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Interaction of ribo- and deoxyriboanalogs of yeast tRNA^{Phe} anticodon arm with programmed small ribosomal subunits of *Escherichia coli* and rabbit liver

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ABSTRACT

A synthetic ribooligonucleotide, r(CCAGACUGm-AAG-AUCUGG), corresponding to the unmodified yeast tRNA^{Phe} anticodon arm is shown to bind to poly(U) programmed small ribosomal subunits of both *E.coli* and rabbit liver with affinity two order less than that of a natural anticodon arm. Its deoxyriboanalogs d(C-CAGACTGAAGATCTGG) and d(CCAGA)r(CUGm-AAG-A)d(TCTGG), are used to study the influence of sugar-phosphate modification on the interaction of tRNA with programmed small ribosomal subunits. The deoxyribooligonucleotide is shown to adopt a hairpin structure. Nevertheless, as well as oligonucleotide with deoxyriboses in stem region, it is not able to bind to 30S or 40S ribosomal subunits in the presence of ribo-(poly(U)) or deoxyribo-(poly (dT) template. The deoxyribooligonucleotide also has no inhibitory effect on tRNA^{Phe} binding to 30S ribosomes at 10-fold excess over tRNA. Neomycin does not influence binding of tRNA anticodon arm analogs used. Complete tRNA molecule and natural modifications of anticodon arm are considered to stabilize the arm structure needed for its interaction with a programmed ribosome.

INTRODUCTION

Aminoacyl-tRNA selection by the programmed ribosome was assumed to be carried out through a direct interaction of the ribosomal decoding centre with the codon-anticodon sugar-phosphate backbone [1,2]. Accordingly, the difference in template activity of ribo- and deoxyribopolynucleotides have been shown [3,4]. To study the influence of tRNA sugar-phosphate backbone modification on tRNA interaction with the ribosome, DNA analogs of unmodified yeast tRNA^{Phe} anticodon arm are tested in this work. The use of this part of tRNA molecule instead of the whole tRNA seems to be justifiable since the anticodon arm of yeast tRNA^{Phe} interacts with poly(U)-programmed 30S ribosomal subunits with association parameters similar to those of the intact tRNA molecule [5, 6]. Small ribosomal subunits of rabbit liver also bind the anticodon arm of tRNA^{Phe} with association constants close to those for the tRNA though with more expressed cooperative effect (unpublished results).

In this paper the binding of synthetic deoxyriboanalogs of tRNA^{Phe} anticodon arm to poly(U)- or poly(dT)-programmed 30S and 40S ribosomal subunits was studied. Unmodified riboanalog of the anticodon arm served as a control. The data obtained show high selectivity of pro- and eukaryotic ribosomes to the presence of modifications in both backbone and bases of tRNA anticodon arm.

MATERIALS AND METHODS

T4 polynucleotide kinase and bacterial alkaline phosphatase were purchased from NPO 'Ferment', USSR. 30S ribosomal subunits of *E.coli* MRE600 were a gift from V.I.Machno. 40S subunits from rabbit liver were obtained according to [7]. Poly(U) was from 'Reanal', Hungary, poly(dT) was from NIKTI BAV, USSR, neomycin sulfate was purchased from Boehringer W.Germany, γ-[^{32}P]ATP from VO 'Isotop', USSR.

Ligands preparation

Enriched tRNA^{Phe} (1.5 nmol/A unit) was prepared from 260 yeast as in [3]. rN_{15} (Fig. 1) was prepared as in [5]. dN_{17}, $drdN_{17}$ and rN_{17} (Fig. 1) were synthesized using the automatic phosphitamide method on 'Victoriya-4M' synthetizer [8]. 5'-phosphorilation of ligands with [^{32}P] was carried out using γ-[^{32}P]ATP and T4-polynucleotide kinase according to [9]. The radioactivity was 27–135 Ci/mmol (rN_{15}), 3–10 Ci/mmol (tRNA^{Phe} dN_{17}, $drdN_{17}$, rN_{17}). The temperature dependence curves of UV absorbtion of dN_{17} in solution were recorded at 260 nm in 20 mM tris/HCl pH7.6, 20 mM $MgCl_2$, 100 mM NH_4Cl using a Cary 215 spectrophotometer, USA. The oligonucleotide concentration was 0.5×10^{-2}; 0.3×10^{-3}; 0.3×10^{-4} M. The molar extinction coefficient was taken as the average of those of nucleotide links, $E_{260}=11240\ M^{-1}cm^{-1}$.

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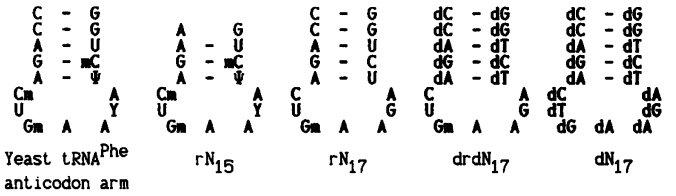

Fig. 1. Structures of yeast tRNA^{Phe} anticodon arm and its analogs.

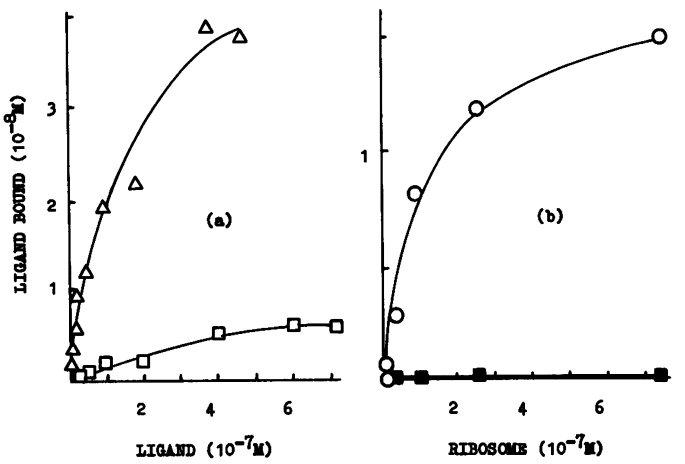

Fig. 2. Binding of ribo- and deoxyriboanalogs of yeast tRNA^{Phe} anticodon arm to poly(U)-programmed 30S ribosomal subunits. △-rN_{15}, □-rN_{17}, ○-tRNA^{Phe}, ■-dN_{17}-$drdN_{17}$. (a)−ribosome concentrations is 5×10^{-8}M, rN_{15} and rN_{17} concentrations are varied as indicated. (b)−tRNA^{Phe} concentration is 5×10^{-8}M, dN_{17} and $drdN_{17}$ concentrations are 3×10^{-8}M, ribosome concentration is varied as indicated.

Ribosome binding

All experiments were carried out in 20 mM tris/HCl pH7.6 20 mM $MgCl_2$, 100 mM NH_4Cl at 20°C. 50−μl assays contained either 5 μg poly(U) or 3 μg poly(dT). Preliminary experiments on binding kinetics have shown that 30 minute incubation is sufficient to reach maximal binding level for rN_{15} and tRNA and 60 minute are enough for rN_{17}. Other conditions are given in figure legends. The amount of material bound by the ribosome was determined by the nitrocellulose membrane technique [10].

RESULTS AND DISCUSSION

rN_{17}, the unmodified riboanalog of yeast tRNA^{Phe} anticodon arm, binds to small ribosomal subunits with affinity two order lower in comparison with the natural arm (Fig. 2a, 3a). This seems to be reasonable since modified nucleotides take part in formation of tertiary structure of yeast tRNA^{Phe} anticodon loop through interaction with magnesium ion coordinated in the loop [11, 12]. Removal of this ion destabilizes 3'-stacked conformation of the anticodon loop [12, 13]. Thus, natural modifications in the isolated anticodon arm are important for its effective binding to the programmed ribosome. On the other hand, binding of the whole yeast tRNA^{Phe} to *E.coli* ribosomes seems to be hardly affected by the absence of the modifications [14].

As for modifications of the anticodon arm bacbone, we have not succeeded to detect complexes of dN_{17} and $drdN_{17}$ with small ribosomal subunits in the presence of either poly(U) (Fig. 2b 3b) or poly(dT) (data are not presented). 10-fold excess of dN_{17} over tRNA^{Phe} does not inhibit the tRNA binding to the ribosome. The deoxyriboanalogs binding is not stimulated by 100 μM neomycin which is known to unblock DNA translation [3 4, 15, 16].

There could be at least three possible reasons why the ribosomes do not bind dN_{17} and $drdN_{17}$. The first is that deoxyriboanalogs do not adopt a hairpin structure. Indeed, dN_{17} and $drdN_{17}$ can form both the intramolecular hairpin and intermolecular dimer, their ratio depending on the oligonucleotide concentration, the length of complementary and noncomplementary regions, their GC-composition [17, 18]. However, the one-step shape of dN_{17} melting curve (Fig. 4)

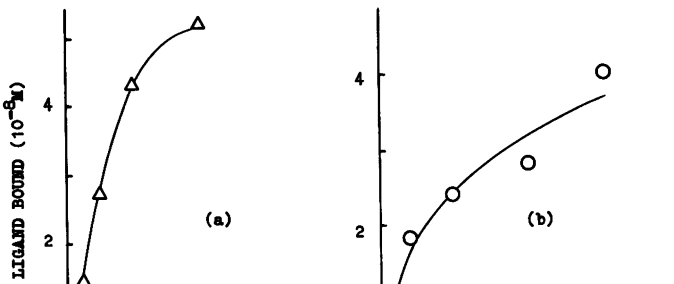

Fig. 3. Binding of ribo- and deoxyriboanalogs of yeast tRNA^{Phe} anticodon arm to poly(U)-programmed 40S ribosomal subunits. △-rN_{15}, □-rN_{17}, ○-tRNA^{Phe}, ■-dN_{17} $drdN_{17}$. (a)−ribosome concentrations is 5×10^{-8}M, rN_{15} and rN_{17} concentrations are varied as indicated. (b)−tRNA concentration is 1×10^{-7}M, dN_{17} and $drdN_{17}$ concentrations are 2.5×10^{-8}M, ribosome concentration is varied as indicated.

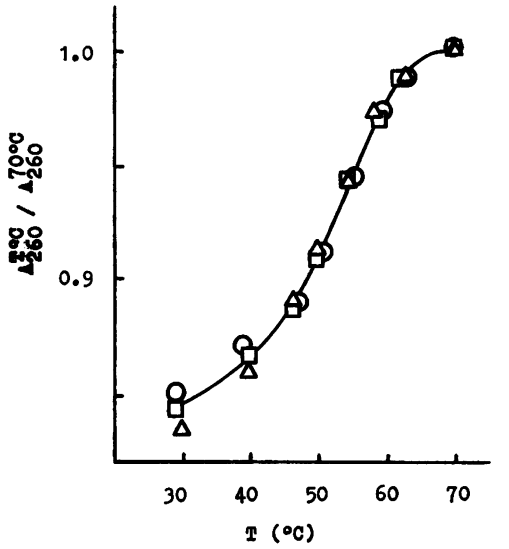

Fig. 4. Normalized melting curve of dN_{17} for different oligonucleotide concentrations (0.3×10^{-4}M (○), 0.3×10^{-3}M (△), 0.5×10^{-2}M (□)).

indicates that only one structure is formed in the solution [18]. Taking into consideration that melting temperature of dN_{17} does not depend on its concentration this structure is obviously the intramolecular hairpin.

The second explanation of poor binding of deoxyribooligonucleotides to the ribosomes is based on the assumption that 2'-hydroxyls in the stem region participate directly in interaction of the anticodon arm with the ribosome. Finally, we cannot exclude that the presence of deoxyriboses in the stem region leads to the loop conformation unsiutable for the ribosomal decoding centre. In the last case the data obtained correspond well to the hypothesis of direct interaction of the ribosomal decoding centre with the codon-anticodon sugar-phosphate backbone of [1,2].

The literture data allow to compare some characteristics of the whole tRNA and its isolated anticodon arm deoxyriboanalogs. $tRNA^{Phe}$ deoxyriboanalogs were found to be active in recognition of aminoacyl-tRNA synthetase [19] and poly(U) dependent binding to 70S ribosomes of *E.coli* [20]. In both processes tRNA anticodon arm is concidered to be functionally significant.

The difference revealed for the whole tRNA analogs and analogs of its isolated anticodon arm may be due to removal of the arm from the complete tRNA structure. Indeed, there are some indications that the entire tRNA molecule influences anticodon loop structure. The isolated anticodon arm of yeast tRNA interacts with a codon in solution with assosiation constant an order of magnitude greater than that of the whole tRNA [21]. Besides, the difference between the two main conformations of the anticodon loop (interacting and noninteracting with cognate codon) is more pronounced when the anticodon arm is uncoupled from the rest of the tRNA structure [22]. Allosteric relationship between the anticodon and other parts of tRNA molecule is discussed in review [23].

The data obtained show high sensitivity of pro- and eukaryotic ribosomes to the modifications in a sugar-phosphate backbone of tRNA anticodon arm. Complete tRNA structure and natural modifications of the anticodon arm are evidently essential for effective interaction with a programmed ribosome, probably, through stabilization of the arm structure.

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ABBREVIATIONS

rN_{15}, r(A A G A Cm U Gm A A Y A 7J m5C U G) riboanalog of yeast $tRNA^{Phe}$ anticodon arm; rN_{17}, r(CCAGACUGmAA-GAUCUGG), riboanalog of yeast $tRNA^{Phe}$ anticodon arm having no modified bases, dN_{17}, d(CCAGACTGAAGATCTG-G) and $drdN_{17}$, d(CCAGA)r(CUGm AAGA)d(TCTGG), deoxyriboanalogs of yeast $tRNA^{Phe}$ anticodon arm having no modified bases.

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