarters from $tRNA_I^{Val}$ molecules prevents the restoration of acceptor activity possibly as a result of structural alteration in the acceptor and anticodon two-strand limbs of the molecule. This conclusion is supported by the observation that half-molecules of $tRNA_I^{Val}$, if applied separately, do not inhibit the process of aminoacylation of the $tRNA_I^{Val}$ molecule [11]. Low activity sometimes found in the experiments 7–11 (table 1) is due to the presence of impurities in the fragments. No activity is obtained when the fragments are carefully purified. Therefore, the purity of each fragment was always analysed.

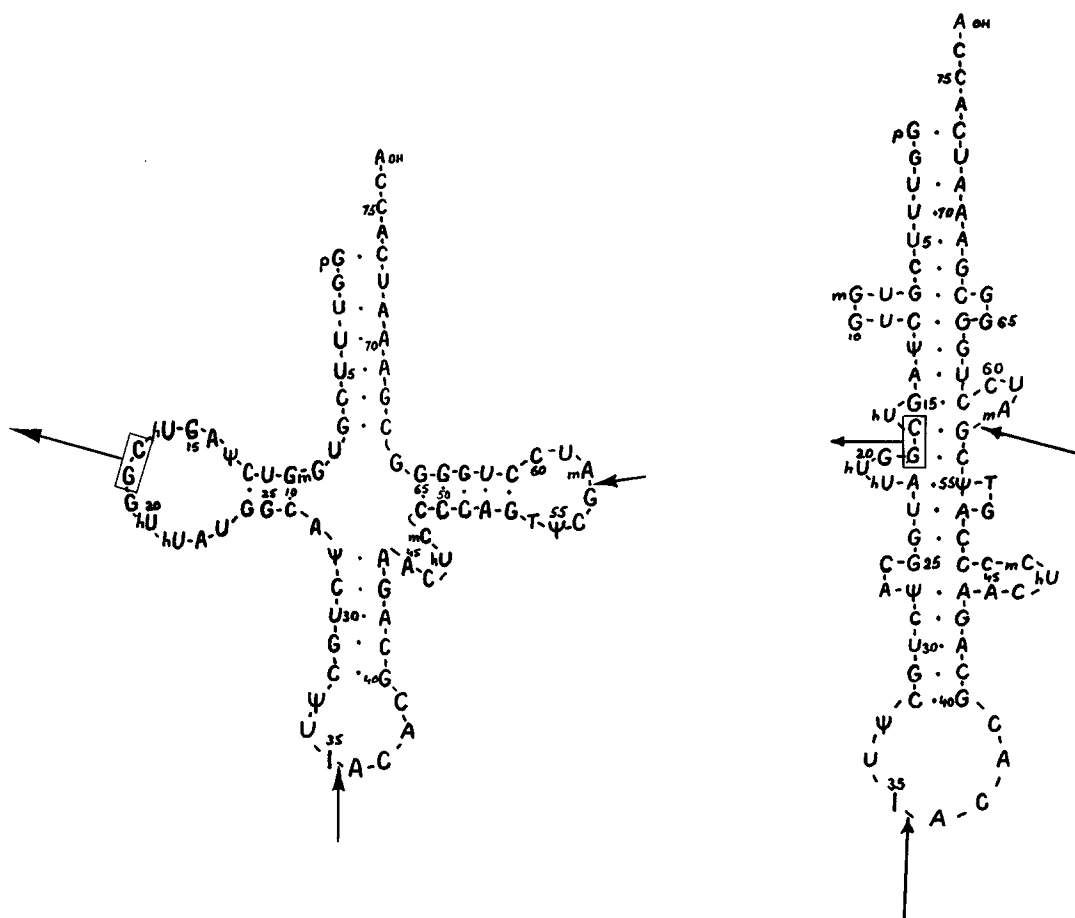

Fig. 1. Clover leaf (a) and one-hairpin (b) models of the active $tRNA_1^{Val}$ molecule. The arrows show split phosphodiester bonds and eliminated dinucleotide C_{17}-Gp.

Imura et al. [10] reported that the acceptor activity of 3′- and 5′-acceptor quarter fragments of yeast $tRNA_2^{Ala}$ was up to 5% and that of 3′-half and acceptor 5′-quarter fragments up to 25%. We could not find any acceptor activity in corresponding systems from yeast $tRNA_1^{Val}$ (table 1, exp. 7–11).

Two $tRNA_1^{Val}$ secondary structure models contain a maximal number of Watson-Crick complementary pairs: Holley's [12] clover leaf model and the one-hairpin model (fig. 1). The observation of self-assembly of four $tRNA_1^{Val}$ quarter fragments is more consistent with the clover leaf model than with the one-hairpin model because in the clover leaf model each quarter interacts with two complementary fragments and thus stabilizes the four-piece aggregate molecule. With the one-hairpin model two broken phosphodiester bonds are almost at the same level of the rod-like molecule and it is difficult to explain how the self-assembly could proceed.

Further studies on the structural basis and mechanism of $tRNA_1^{Val}$ acceptor activity with the "dissected molecule" method are in progress.

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