ENZYMATIC AMINOACYLATION OF DISSECTED MOLECULES OF BAKER'S YEAST VALINE tRNA 1

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1. Introduction

Although the function of tRNA and the role of specific regions of the various tRNA molecules in protein synthesis have been studied intensively, relatively little progress has been made in this field. Thus, the anticodons of valine [1], tyrosine [2] and formylmethionine [3] tRNA's have been established by direct experiments. However, attempts to locate the aminoacyl tRNA synthetase recognition sites have not been successful. It has been shown that the terminal sequence C-C-A_{OH} is indispensable for aminoacylation [4] but the role of other regions of the tRNA molecule remained rather ambiguous. The anticodon, most probably, has no significance in the recognition of aminoacyl tRNA synthetase [1,3,5–7]. In 1967 we have reported data on the enzymatic aminoacylation of $tRNA_1^{Val}$ halves [8] and on the functional topography of tRNA by using the "dissected molecule" method. Here we describe the results of further investigations on aminoacylation of valine tRNA fragments.

2. Methods

$tRNA_1^{Val}$ fragments were prepared by partial digestion of $tRNA_1^{Val}$ 3′- and 5′- halves with guanylo-RNase (EC 2.7.7.26) from *Actinomyces aureoverticillatus* Kras et Di Shen and pancreatic pyrimidylo-RNase (EC 2.7.7.16). The fragments were isolated and purified by ion exchange chromatography on a DEAE-cellulose column at pH 8.0 and pH 3.3 in 7M urea [9].

The fragments were identified by the method previously described [9] or by two dimensional thin layer cellulose chromatography of complete quanylo- or pyrimidylo-RNase digests of the fragments. The assay mixture for the estimation of acceptor activity of $tRNA_1^{Val}$ fragments contained the following components (μmoles) in a total volume of 0.5 ml: Tris-HCl buffer, pH 7.5, 50; $MgCl_2$ 10; KCl 10; EDTA 1; ATP 5; C^{14}-labelled valine (specific activity 105 $\mu C/\mu$mole) 1; partially purified yeast aminoacyl tRNA synthetase 0.2 mg (protein); and specified amounts of $tRNA_1^{Val}$ fragments.

$tRNA_1^{Val}$ fragments were dissolved in water and after the addition of all the components except the enzyme the mixture was preincubated at 15° for 20 min. After addition of the enzyme the mixture was incubated for 30 min at 15°. The reaction was stopped with the addition of 0.5 mg of Cetavlon (cetyltrimethylammonium bromide) dissolved in 0.1 ml of 0.75 M phosphate buffer, pH 5.0. The precipitate was collected on a microcellulose filter, washed with 50 ml of 0.03% Cetavlon solution, dried and counted in a scintillation counter with 50–60% efficiency.

3. Results

The primary structure of $tRNA_1^{Val}$ has been established earlier [10]. Bayev et al. [8] have found that the halves of $tRNA_1^{Val}$ obtained after the cleavage of the phosphodiester bond between I_{35} and A_{36} in the anticodon region did not possess the ability to be aminoacylated and did not inhibit the enzymatic aminoacylation of the intact $tRNA_1^{Val}$ either. Never-

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Table 1

Acceptor activity of $tRNA_1^{Val}$ fragments. Incubation mixture (see Methods) contained $tRNA_1^{Val}$ 0.09 A_{260} unit, 5′- or 3′-half 0.05 A_{260} unit, and fragments 0.03 A_{260} unit each. Halves and other shorter fragments of $tRNA_1^{Val}$ are denoted by H and F respectively. Indices at H and F represent the original numbers of the corresponding nucleotides beginning from the 5′ end of the $tRNA_1^{Val}$ molecule.

$tRNA_1^{Val}$ fragments Halves : Fragments	^{14}C-valine incorporation c.p.m.
1 $tRNA_1^{Val}$	14500
2 $H_{1-35} + H_{36-77}$	14500
3 $H_{1-32} + H_{36-77}$	13800
4 $H_{1-35} + H_{41-77}$	33
5 $H_{1-35} + H_{41-77} + A-C-A-C-G_p$	400
6 $H_{1-35} + F_{41-57}$	171
7 $H_{1-35} + F_{58-77}$	208
8 $H_{1-35} + F_{36-53}$	99
9 $H_{1-35} + F_{36-57}$	879
10 $H_{36-77} + F_{19-35}$	198
11 $H_{1-35} + F_{41-57} + F_{58-77}$	151
12 $H_{1-35} + F_{36-57} + F_{58-77}$	14925
13 $H_{1-35} + F_{36-53} + F_{58-77}$	5080

theless, upon mixing the two halves form an aggregate exhibiting an acceptor activity equal to that of intact $tRNA_1^{Val}$. Data concerning the acceptor activity of $tRNA_1^{Val}$ fragments other than the halves described above are summarized in table 1.

We suppose that the restoration of acceptor activity is preceded by the self-assembly of the fragments in the aggregate tRNA molecules. Two kinds of aggregate molecules can be distinguished: (1) those with one or more split phosphodiester bonds (dissected molecules), (2) aggregate molecules lacking some part of the polynucleotide chain (incomplete molecules).

The incomplete aggregate molecule, with excised trinucleotide $\psi_{33}-U-I_p$ in the anticodon region, as shown in fig. 1, exhibits the same acceptor activity as a dissected molecule formed by the two halves (table 1, exp. 3).

The dissected aggregate molecule with two split phosphodiester bonds at I_{35} and G_{57} exhibits full acceptor activity. A more incomplete molecule, devoid of the ubiquitous tetranucleotide sequence $T_{54}-\psi-C-G_p$, has only 35% activity (table 1, exp. 12 and 13). The incomplete aggregate molecule, devoid of the pentanucleotide $A_{36}-C-A-C-G_p$, is fully inactive when composed of two or of three fragments (table 1, exp. 4 and 11). The activity is not restored if the pentanucleotide $A_{36}-C-A-C-G_p$ is added to the incubation mixture (table 1, exp. 5).

Other fragment combinations shown in table 1 are also inactive (exp. 5–10).

The results of the functional study of $tRNA_1^{Val}$ fragments are summarized in fig. 1. $tRNA_1^{Val}$ is presented in the clover leaf model with two split internucleotide bonds. However, we do not assume this to be the real shape of $tRNA_1^{Val}$ aggregated molecules.

4. Discussion

The removal of three nucleotides $\psi_{35}-U-I_p$ from the seven constituting the anticodon loop of $tRNA_1^{Val}$ (No. 33–39), including the first anticodon nucleotide I_{35}, does not impair the acceptor activity of the molecule. There is some evidence that the other nucleotides

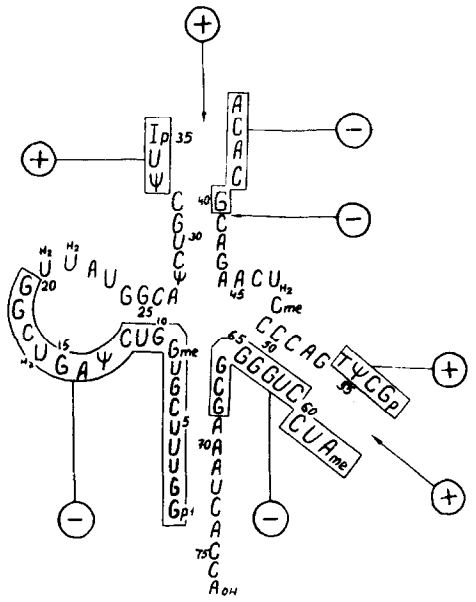

Fig. 1. Acceptor activity of dissected $tRNA_1^{Val}$ molecules. ⊕ aminoacylation; ⊖ no aminoacylation; → cleavage of phosphodiester bond; ▭ eliminated sequences.

of the anticodon loop are also not necessary for the interaction between tRNA and aminoacyl tRNA synthetase. The excision of $\mathcal{9}$ in $tRNA_{yeast}^{Phe}$ [11] as well as the modification of $tRNA_{yeast}^{Ala}$ [7], located in positions, corresponding to the 38th and 39th nucleotides of $tRNA_1^{Val}$ does not impair the acceptor activity either.

The inactivation observed after the removal of the pentanucleotide A_{36}–C–A–C–G_p is due possibly to the lack of the guanylic acid residue G_{40}. The latter may be a component of the recognition site or it is indispensable for the stability of the conformation of the anticodon branch which seems to be of importance for the acceptor activity of the whole molecule.

The elimination of the ubiquitous tetranucleotide sequence T_{54}–ψ–C–G_p does not inactivate $tTNA_1^{Val}$. It has been shown, however, that the 3′-half, which contains the tetranucleotide as a component, is bound to the 50S ribosomal sub-unit [5]. One can assume that the tetranucleotide T_{59}–ψ–C–G_p does not participate in the tRNA – aminoacyl-tRNA-synthetase interaction but it is necessary for binding with the 50S ribosomal sub-unit.

The results of these investigations have been reported at the 6th FEBS Meeting (Madrid, 1969) [12].

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