THE ROLE OF ANTICODON IN THE ACCEPTOR FUNCTION OF tRNA*)

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It has been found that a mixture of two $tRNA_1^{val}$ halves accepts valine to the same extent as do the intact molecules (Bayev, et al., 1967).

In the present studies a part of the central region of tRNA molecule has been removed, and the effect of this modification upon valine-, serine- and tyrosine-acceptor function of tRNA was investigated.

EXPERIMENTAL

Total tRNA was prepared from baker's yeasts by the phenol-cetavlon method (Mirzabekov, et al., 1965), and treated with crude pH5 enzyme for regeneration of the C-C-A_{OH} end. The partial digestion of tRNA and separation of the digested tRNA on DEAE-cellulose were performed as described (Axelrod, et al., 1967).

The acceptor activity determination and isolation of crude pH5 enzymes fraction from rat liver was performed according to (Holley, et al., 1961) with minor modifications.

The following labelled amino acids were used for the acceptor activity determination: serine-C^{14}, valine-C^{14}, tyrosine-

*) Abbreviations: tRNA = transfer RNA; A,G,U,C,I = adenosine, guanosine, uridine, cytidine, inosine respectively; small p or hyphen = phosphate residue.

C^{14} with the activity 0.33, 0.89, and 0.64 mc per mg respectively.

RESULTS

The overall course of the experiment is presented in Fig. 1. Total tRNA molecules were cleaved into two large fragments (halves) with guanylo-ribonuclease (EC 2.7.8.2b) from Actinomyces aureoverticillatus (Tatarskaya, et al., 1966) at 0° in the presence of Mg^{++} (step I). Separation of the digested tRNA on DEAE-cellulose affords two fractions: the intact tRNA (peak 2) and the mixture of halves of all individual tRNA's which are sensitive to guanylo-ribonuclease action (peak 1). The half fraction consisted of mixed 3'- and 5'-fragments was used in the experiments. The 5'-halves contained a 3'-terminal phosphate residues which were formed by cleavage of phosphodiester bond in the central part of tRNA molecules. To remove this phosphate residue and adjacent nucleoside halves of tRNA were subjected to alkaline phosphatase digestion (step II) (Harkness and Hilmoe, 1962) followed by sodium periodate treatment (step III) as described (Aubert, et al., 1967) and by the second alkaline phosphatase digestion (step IV).

The yield of terminal phosphate amounted to 105% of theoretical value for intact tRNA (Fig. 1, peak 2) and 90% for the halves (Fig. 1, peak 1). The yield of adenine was 96–98% and guanine plus hypoxantine 86–88% of the theoretical value (Fig. 2). At each of the steps acceptor activity of the half fraction was measured and compared with that of the control sample (Table 1). The control preparations were not digested with alkaline phosphatase prior to the oxidation (Fig. 1). Their incubation in lysine buffer after periodate oxidation affords only adenine of the C-C-A_{OH} terminus. In experimental samples

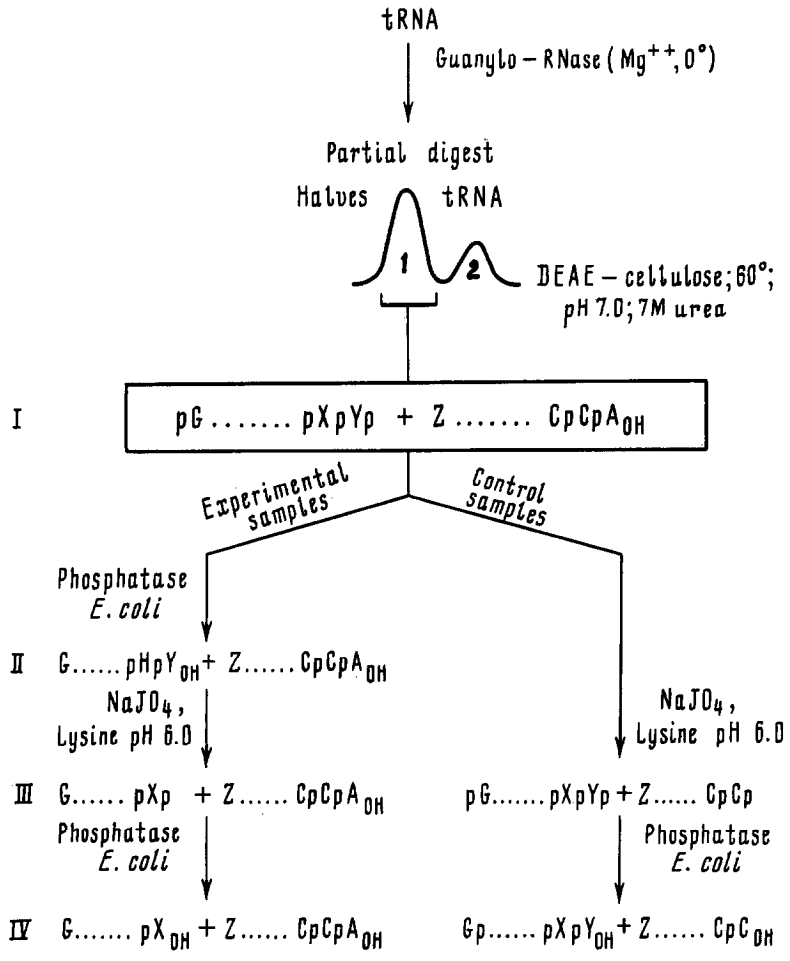

Figure 1. Removal of a fragment from the central part of tRNA molecule. The tRNA molecule is shown schematically.

(Fig. 2) not only terminal adenosine, but also nucleosides from the central region of tRNA were removed. At step III there is a practically complete loss of the acceptor activity (Table I) both of experimental and control samples. Subsequent treatment with alkaline phosphatase (Fig. 1, step IV) resulted in the recovery of the acceptor activity (Table I).

DISCUSSION

The modifications of the central part of tRNA molecules

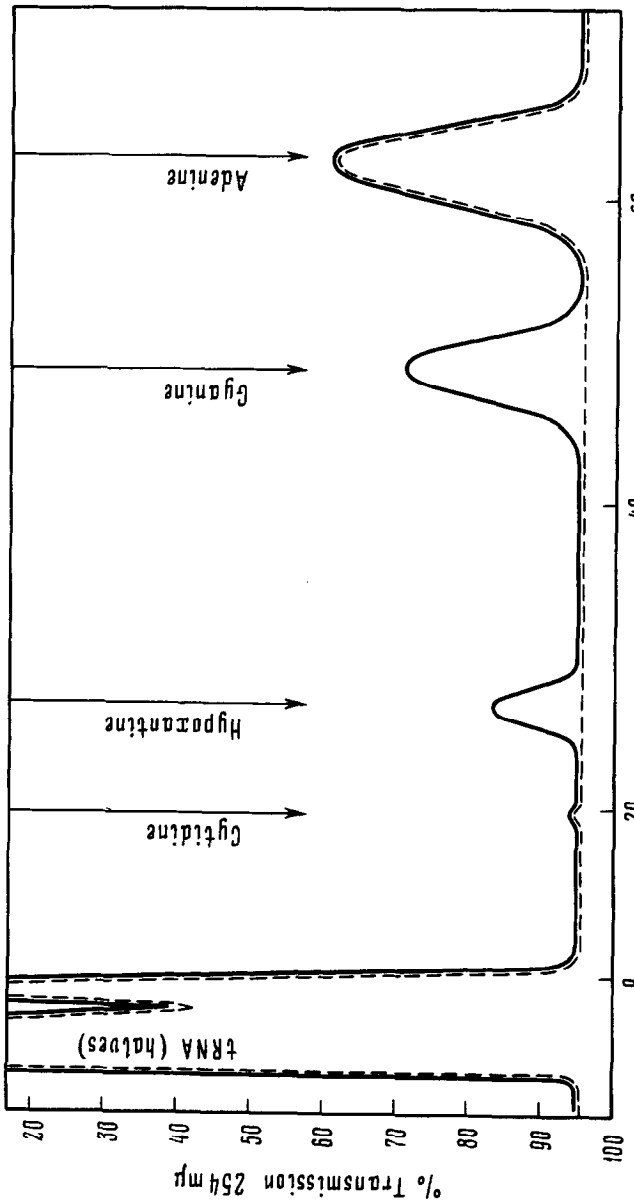

Figure 2. Chromatography of the tRNA periodate oxidative degradation products by the method of Aubert, et al. on Sephadex G-10. Column 0.8x23 cm; buffer contains: 0.1 M NaCl, 0.02 M Tris-HCl pH 8.5, 2% isoamyl alcohol, 1% methanol. Elution rate is 6 ml per hr, fraction volume 1.43 ml. Arrows indicate the positions of the components of a model mixture, containing tRNA and bases. The products corresponding to the peak with maximum in the ninth fraction are formed by reduction of periodate with ethylene glycol.

- - - - tRNA or its halves (9 mg) not treated with phosphatase prior to the periodate oxidation.

——— Halves (9 mg) treated with phosphatase prior to the periodate oxidation.

and their acceptor termini produce independent effects upon acceptor function of tRNA and for this reason are discussed separately.

Modification of the C-C-A_{OH} end. The first treatment with alkaline phosphatase (Fig. 1) doest not change the acceptor activity of the mixed halves with respect to valine and tyrosine, but the serine-acceptor activity increases considerably (Table I). This may be well explained by the fact that serine tRNA contains G-C-C-A_{OH} as the terminal sequence. According to the data of Zachau, et al., (1966), T_1-ribonuclease at 0° in the presence of Mg^{++} splits off the C-C-A_{OH} terminal sequence in serine tRNA molecules producing a 3'-phosphoryl group at the newly formed Gp terminus. The 3'-phosphoryl prevents reconstitution of the C-C-A_{OH} terminal sequence (Berg, et al., 1962). The periodate treatment results in removal of guanosine from the 3'-terminus of serine tRNA and adenosine from the valine and tyrosine tRNA's and produce a new 3'-end phosphate group (step III). The acceptor activity of all the tRNA's decreases after this treatment, but restores owing to a new alkaline phosphatase dephosphorylation. The fact might be a indication that the above mentioned G residue in the case of serine tRNA is not obligatory one for either restoration of the C-C-A_{OH} terminal sequence or the aminoacylation of serine tRNA.

Excision of fragments in the central region. Removal of phosphate group from the central region of tRNA molecule (Fig. 1) and subsequent scission of a 3'-nucleoside and a second phosphate group affected acceptor function of valine and serine tRNA's in a very small extent (Table 1). In the case of tyrosine, serine and valine tRNA's this fragment belongs to their anticodon moieties GΨA, IGA, and IAC, respectively. Hence the

bond break in the anticodon and even removal of its considerable part does not deprive above mentioned tRNA's of its capacity to be enzymatically aminoacylated, and consequently anticodon is not the site responsible for the recognition aminoacyl-tRNA-synthetases. It should be mentioned too this modification does not affect the enzymic regeneration of the C-C-A_{OH} end sequence.

Table I. Acceptor activity of tRNA halves after four-step treatment

Step Fig. 1	cpm 10^{-3}/ mg					
	Valine		Tyrosine		Serine	
	exper.	control	exper.	control	exper.	control
I	366	-	257	-	4.8	-
II	374	-	239	-	15.2	-
III	33.7	39.7	-	-	1.8	3.6
IV	290	305	125	168	9.8	10

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