STEPWISE REMOVAL OF FIVE NUCLEOTIDES FROM 5'-HALF OF YEAST VALINE TRANSFER RNA AND ITS ACCEPTOR ACTIVITY

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The removal of nucleosidediphosphate from the central part of baker's yeast tRNA by stepwise oxidative degradation of partially digested molecules does not interfer with valine, tyrosine, serine acceptor activity and with enzymatic reconstitution of the tRNA C-C-A_{OH} terminal sequence (1). The same is true for tyrosine specific tRNA (Torulopsis utilis) (2).

We have carried out the above mentioned experiment with a mixture of 3'- and 5'-halves of baker's yeast total tRNA (1). Now the studies are extended on specific $tRNA_1^{Val}$. Five nucleotides have been split off from 5'-half of $tRNA_1^{Val}$ molecule by periodate oxidative degradation and it has been shown that the mixture of the 5'-half deprived of 1-5 nucleotides and an intact 3'-half restores the acceptor function without essential losses.

METHODS

Total tRNA and $tRNA_1^{Val}$ were prepared from baker's yeast (3). pH 5 enzyme fraction was isolated from baker's yeast by the method (4) modified according to (5). Acceptor activity determination was performed as described previously (6) incu-

bation was carried out for 30 min at 30°. Valine-C^{14} with the activity 0,61 mC per mg was used for acceptor activity assay. $tRNA_1^{Val}$ (90% pure) was cleaved into halves with guanylo-RNase (EC 2.7.7.26) from Actinomyces aureoverticillatus (7) at 0° in the presence of Mg^{2+} (8). $tRNA_1^{Val}$ halves were separated on a DEAE-cellulose column in 7 M urea as described in (9), freed from salts and urea by dialysis, concentrated in evaporator and by ethanol precipitation in the presence of 1/10 volume of 1 M sodium acetate pH 5.

The stepwise degradation (10) includes for one cycle the following procedure: 1) The digestion with E. coli alkaline phosphatase (11) for 1 hour at 60° in 0,1 M Tris-HCl buffer pH 8,3 (12). 5'-half is separated from alkaline phosphatase by cetyltrimethylammonium bromide (cetavlon) precipitation followed by 3 ethanol precipitations. 2) The oxidation of 5'-half with 0,03 M sodium periodate for 20 min at 18°. Oxidized 5'-half is precipitated 3 time with two volume of ethanol in the presence of 1/10 volume of 1 M sodium acetate pH 5 to eliminate sodium periodate. 3) The scission of the terminal oxidized nucleoside by incubation of 5'-half for 2 hours at 45° in the presence of 0,5 M D,L-lysine pH 8,0. Then 5'-half is precipitated twice with ethanol. Degradation products dissolved in supernatant ethanol are identified by chromatography on Sephadex G-10 (13) (Fig. 2) and by UV spectrophotometry. The 5'-half after the first cycle of oxidation is subjected to the second cycle etc. The aliquots are taken away after each cycle of degradation for acceptor activity assay.

RESULTS AND DISCUSSION

The course of the experiment in its main features

TABLE I

Acceptor activity of mixture of $tRNA_1^{Val}$ 5'-half or its periodate oxidative degradation products with 3'-half */

$tRNA_1^{Val}$ and its fragments	cpm for 1 min/A_{260} unit
$tRNA_1^{Val}$	196600
pGGUUUCGUGGUCΨAGUCGGUUAUGGCAΨCUGCΨUJ$_p$ (me, H_2H_2)	132700
GGUUUCGUGGUCΨAGUCGGUUAUGGCAΨCUGCΨU (me, H_2H_2)	132900
GGUUUCGUGGUCΨAGUCGGUUAUGGCAΨCUGC (me, H_2H_2)	130000
GGUUUCGUGGUCΨAGUCGGUUAUGGCAΨCUGC (me, H_2H_2)	123600
GGUUUCGUGGUCΨAGUCGGUUAUGGCAΨCUG (me, H_2H_2)	106300
GGUUUCGUGGUCΨAGUCGGUUAUGGCAΨCU (me, H_2H_2)	96720
5'-half or its fragments without 3'-half	3500

*/ The reaction mixture for the acceptor activity determination contained 0,05 A_{260} unit of 5'-half or its fragment and 0,06 A_{260} units of 3'-half.

was the same one as in the case of total tRNA (1). Yeast $tRNA_1^{Val}$ whose primary structure hase been described previously (14) was split with guanylo-RNase at the phosphodiester bond between I_{35} and A_{36} in the anticodon. The resulting

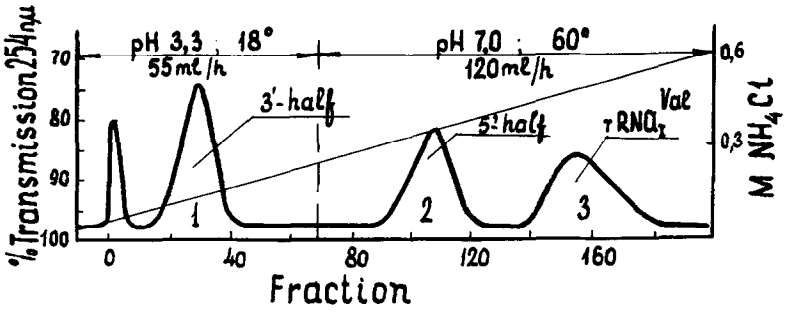

Fig. 1. Chromatography of $tRNA_1^{Val}$ partial digest on DEAE-cellulose column.
348 A_{260} units of partial digest (see Methods) were applied on 1,8 x 22 cm column of DEAE-cellulose (0,7 meq/g). Eluent 0,1 M NH_4Cl in 7 M urea ÷ 0,55 M NH_4Cl in 7 M urea (2500 ml each).

3'- and 5'-halves of $tRNA_1^{Val}$ molecules were separated by column chromatography (9) and isolated as pure preparations (Fig. 1). Only 5'-half was subjected to periodate oxidative degradation as described in "Methods". The total number of periodate oxidation cycles was five. After each cycle the aliquotes were taken away and mixed with an equimolar amount of 3'-half. The acceptor activity of the mixture for valine was measured and the results are shown in Table I.

The data indicate that the removal of the fragment G-C-ψ-U-J_p from the central part of $tRNA_1^{Val}$ molecules decreases the acceptor activity only slightly. Examination of the split bases indicates that at each cycle the degradation reaction proceeds quantitatively.

Some side reactions appear to proceed in the course of the degradation due to the action of periodate and lysine. It is particularly clear at the last cycle of the degradation (Fig. 2). The product, obtained after fifth cycle of the degradation was purified by chromatography on DEAE-cellulo-

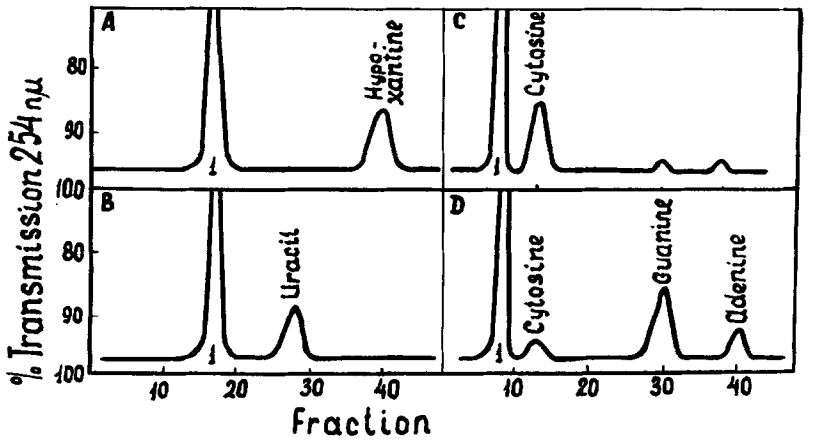

Fig. 2. Chromatography of the degradation products of $tRNA_1^{Val}$ 5'-half on Sephadex G-10 (particle size 40-120 μ)
A,B,C,D chromatography of the material after 1,2,4 and 5 cycles of degradation. After the third degradation cycle (Ψ_p scission) the chromatography profile became ambiguous because $NaIO_4$ destroys pseudouridine with the formation of several degradation products (16). However, the fourth degradation (C) gave a proper amount of cytosine, it is an indirect indication on the quantitative scission of Ψp residue at the preceeding step of the degradation.

A and B: 0,85 x 80 cm column, elution rate 3,4 ml/h, fraction volume 1,26 ml.

C and D: 0,35 x 40 cm column, elution rate 1 ml/h, fraction volume 0,37 ml.

Peak 1 on A,B,C,D - products of periodate reduction by ethylenglycol. 28,21,11,10 A_{260} units were applied on the column at A,B,C,D respectively.

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se in 7 M urea at 60° (Fig. 3). The fraction 2 was assayed for valine acceptance (Table I) and then completely digested with guanylo-RNase. Oligonucleotides of the digest were separated with twodimensional thin layer chromatography on cellulose in tert.butanol-isobutyric acid (15). Resulting fingerprints revealed the absence of the fragment G-C-Ψ-U--J_p in the 5'-half after five cycle degradation.

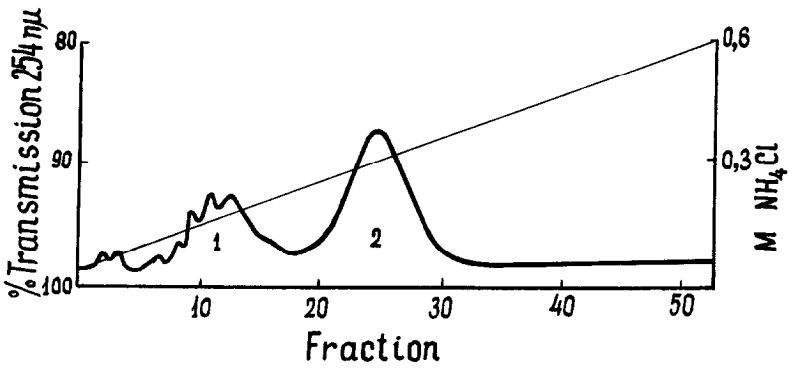

Fig. 3. Chromatography of $tRNA_1^{Val}$ 5'-half after five step degradation on DEAE-cellulose column. 12 A_{260} units of the material are put on a 0,5 x 3,5 cm column, eluent 0,02 M - 0,6 M NH_4Cl in 7 M urea pH 7,0 (70 ml each), elution rate 4,5 ml/h, fraction volume 1,6 ml, temperature 60 °.

One can draw two conclusions from the experiments. First, the sequence G-C-ψ-U-J_p appears to be nonessential for valyl-tRNA-ligase recognition, i.e. it is not a ligase recognition site. Elimination of the sequence do not interfere with active conformation of tRNA and thus has no indirect influence on the recognition. Second, the same is true for the recognition of nucleotidyl-tRNA-transferase. $tRNA_1^{Val}$ used in these studies is deprived of the terminal adenosine and the regeneration of C-C-A_{OH} end is necessary for the subsequent aminoacylation.

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