# EFFECT OF NUCLEOTIDE ADJACENT TO 3'-END OF CODON TRIPLET ON RIBOSOMAL BINDING OF AMINOACYL-tRNA

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Summary. Several oligonucleotides such as GpCpCpN (N: U, C or A) and GpCpCpNpN (NpN: UpU or ApA) which contain one of the codons for alanine at 5'-end, were compared by estimating the stimulation of binding of alanyl-tRNA to the ribosomes. GpCpCpA or GpCpCpApA did not reveal any enhanced activity, though they contained an adenosine residue adjacent to the 3'-end of the codon triplet sequence which can pair with the common U-residue adjacent to the 5'-end of the anticodon in alanyl-tRNA, analogously to such interaction of ApUpGpA with $tRNA_F^{Met}$ in the absence of ribosomes as was observed by Uhlenbeck et al..

Introduction. The whole nucleotide sequence of over a dozen tRNAs has been determined and each of them contains several common sequences and nucleotides which seem to be related to the common function of tRNAs[1]. The uridine (U) residue adjacent to 5'-end of the anticodon sequence is one of the nucleotides common to all these tRNA molecules.

A few speculations about the function of this U-residue have been made[2, 3], but no communication based on the chemical experiments concerning with this problem has been encountered except the recent one by Uhlenbeck et al.[4], who showed, by use of dialysis equiblibrium method, that in the absence of ribosomes, the U-residue at the 5'-side of the anticodon sequence of $tRNA_F^{Met}$ is able to pair with aenosine (A) adjacent to the 3'-end of the corresponding codon in a tetranucleotide, ApUpGpA.

It should be interesting to see whether such interaction between the common U-residue in the tRNA and the A-residue in such oligomer is also observable in the complex formation between aminoacyl-tRNA and ribosomes or not, because in the natural protein synthesis, contrary to a simple mixture of the oligomer and tRNA, a definite matching between anticodon in a tRNA

and the corresponding codon in the messenger must be required.

In this paper, we communicate the results of research in which the binding activities of oligonucleotides containing several kinds of nucleoside adjacent to the 3'-end of one of the alanine codons (GpCpC), such as GpCpCpN (N represents U, C and A) and GpCpCpNpN (NpN represents UpU and ApA) were compared according to the technique of Nirenberg and Leder[5].

## EXPERIMENTAL AND RESULTS

### Materials

Baker's yeast alanine tRNA was kindly supplied by Dr. Chambers of New York University, the purity of which was about 70 % with respect to its amino acid accepting activity. Ribosomes were prepared from E. coli B as described by Nishimura et al.[6] except that the ribosomes were washed twice with 2 M $NH_4Cl$ by gentle suspension.

Preparation of aminoacyl-tRNA synthetase and [$^{14}$C]-alanyl-tRNA were described previously[7]. [$^{14}$C]-Alanine was the product of New England Nuclear Gorp., and its specific activity was 135 μC/μ mole.

Polynucleotide phosphorylase from Micrococcus lysodeikticus was purified according to the procedure of Fitt[8] and converted to be primer-dependent by partial digestion with trypsin as described by Singer[9].

### Preparation of oligonucleotides

GpCpC was prepared by enzymatic condensation of guanosine-2',3'-cyclic phosphate with CpC, using ribonuclease $T_1$ as reported previously [10] GpCpCpN and GpCpCpNpN were prepared by addition of the nucleoside diphosphate, NDP, to GpCpC under catalysis of primer-dependent polynucleotide phosphorylase. The typical reaction mixture in the synthesis of a tetramer contained 60 μ moles of Tris-HCl buffer, (pH 9.0), 2 μ moles of $Mg(OAc)_2$, 1.1 μ moles of GpCpC, 1.8 μ moles of nucleoside diphosphate, 200 μ moles of NaCl, and 0.06 mg of polynucleotide phosphorylase in a final volume of 0.2 ml. After 12 hours' incubation at 37°C, 0.02 mg of E. coli alkaline phosphatase was added to the mixture to degrade unreacted nucleoside

Table 1. Characterization of oligonucleotides

| Oligo-nucleotide | Base Analysis | | |
|---|---|---|---|
| | RNase | Hydrolysis product | Molar Ratio |
| GpCpCpC | RNase $T_1$ & A | Gp, Cp, C | 1.08/0.98/1.00 |
| GpCpCpU | RNase $T_1$ & A | Gp, Cp, U | 1.00/2.09/0.91 |
| GpCpCpC | RNase $T_1$ & A | Gp, Cp, C | 0.96/2.00/1.02 |
| GpCpCpA | RNase $T_1$ & A | Gp, Cp, A | 1.01/1.90/1.00 |
| GpCpCpG | RNase $T_2$ | Gp, Cp, G | 1.00/2.09/0.91 |
| GpCpCpUpU | RNase $T_2$ | Gp, Cp, Up, U | 1.00/2.10/1.11/0.88 |
| GpCpCpApA | RNase $T_2$ | Gp, Cp, Ap, A | 0.99/1.97/1.07/1.00 |

diphosphate, and the incubation was continued for additional one hour.

The products were separated by ascending paper chromatography as described by Leder et al.[11] The oligonucleotide thus obtained was characterized by base analysis using a combination of pancreactic ribonuclease and ribonuclease $T_1$ or ribonuclease $T_2$. Table 1 indicates that the olibonucleotides were pure enough to submit to the following research.

Binding of alanyl-tRNA to the ribosomes in the presence of oligonucleotides

Fig. 1 shows that under the conditions used in the assay, the binding of [$^{14}$C]-alanyl-tRNA increased proportionally to the amount of the alanyl-tRNA added. In the following experiments, 10 μμ moles of [$^{14}$C]-alanyl-tRNA was added against 2.0 $A_{260\ m\mu}$ units of ribosomes and 1 mμ mole of an oligonucleotide. The binding efficiencies of various tetramers, GpCpCpN (N represents U, C and A) were compared by increasing the amount of each tetramer in the assays taking that of GpCpC as a reference (Fig. 2).

As shown in Fig. 2, the stimulation of the binding of alanyl-tRNA to the ribosomes by GpCpCpU, GpCpCpC and GpCpCpA were similar and resembled that by GpCpC.

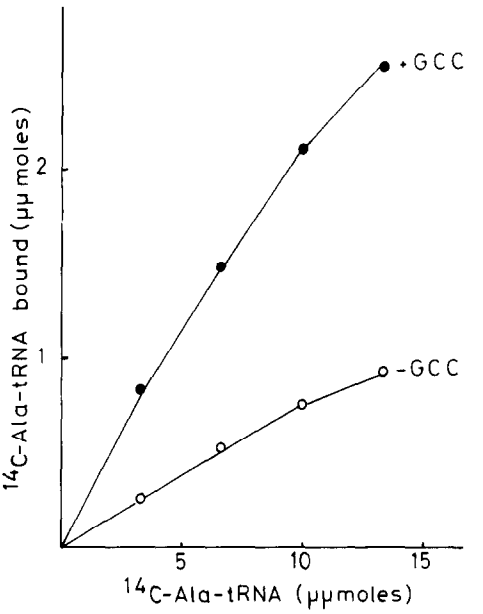


Fig. 1. Relation between amount of [$^{14}$C]-alanyl-tRNA and its binding activity to the ribosomes in the presence ( ● ) and absence of GpCpC ( o ). Each reaction mixture (50 μl) contained 0.1 M Tris-acetate (pH 7.2), 0.05 M $NH_4Cl$, 2.0 $A_{260}$ mμ units of E. coli ribosomes, 0.02 M $Mg(OAc)_2$ 1 mμ mole of GpCpC and appropriate amount of the [$^{14}$C]-alanyl-tRNA. The mixture was incubated at 24°C for 20 min.

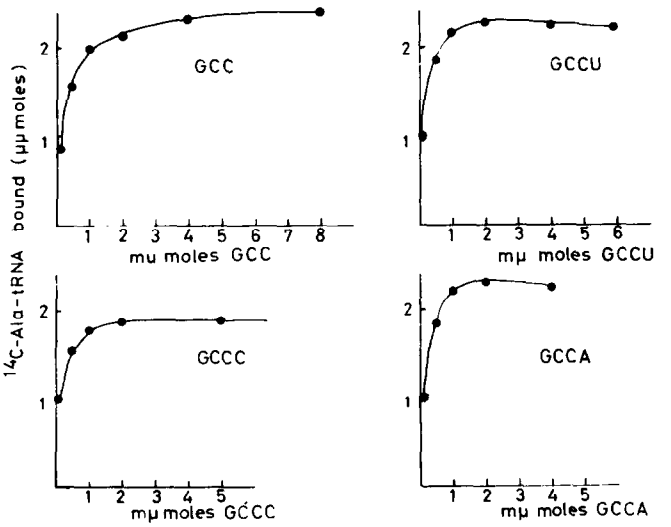


Fig. 2. Relation between amount of oligonucleotides and [$^{14}$C]-alanyl-tRNA bound to ribosomes. The composition of reaction mixtures was the same as mentioned in legend of Fig. 1 except that they contained 10 μμ moles of [$^{14}$C]-alanyl-tRNA and appropriate amount of the oligonucleotides. The mixtures were incubated at 24°C for 20 min. The amounts of oligonucleotides used were calculated from $A_{260}$ mμ of the constituent nucleotide residues, with no special consideration about the hypochromicity.

Next, the effect of the $Mg^{++}$ concentration on the binding of [$^{14}$C]-alanyl-tRNA to the ribosomes was estimated and the results are given in Fig. 3. There were no remarkable differences among the $Mg^{++}$ concentration-dependent stimulations of the binding by oligonucleotides GpCpC, GpCpCpU, GpCpCpC, and GpCpCpA.

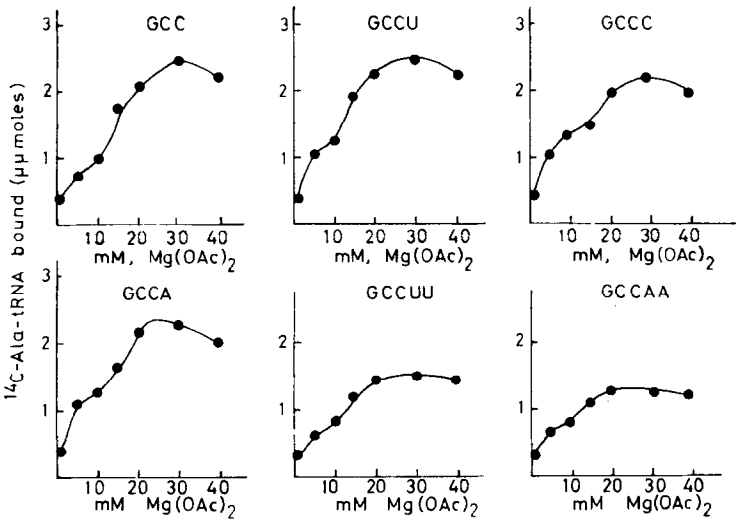


Fig. 3. Relation between $Mg^{++}$ concentration and [$^{14}$C]-alanyl-tRNA binding to the ribosomes. The composition of reaction mixtures was the same as mentioned in legend of Fig. 1 except that they contained 10 μμ moles of [$^{14}$C]-alanyl-tRNA and appropriate concentration of the $Mg(OAc)_2$ The mixtures were incubated at 24°C for 20 min.

## DISCUSSION

The tetranucleotides, GpCpCpN, and pentanucleotides, GpCpCpNpN, which contained one of the codon triplet sequences for alanine in the 5'-end of the chains were compared by estimating the stimulations of binding of [$^{14}$C]-alanyl-tRNA to ribosomes under several conditions by these oligomers. It was found that there was no remarkable difference among the efficiencies for GpCpCpU, GpCpCpC, and GpCpCpA.

It is noteworthy that GpCpCpA which contained adenosine (A) residue at 3'-end did not show any increased binding compared to GpCpCpU or GpCpCpC, since the former tetramer was expected to match more tightly

to the anticodon loop of alanyl-tRNA and show a larger efficiency in binding alanyl-tRNA to the ribosomes in consideration of the observation by Uhlenbeck et al.[4] that the adenosine residue contained adjacent to 3'-end of a codon trinucleotide could pair with U-residue adjacent to 5'-end of the anticodon sequence of tRNA.

Our results, however, were obtained in a double interaction of the oligonucleotide with alanyl-tRNA and the ribosomes to form the complex, while those of Uhlenbeck et al.[4] were obtained in a simple interaction between tRNA and oligonucleotides. Thus the discrepancy might be caused by some special feature of the complex formation which prevents the pairing of the common U-residue with the A-residue in the oligonucleotide adjacent to 3'-end of the codon triplet. One possibility is that a conformational change of tRNA at the location of the common U-residue occurs by the aminoacylation of tRNA. Another possibility concerns some restriction in fitting a single trinucleotide unit of a template oligonucleotide as a codon on the aminoacyl-tRNA binding site of the ribosomes.

The lower activity of pentanucleotides, GpCpCpNpN, compared to that of the tetramers shown in Fig. 3, might be explained by a lower efficiency for longer oligomers to utilize the proper codon triplet contained in those pentamers at the binding site of ribosomes.

The results obtained so far thus indicate that, in aminoacyl-tRNA-ribosome complex, the common U-residue adjacent to the 5'-end of the anticodon sequence must be comformed in such a way that it can hardly pair with the complemental base present adjacent to 3'-end of the codon trinucleotide sequence.

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