HYBRIDIZATION OF HETEROLOGOUS FRAGMENTS OF tRNA FROM YEAST, RAT LIVER AND E. COLI

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1. Introduction

Recently a study by the "dissected molecules" method proposed by Bayev et al. [1] of the functional topography of transfer RNA's has been undertaken in our laboratory. It has been demonstrated that yeast $tRNA_1^{Val}$ halves obtained after cleavage of the tRNA molecule at the anticodon site are capable of a self-assembly to form an aggregate, a dissected molecule, that retains the acceptor function but is devoid of the adaptor activity. It has been found thereafter that the acceptor activity of the dissected molecules is retained even after the removal of several nucleotides from the anticodon region, from the thymidylic and dihydrouridylic loops. Also, aggregate molecules reconstituted from four quarter-fragments proved to possess acceptor activity [2–5]. Along with the approach outlined above a study of the functional activity of aggregate molecules with some nucleotide sequences replaced by structurally different fragments, obtained e.g., from other tRNA molecules seems promising for the identification of the active center of tRNA molecules.

The present communication reports the results of the study of the acceptor activity of mixtures of the 3′-half of $tRNA_1^{Val}$ with unfractionated 5′-halves obtained from tRNA of yeast, rat liver and *E. coli*. It was found that yeast $tRNA_1^{Val}$ molecules with 5′-halves substituted by 5′-halves from rat liver tRNA (halves apparently not even belonging to valine tRNA's), possess the ability to be aminoacylated. This the first demonstration of the activity of tRNA hybrid molecules.

The probability of the heterologous tRNA half hybridization appears to be not high, as it follows from comparison of tRNA primary structures. Therefore we have prefered to use the total mixture of 5′-halves, and not individual tRNA halves.

2. Methods

Transfer RNA's and unfractionated aminoacyl-tRNA-synthetases were obtained from yeast [6, 7], rat liver [8, 9] and *E. coli* B respectively [9, 10]. The 3′-half of yeast $tRNA_1^{Val}$ was isolated as described [11]. For the preparation of 5′-halves, tRNA was cleaved by limited hydrolysis with guanylo-ribonuclease of *Actinomyces aureoverticillatus* at 0° in 0.02 M Tris-HCl buffer pH 7.5, in the presence of $MgCl_2$ (0.01 M). The hydrolysate thus obtained consisted mainly of intact tRNA and tRNA cleaved into halves (about 50%). This mixture was treated with periodate to oxidize the vicinal glycol grouping of the 3′-terminal nucleoside of the acceptor end and passed through a column containing polyacrylic acid hydrazide melted into agar gel in 0.075 M sodium acetate, pH 5.6, 0.05M magnesium acetate at 0° according to Knorre et al. [12]. The column was washed with the same buffer and then with water at 20°. The 3′-halves of tRNA molecules cut at the anticodon loop and bound covalently to the hydrazide-agar gel at the oxidized terminal nucleoside of the 3′-halves were dissociated by passing 7 M urea in water at 30° through the column. Under these conditions, the 5′-halves were eluted from column whereas the 3′-halves and the intact tRNA remained bound to the hydrazide-agar gel.

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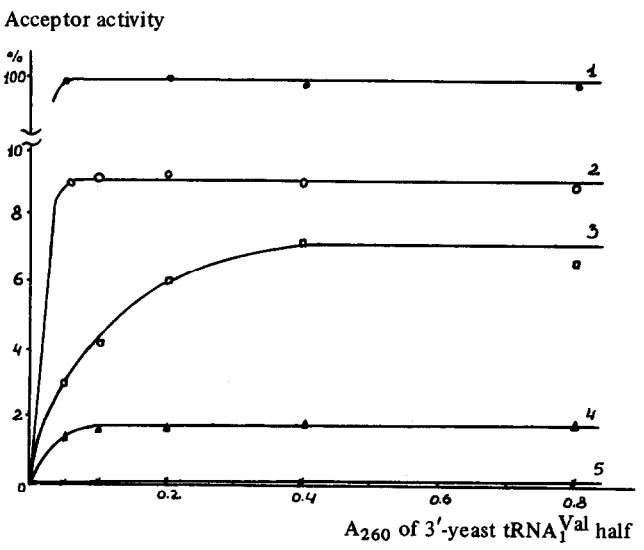

Fig. 1. Acceptor activity of hybrid tRNA molecules formed by mixing of 3′-half of yeast $tRNA_1^{Val}$ with total (unfractionated) preparations of 5′-halves of different tRNA's. (1) Activity of the homologous mixture of 3′- and 5′-halves of yeast $tRNA_1^{Val}$ assumed to be 100%; (2) 5′-halves of total yeast tRNA (yeast enzyme), (3) and (4) 5′-halves of rat liver tRNA (3-rat liver enzyme, 4-yeast enzymes), (5) 5′-halves of *E. coli* tRNA (*E. coli* and yeast enzymes).

An appropriate amount of yeast $tRNA_1^{Val}$ 3′-half was added to 0.2 A_{260} unit unfractionated 5′-halves in 0.4 ml of 0.05 M Tris-HCl buffer (pH 7.5), 0.01 M $MgCl_2$, 0.01 M KCl and 0.001 M EDTA. The mixture was heated for 1 min at 100° and gradually cooled, during 60 min, to 15°; C^{14}-labelled amino acids, ATP and crude aminoacyl-tRNA-synthetase were added to the reaction mixture which was incubated for 1 hr at 15° [6]. Under these conditions, maximal aminoacylation of the mixture of heterologous halves was reached in less than 30 min.

3. Results and discussion

The original guanylo-ribonuclease digest of tRNA when chromatographed on DEAE-cellulose in 7 M urea at 60° [11] was eluted in two separate peaks, the first of these contained a mixture of 3′- and 5′-halves whereas the second one corresponded to tRNA not cleaved at the anticodon loop. Under the same conditions the preparation of the 5′-halves obtained as described above was eluted as a single peak in the region of half molecules and did not contain any detectable amount of intact tRNA. The 3′-halves, unlike the 5′halves contains thymidylic acid in the ubiquitous tetranucleotide T-Ψ-C-G. No thymidylic acid was found in the 5′-halves fraction. The sensitivity of the analytical method enable us to claim an at least 90% purity of the preparation of 5′-halves. The amount of the dissected tRNA molecules having valine acceptor activity in the 3′- + 5′-halves fraction obtained by chromatography on DEAE-cellulose was 9% for yeast tRNA, 0.7% for rat liver tRNA and 0.1% for that of *E. coli*. These values must correspond to the content of 5′-valine halves in the unfractionated preparation of 5′-halves isolated by the hydrazide-agar procedure.

The acceptance of valine by tRNA's of yeast, rat liver and *E. coli* and a mixture of 3′- and 5′halves of yeast $tRNA_1^{Val}$ was approximately the same (± 15%) in the presence of synthetases from yeast or rat liver. Heterologious aminoacylation of yeast and rat liver tRNA with the enzyme from *E. coli* was about 15% and 30% of the homologous amino-acylation, respectively.

The 3′-halves of yeast $tRNA_1^{Val}$ and the 5′-halves unfractionated tRNA's of yeast, *E. coli* and rat liver do not exhibit any acceptor activity, if tested separately. However, the acceptor activity was found to be restored after mixing the 3′-half of yeast $tRNA_1^{Val}$ with the unfractionated 5′-halves of tRNA from yeast and rat liver (fig. 1). The extent of aminoacylation with valine of the mixture of yeast tRNA halves corresponded to a content of 5′-valine halves equal to 9% in the unfractionated yeast 5′-halves preparation. A high specificity of aggregation of the yeast valine tRNA 3′- and 5′-halves in the presence of nonvaline yeast tRNA 5′-halves was demonstrated earlier [7]. The same value was obtained by estimating the valine acceptor activity of the DEAE-cellulose fraction of 3′- + 5′-halves of unfractionated yeast tRNA. The amount of valine accepted by the mixture of $tRNA_1^{Val}$ 3′-half with rat liver 5′-halves in the presence of yeast and liver synthetases differed more than three times and corresponded to a content of 2% and 7% of "active" 5′-halves in the unfractionated 5′-halves preparation. As the content of valine halves in this preparation did not exceed 0.7% (evaluated from the activity of the 3′- and 5′-halves mixture obtained by chromatorgraphy on DEAE-cellulose), it seems that yeast and to a still greater extent rat liver valyl-tRNA-synthetases are able to catalyze the binding of valine to hybrid mole-

cules assembled from the 3′-yeast valine half and rat liver 5′-non-valine tRNA fragments. It seems also that there are several non-valine 5′-halves capable of the formation of hybrid molecules, some of these being acylated only with yeast, and the others with both yeast and rat liver enzymes.

However, the mixture of 3′-half of $tRNA_1^{Val}$ plus 5′-halves of total rat liver and yeast tRNA's did not display any acceptor activity with crude yeast or liver enzymes in the presence of the following aminoacids: alanine, leucine, serine, tyrosine, pheylalanine, lysine, histidine and aspartic acid.

A mixture of yeast $tRNA_1^{Val}$ 3′-half plus 5′-halves from *E. coli* was inactive in the presence of both yeast and *E. coli* enzymes (fig. 1). However, it is not surprising that no active aggregate formation from combined valine halves of tRNA's from yeast and *E. coli* was found since these tRNA's have highly different structures [13, 14].

Studies on the nucleotide sequences on fragments from rat liver tRNA that form active hybrid molecules with the 3′-half of $tRNA_1^{Val}$ from yeast and a comparison of their structures with the known structure of yeast $tRNA_1^{Val}$ 5′-half [13] may give additional information on the active site of tRNA and may be, particularly, useful to check the validity of our proposal [5, 7] about the necessity of the intact helical anticodon and acceptor arms of $tRNA_1^{Val}$ for its acceptor activity.

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