# FORMATION OF COMPLEXES BETWEEN tRNA AND TRINUCLEOTIDES

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## 1. Introduction

The formation of complementary complexes between exposed sites of tRNA and oligonucleotides has been demonstrated in the laboratory of Prof. P. Doty [1–4]. The binding constants of individual oligonucleotides were determined in these studies by the equilibrium dialysis method to characterize the exposed sites of 5 S RNA [2] and of several tRNA's [3–5]. The major technical limitation of the method is the scarcity of synthetic oligonucleotides.

The present communication describes a modification of the approach. The use is proposed for binding experiments of statistic isoplith oligonucleotides mixtures that can be obtained by fractionation of oligonucleotides formed by the action of non-specific nucleases like cobra venom endonuclease (preparation of the enzyme is described in [6]). RNA-oligonucleotide complexes can be separated by gel-chromatography from the non-bound oligonucleotides. Fractionation of the oligonucleotides prepared by decomposition of the complex and their structural analysis will give information on the binding sites if the primary structure of RNA under investigation is known. The present communication is concerned with application of the new approach to a study of the complexes between tRNA and trinucleotides. Trinucleotides complementary to the three last nucleotides preceding the 3′-terminal adenosine residue of tRNA form an unusually stable complex with all tRNA's.

## 2. Materials and methods

Unfractionated baker's yeast tRNA was obtained in the Technological Laboratory of this Institute as described earlier [7]; it contained about 50% of intact terminal adenosine residues. Phenylalanine and serine yeast tRNA's were generously provided by Dr. H.G. Zachau (Munich University, BRD).

5 S RNA was a generous gift by Dr. M. Saarma (this Institute). [$^{32}$P]RNA used for the preparation of oligonucleotides was obtained from *E. coli* grown on [$^{32}$P]phosphate-containing medium (20 mCi per liter of cultural medium). Cobra venom endonuclease was obtained as described earlier [6]. [$^{32}$P]RNA was digested during 6 hr at 30° in the following reaction mixture (40 ml): 2 mM Tris-HCl, pH 7.8; 50 mM NaCl; 10 mM $MgCl_2$; 1200 $A_{260}$ units of [$^{32}$P]RNA (2 Ci/mole); 200 activity units of endonuclease; pH of the medium during the incubation was kept constant by addition of 0.05 N NaOH. Isoplith oligonucleotide fractions were obtained by chromatography of the digest on TEAE-cellulose (1.2 × 50 cm) in 7 M urea at pH 6.5 using 1.5 l of linear sodium chloride gradient (0.05–0.25 M). Isoplith oligonucleotide mixtures were desalted using DEAE-Sephadex by the method described in [8].

The tRNA–trinucleotide complexes were obtained in the buffer employed by Uhlenbeck and Doty [1]. Prior to gel-chromatography, the incubation mixtures were kept for 2 hr at 0°. The structures of [$^{32}$P]trinucleotides were elucidated by subjecting them to phosphodiesterase and alkaline hydrolysis. Aliquots of desalted fractions were incubated in 50 mM sodium phosphate, pH 8.8, containing 10 mM $MgCl_2$; 0.3 ml

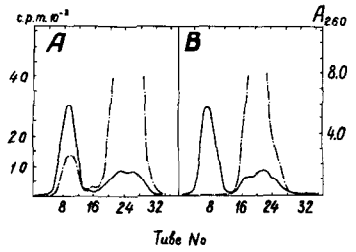


Fig. 1. Gel-chromatography of the complexes of [$^{32}$P]trinucleotides with 5 S RNA (A) and tRNA (B) on Sephadex G-50 (0.9 × 85 cm) at 0°. The mixture of [$^{32}$P]trinucleotides (7 $A_{260}$ units, 4 × $10^6$ cpm) and RNA ( 6 $A_{260}$ units) was incubated in 0.2 ml 1 M NaCl, 10 mM $MgCl_2$, 10 mM sodium phosphate, pH 7.0, for 2 hr at 0° and applied to the column. Gel-chromatography was performed in the same buffer at flow rate 3.5 ml/hr. Fraction volume was 2.2 ml. (——) $A_{260}$; (–·–·–·) radioactivity.

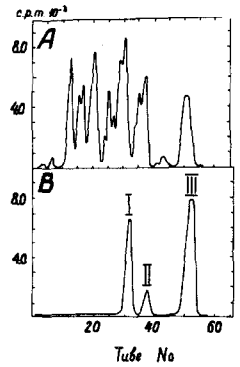


Fig. 2. Chromatography of trinucleotides on TEAE-cellulose column (0.6 × 40 cm). Elution was performed with 500 ml of linear NaCl gradient (0.05 to 0.2 M) in 7 M urea, 0.05 N HCOOH (pH 3.5). Flow rate 12 ml/hr, fraction volume 3.7 ml. A) Pattern of the starting trinucleotides mixture. B) Pattern of the trinucleotides of fractions no. 35–46 (fig. 1). Before application to column, the fraction was diluted with 20 vol of water. The column was washed with 50 ml of 0.05 M NaCl in 7 M urea, 0.05 M HCOOH, pH 3.5 before application of gradient.

of reaction mixture containing 25 activity units of phosphodiesterase isolated by the method previously described [6] and 18 $A_{260}$ units of carrier non-radioactive trinucleotides obtained by the action of cobra venom endonuclease on non-radioactive RNA. The products of phosphodiesterase digestion were fractionated on Dowex 1 × 4 (200–400 mesh) columns (0.3 × 10 cm) using 200 ml linear gradient of sodium acetate, pH 4.4 (0.02–2.0 M); the flow rate was 10 ml/hr, the fraction volume 2 ml. The mononucleotides were identified by their UV-spectra, and their content evaluated by counting the radioactivity. Alkaline hydrolysis of aliquots of fractionated [$^{32}$P]oligonucleotides was performed in 0.5 ml 0.5 M NaOH with 30 $A_{260}$ units of non-radioactive trinucleotides for 16 hr at 37°. The hydrolysates were neutralized with Dowex-50 $H^+$ (0.8 × 1 cm) and subjected to chromatography on Dowex 1 × 4 under the conditions described above, but with 400 ml of sodium acetate linear gradient from 0.02 to 3.0 M.

### 3. Results and discussion

Cobra venom endonuclease does not exhibit any specificity towards base residues [6], and it is believed that all the short oligonucleotides obtained by its action are present in the isopliths in statistical amounts. If this is so, isoplith mixtures can be applied universally to detect any complementary sequences in RNA.

Fig. 1 illustrates the different stabilities of the complexes of trinucleotides with tRNA and with ribosomal RNA. It is clearly seen that stable complexes are formed only with tRNA. Depending on the composition of the incubation mixtures, stoichiometry of the binding is 0.3–0.8 moles of trinucleotides per mole of tRNA.

Fig. 2 shows the chromatographic pattern obtained in acidic medium of the trinucleotides bound by tRNA compared with that of the whole starting trinucleotides mixture. Obviously, the stable binding of trinucleotides with tRNA is highly selective in spite of the fact that unfractionated tRNA was used in the experiments.

Table 1 shows the results of structural analysis of the firmly bound trinucleotides.

It is seen that peaks I and II contain the practically homogeneous trinucleotides pGpGpC and pGpGpA. Peak III corresponds to a mixture of three trinucleotides pGpGpU, pGpGpG and pUpGpG.

The structures of the trinucleotides forming the stable complex suggest that they are bound by the non-helical sequence at the acceptor terminus adjacent to terminal adenosine. To confirm this, trinucleotides

Table 1
Results of the analysis of trinucleotides bound by tRNA.

| Frac. no.* | Phosphodiesterase hydrolysate pC | pA | pU | pG | Alkaline hydrolysate Cp | Ap | Up | Gp | pCp | pAp | pUp | pGp | Trinucleotides identified |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 0.4 | – | 0.1 | 1.0 | – | – | – | 1.0 | – | – | – | 1.0 | pGpGpC |
| II | – | 0.6 | 0.1 | 1.0 | – | – | – | 1.0 | – | – | – | 1.0 | pGpGpA |
| III | – | – | 0.3 | 1.0 | – | – | 0.1 | 1.0 | – | – | 0.2 | 1.0 | pGpGpU + pGpGpG + pUpGpG |

* The fractions were obtained as shown in fig. 2B.

The content of guanylic acid and of guanosine diphosphate was assumed to be equal to I. Dashes indicate contents smaller than 0.1. Conditions of the hydrolysis and chromatographic separations are described under Materials and methods.

bound by yeast phenylalanine and serine tRNA's, whose sequences at this site are different, were studied.

Fig. 3 shows the chromatographic patterns of trinucleotides firmly bound by $tRNA^{Phe}$ and $tRNA^{Ser}$. Results of the structural analysis of these trinucleotides are shown in table 2.

As expected, the major trinucleotide bound by $tRNA^{Phe}$ was pGpGpU while that bound by $tRNA^{Ser}$ was pGpGpC, i.e., the trinucleotides appeared complementary to the sequences of the acceptor termini . . . ApCpCpA ($tRNA^{Phe}$ [9]) and . . . GpCpCpA ($tRNA^{Ser}$ [10]).

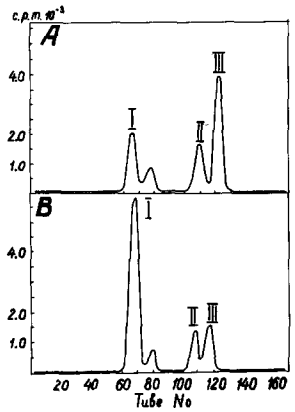


Fig. 3. Chromatographic patterns of the trinucleotides bound by $tRNA^{Phe}$ (A) and $tRNA^{Ser}$ (B) obtained on DEAE-Sephadex A-25 columns (0.8 × 80 cm). The polymer fraction after gel-chromatographic isolation of complex was diluted with 20 vol of water and applied to the column. Elution with 1 l of NaCl linear gradient (0 to 0.15 M) in 7 M urea. In the reservoir the pH of solution was adjusted with HCl to 3.5, in the mixer to 2.7. Flow rate 30 ml/hr, fraction volume 5.8 ml. Radioactivity was measured by Cherenkov effect.

Table 2
Structure of the trinucleotide bound by $tRNA^{Phe}$ and $tRNA^{Ser}$.

| Frac. no. in fig. 3 | Trinucleotide identified $tRNA^{Phe}$ (fig. 3A) | $tRNA^{Ser}$ (fig. 3B) |
|---|---|---|
| I | pGpGpC | pGpGpC |
| II | pGpGpG | pGpGpG |
| III | pGpGpU | pGpGpU |

The binding of trinucleotides by the trinucleotide sequences of tRNA's adjacent to terminal adenosine residues is unusually firm and specific, most probably because the bound trinucleotides extend the perfect double helix.

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