COMPETITIVE INHIBITION OF THE ACCEPTOR ACTIVITY OF $tRNA^{Tyr\ II}_{E.coli}$ BY A COMBINATION OF OLIGO G AND A CCA TERMINATED NINETEEN RESIDUE OLIGONUCLEOTIDE OF $tRNA^{Tyr\ II}_{E.coli}$

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1. Introduction

We have described [1] a specific competitive inhibition of the acceptor activity of yeast $tRNA^{Phe}$ by the combination of the 3′-half and a pG-terminated fragment, 15 nucleotides long, from the 5′-end. The individual fragments were not inhibitory. These results could be considered in connection with those of Schulman and Chambers [2] who suggested that the specific recognition site for yeast alanyl-$tRNA^{Ala}$ synthetase is located in the acceptor stem of $tRNA^{Ala}$. Using *E. coli* $tRNA^{Tyr\ II}$ we further investigated whether the stem of the clover leaf could be a common specific recognition site of tRNAs. *E. coli* $tRNA^{Tyr\ II}$ was chosen for two reasons: (1) one can easily obtain a –CCA containing quarter of the molecule after complete ribonuclease T_1 digestion because the first G (beginning from the CCA) is in the 20th position, (2) the stem is composed of G–C pairs, with the exception of one A–U pair, and is easily imitated by combining the –CCA containing nineteen residue oligonucleotide (called hereafter 3′-fragment) and oligo G. The present communication describes the competitive inhibition of the acceptor activity of *E. coli* $tRNA^{Tyr\ II}$ by such a combination, while the individual fragments are not inhibitory. This finding strengthens the assumption that the stem of the clover leaf is one of the common recognition sites of the tRNAs for the synthetases.

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2. Materials and methods

Ribonuclease T_1 was purchased from Sankyo Corp. (Japan). *E. coli* $tRNA^{Tyr\ II}$ was purified as previously described [3]. Oligo G (chain length 10–12 nucleotides) was prepared by hydrolyzing 100 mg poly G in 25 ml 8 M NH_4Cl, 12 hr at 37°; the polymer was separated from oligonucleotides by filtration on a G-50 Sephadex column; oligonucleotides were separated on a DEAE-cellulose DE-52 column (2.5 × 16 cm) by elution with a gradient 0.01–0.5 M and 0.5–0.8 M of NH_4bicarbonate, and then lyophilized to eliminate the bicarbonate; finally they were desalted on a P2 column (3 × 45 cm), and characterized by end group analysis. The acceptor activity was determined as follows, according to Ingram and Pierce [4]: after incubation of the mixtures as indicated in table 1, aliquots of 0.05 or 0.075 ml were spotted on Whatman DE-81 paper, washed for 2 hr with 8.7% acetic acid in 2.5% formic acid, dried, and counted in a 5 ml toluene-based liquid scintillator containing 0.3 g 1.4-bis(*p*-phenyloxazolyl-2)-benzene, and 5 g 2.5-diphenyloxazole per liter, in a Packard liquid scintillation counter (1 μCi = 1124,000 cpm). Purified tyrosyl-tRNA-synthetase was a gift from Dr. Schusterman (Paris) and was prepared according to Calender and Berg [5]; the fraction used was the one purified through electrophoresis on polyacrylamide gel (specific activity, tested by exchange: 500–700). The complete ribonuclease T_1 digestion and the chromatographic isolation of the nineteen residue oligonucleotide containing the –CCA terminus (3′-fragment) was performed as described by Seno et al.

Table 1
Inhibition of the acceptor activity of *E. coli* $tRNA^{Tyr\ II}$ by the combination of oligo G and 3′-fragment of this tRNA.

	cpm / assay	Inhibition (%)
E. coli $tRNA^{Tyr\ II}$		
alone (control)	700	0
+ 3′-fragment	720	−3
+ oligo G	670	4.3
+ 3′-fragment + oligo G	330	53
+ oligo G + 3′-half of $tRNA^{Phe}_{yeast}$	705	0

Inhibition mixture (0.1 ml): tris-HCl buffer, 100 mM (pH 7.5); $MgCl_2$, 5 mM; ATP, 2 mM; KCl, 5 mM; β-mercaptoethanol, 5 mM; bovine serum albumin, 5 μg; $tRNA^{Tyr\ II}$, 0.2 A_{260}; 3′-fragment, 0.5 A_{260}; oligo G, 0.25 A_{260}; ^{14}C-tyrosine, 0.01 μmole (S.A. = 180 mCi/mmole); tyrosyl-tRNA-synthetase, 0.05 μg protein.

In the assay of $tRNA^{Tyr\ II}$ + 3′-fragment + oligo G, the concentrations of 3′-fragment and oligo G were 0.25 A_{260} and 0.086 A_{260}, respectively. Incubation 2 min at 10°. 0.05 ml of incubation mixture was spotted on Whatman DE 81 paper and the amino acid separated was counted.

[6], except for the amount of *E. coli* $tRNA^{Tyr\ II}$ hydrolyzed which was, in our case, 200 A_{260} units.

3. Results

3.1. *Chromatographic separation of the 3′-fragment after complete ribonuclease T_1 digestion of E. coli $tRNA^{Tyr\ II}$*

The results are presented in fig. 1. We obtained exactly the same peaks as Seno et al. [6] where peak 7 was characterized as a –CCA terminated oligonucleotide of nineteen residues. In other experiments the *E. coli* $tRNA^{Tyr\ II}$ was charged with ^{14}C-tyrosine before treatment with RNAse T_1. After hydrolysis the products were passed through on DEAE Sephadex A-25 column in the presence of 7 M urea (pH 3.0). Peak 7 was excluded at the beginning of the gradient and contains all the radioactivity. Upon electrophoresis in polyacrylamide gel, peak 7 migrates faster than the 3′- and 5′-halves of yeast $tRNA^{Phe}$. These results are in accordance with the identification of peak 7 as the –CCA terminated oligonucleotide of nineteen residues long (3′-fragment) as characterized by Seno et al. [6].

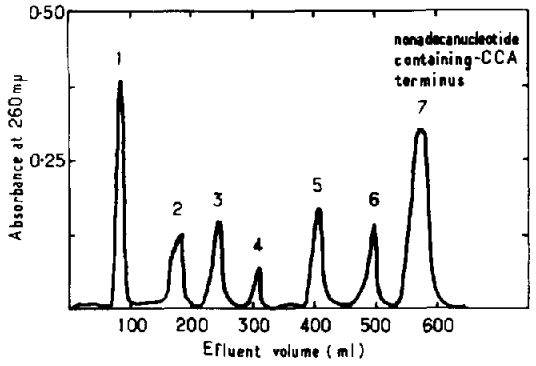

Fig. 1. Chromatographic isolation of 3′-fragment (oligonucleotide of nineteen residues containing the –CCA terminus) after complete ribonuclease digestion of *E. coli* $tRNA^{Tyr\ II}$*. The conditions of ribonuclease T_1 digestion and of column chromatography were identical to those described by Seno et al. [6]. After hydrolysis of 200 A_{260} units of *E. coli* $tRNA^{Tyr\ II}$ the products are passed on a DEAE-Sephadex A-25 column (0.5 × 150 cm); linear gradients of 400 ml each, 0.14 M and 0.7 M NaCl in 7 M urea (pH 7.4).

* This tRNA contained 60% acceptor activity for tyrosine and was contaminated by 30% acceptor activity for serine.

3.2. *Specific inhibition of the acceptor activity of E. coli $tRNA^{Tyr\ II}$ by combination of the 3′-fragment and oligo G*

Table 1 shows the inhibition of the acceptor activity of *E. coli* $tRNA^{Tyr\ II}$ by the combination of the 3′-fragment and oligo G. It can be seen that neither the 3′-fragment nor oligo G alone inhibits the acceptor activity, whereas the combination of both has a marked inhibitory effect. The inhibition is specific and this combination has no effect on the acceptor activity of yeast $tRNA^{Phe}$ or yeast $tRNA^{Lys}$, in the presence of their respective synthetase. In addition, the combination of oligo G with the chemically obtained 3′-half of yeast $tRNA^{Phe}$ [1], has no effect on the acceptor activity of *E. coli* $tRNA^{Tyr\ II}$.

Fig. 2 shows the Dixon plot of the inhibition of the acceptor activity of *E. coli* $tRNA^{Tyr\ II}$, at 10°, in

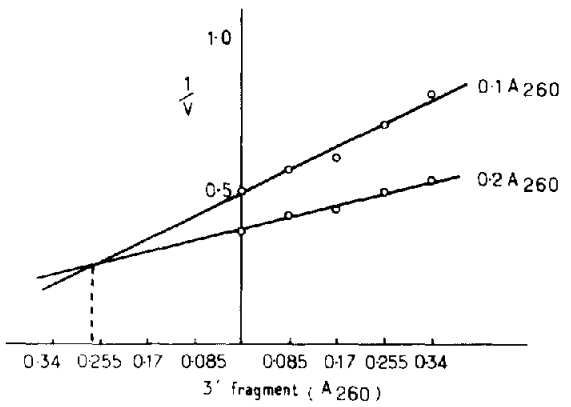

Fig. 2. Dixon plot of inhibition of *E. coli* $tRNA^{Tyr\ II}$ charging by combination of the 3′-fragment and oligo G. Charing conditions identical to those described in table 1. The concentrations of *E. coli* $tRNA^{Tyr\ II}$ were as indicated. V is expressed in pmoles charged tRNA per 2 min. Equal quantities of A_{260} units of 3′-fragment and oligo G were added.

the presence of various amounts of 3′-fragments and oligo G; it can be seen that the combination of both acts as competitive inhibitor.

Attempts to charge the combination of the 3′-fragment and oligo G were unsuccessful, even in the presence of intact *E. coli* $tRNA^{Tyr\ II}$ and after different pretreatments of the fragments.

4. Discussion

The fact that the combination of the 3′-fragment and oligo G (chain length 10–12 nucleotides) which imitates the natural stem of the clover leaf model of *E. coli* $tRNA^{Tyr\ II}$ specifically inhibits the acceptor activity of this tRNA, strengthens the conclusion that the stem could be a common recognition site of the tRNAs for their synthetase. It is interesting to note that the A–U base pair in position 7 from the 3′-end, which cannot be formed by the combination of the 3′-fragment and oligo G, is not necessary for the recognition of the stem; this differs from the case of yeast $tRNA^{Ala}$ [2].

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