EFFECTIVE BINDING OF OLIGONUCLEOTIDES TO THE ANTICODON OF A tRNA WITHOUT STIMULATION OF tRNA BINDING TO 30 S RIBOSOMES

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1. Introduction

It has been shown that complementary tetranucleotides only bind effectively to the C–C–A end and the anticodon region of the tRNA [1,2]. Extensive studies have been performed using either oligonucleotides and cognate tRNAs or tRNA pairs with complementary anticodons to elucidate the structural details for codon-anticodon recognition [3–5,7].

It was concluded from the more efficient binding of U–U–C–A to $tRNA^{Phe}$ and the 100-fold increased rate association constant as compared to U–U–C that the anticodon loop of a tRNA may change its conformation on binding to the codon [3]. The increased complex formation with U–U–C–A cannot be explained by a higher population of the stacked conformer in the tetranucleotide as compared to the trinucleotide, because U–U–C–C shows a lower association constant as compared to U–U–C–A [2].

Since the most efficient complex formation was obtained with tetranucleotides terminating at the 3′-end with adenosine, it has been speculated, that a fourth base pair is formed between the invariant uracil U33 of the tRNA and the adenine of the oligonucleotide [6]. Recent results, however, support a stacking-type interaction between the fourth base in the tetranucleotide and either the 5′-nucleotide in the anticodon or the constant U33 in the tRNA [7].

We were interested to see, whether this increased association between $tRNA^{Phe}$ and U–U–C–A as compared to the natural codons U–U–U and U–U–C, is reflected in the coded Phe-tRNA binding to ribosomes. We present here evidence that tetranucleotides terminating in adenosine, show efficient binding to their cognate tRNA but are inactive as codons.

2. Experimental

2.1. *Materials*

$tRNA^{Lys}_{E.\,coli}$ and $tRNA^{Phe}_{yeast}$ were purchased from Boehringer, Mannheim. Dinucleoside phosphates and nucleoside 5′-diphosphates were supplied by Pharma Waldhof, Düsseldorf. All radioactively labeled compounds came from Amersham-Buchler, Braunschweig, and *E. coli* MRE 600 type were from Merck, Darmstadt. Dialysis membranes were a gift from Iris 3069 Rhône-Poulenc, C., Paris.

2.2. *Preparation of the components*

30 S ribosomal subunits were isolated from 70 S tight couples [8] by zonal centrifugation and were reactivated prior to use as in [9]. $tRNA^{Lys}_{E.\,coli}$ was charged with [^{3}H]lysine (1 Ci/nmol) using a partially-purified synthetase preparation. $tRNA^{Phe}_{yeast}$ was aminoacylated with [^{3}H]phenylalanine (1 Ci/mmol) by a 100 000 × *g* supernatant from baker's yeast. The various oligonucleotides were prepared with primer-dependent polynucleotide phosphorylase (EC 2.7.7.8) using dinucleoside phosphates as primers [9].

2.3. *Experimental conditions*

The oligonucleotide stimulated binding of aa-tRNA to 30 S ribosomal subunits was followed by

the method in [11] at optimal ribosomal activity as described [10]. The buffer used was: 50 mM Tris–HCl, pH 7.2; 100 mM NH_4Cl, 1 mM 1,4-dithioerythritol and 10 mM $Mg(OAc)_2$.

The complex formation between tRNA and oligonucleotides and the association between oligonucleotides and 30 S ribosomes were measured in the same buffer by equilibrium dialysis as in [1]. The experimental error in the evaluation of the association constants is estimated to be 20% for K-values above 1000 M^{-1}, and to be 50% for K-values below 1000 M^{-1}.

3. Results

3.1. *Determination of the association constants between $tRNA^{Phe}_{yeast}$ or $tRNA^{Lys}_{E.coli}$ and their cognate oligonucleotides*

The complex formation between the tRNAs and the oligonucleotides, complementary to their anticodon sequence, was followed by equilibrium dialysis at high tRNA concentrations (20 μM) and at varying but low oligonucleotide concentrations (0.1–1.0 μM). The association constant was determined by the following equation:

$$K_a = \frac{(\text{complex})}{(\text{codon}_{free})\,(\text{tRNA-complex})}$$

Equilibrium between the compartments was reached within 6 h at 0°C. The data obtained with $tRNA^{Phe}$ and $tRNA^{Lys}$ are summarized in table 1. For the $tRNA^{Phe}$ system there is good agreement with the data taken from the literature (table 1). It seems to be evident that well stacked oligonucleotides like oligoadenylates show higher association constants as compared to oligouridylates. Both tetranucleotides A_4 and U–U–C–A, which terminate in adenosine show an association constant one magnitude higher. That this is not only due to a higher population of the stacked conformes follows from the reduced complex formation A_5 X $tRNA^{Lys}$. Whether the increased affinity of A_4 and U–U–C–A is due to an additional base pair between U33 (tRNA) and the terminal adenosine, or to a stacking interaction with the 5′-base in the anticodon is not known.

Table 1
Molar association constants for oligonucleotides complementary to the anticodon region of $tRNA^{Phe}_{yeast}$ and $tRNA^{Lys}_{E.coli}$

Oligonucleotide	K_a (M^{-1}) measured	Published data
U–U–U	4×10^2	4×10^2 [2]
U–U–U–U	2.7×10^3	2×10^3 [2]
U–U–U–U–U	5×10^2	4×10^2 [5]
U–U–C	1.4×10^3	1.8×10^3 [3]
U–U–C–C	7.6×10^3	8.5×10^3 [2]
U–U–C–A	4.1×10^4	7.4×10^4 [2]
A–A–A	1.8×10^4	
A–A–A–A	1.7×10^5	
A–A–A–A–A	4.2×10^4	

K_a was measured in 50 mM Tris–HCl, pH 7.2; 100 mM NH_4Cl, 1 mM 1,4-dithioerythritol, 10 mM $Mg(OAc)_2$ at 0°C by equilibrium dialysis (chamber vol. 100 μl)

3.2. *Coding properties of the various oligonucleotides*

It seems to be a reasonable assumption that the high efficiency of U–U–C–A and A_4 in complex formation with their respective tRNA should be reflected in a better stimulation of aa-tRNA binding to ribosomes, i.e., either in the amount of aa-tRNA bound or in the amount of oligonucleotide needed to obtain saturation. In order to restrict binding to codon–anticodon-dependent effects thereby avoiding codon-independent binding sites on the 50 S subunits, 30 S ribosomal subunits, not 70 S ribosomes, were used for these studies. Figure 1 shows the oligouridylate-coded binding of the Phe-tRNA to 30 S ribosomes. No major difference can be seen in the relationship between the tRNA X oligonucleotide association constant and the amount of aa-tRNA bound. When the coding properties of U–U–C–A were examined a surprising effect emerged. In spite of its high association constant with $tRNA^{Phe}$, U–U–C–A is practically inactive in the stimulation of Phe-tRNA binding to 30 S ribosomes (fig.2). In the oligoadenylate Lys-tRNA system (fig.3) a corresponding effect can be demonstrated. A_4 shows by far the highest association constant with $tRNA^{Lys}$ but has a reduced codon–anticodon activity as compared to A_3 and A_5.

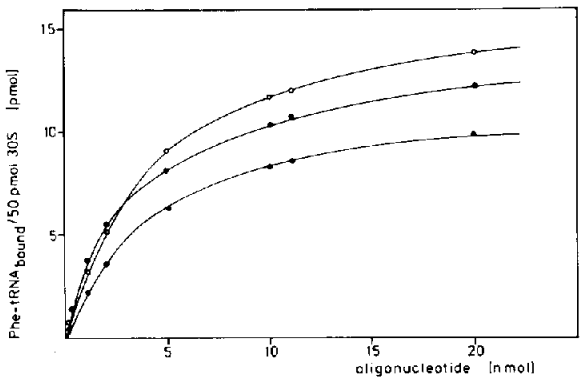

Fig.1. Oligouridylate-dependent binding of [^{3}H]Phe-tRNA^{Phe} to 30 S ribosomes. The incubation mixture contains in total vol. 100 μl: buffer (50 mM Tris–HCl, pH 7.2, 100 mM NH_4Cl, 1 mM 1,4-dithioerythritol, 10 mM $Mg(OAc)_2$); 50 pmol 30 S ribosomes; 60 pmol [^{3}H]Phe-tRNA, and oligonucleotide as indicated. The samples were incubated at 0°C for 40 min. The amount of [^{3}H]Phe-tRNA^{Phe} bound was measured by nitrocellulose filter assay. Blanks (without ribosomes) were subtracted. (●-●) U_3; (○-○) U_4; ●-● U_5.

3.3. *Binding of the oligonucleotides to 30 S ribosomes*

The reduced capability of U–U–C–A or A_4 to stimulate aa-tRNA binding could be explained by a weaker binding of these two tetranucleotides to the 30 S ribosome as compared to the other oligonucleotides.

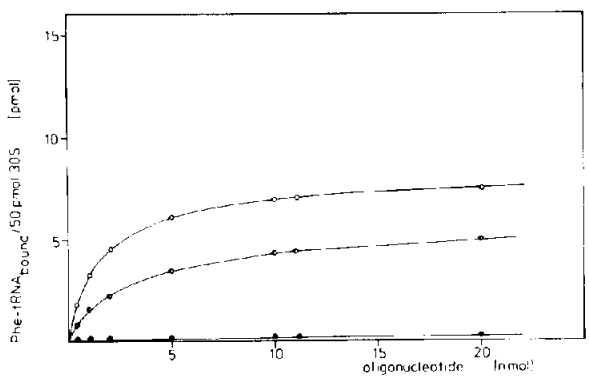

Fig.2. Oligonucleotide-dependent binding of [^{3}H]Phe-tRNA^{Phe} to 30 S ribosomes. Experimental conditions were the same as in fig.1 legend. (○-○) U–U–C; (●-●) U–U–C–C; (●-●) U–U–C–A.

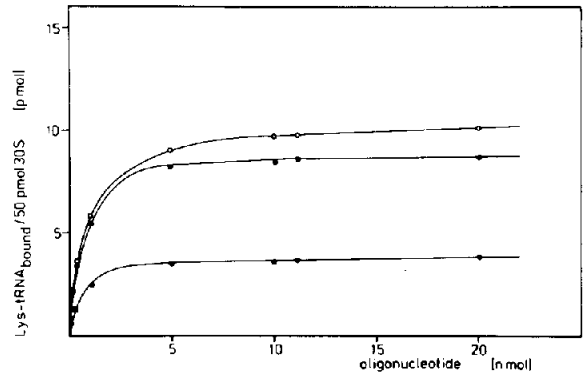

Fig.3. Oligoadenylate-dependent binding of [^{3}H]Lys-tRNA^{Lys} to 30 S ribosomes. Experimental conditions were the same as in fig.1 legend. (○-○) A_3; (●-●) A_4; (●-●) A_5.

It may be concluded from table 2 that the association constants between the various oligonucleotides and the 30 S cannot explain the reduced codon function of A_4 and U–U–C–A. With both series U_n and A_n there is a linear increase in the binding constant with the chain length of the oligonucleotides.

4. Discussion

Complex formation between a tRNA and an oligonucleotide complementary to the anticodon region has been examined by several groups, and the mea-

Table 2
Molar association constants for oligonucleotides to 30 S ribosomes

Oligonucleotide	K_a (M^{-1}) measured
U–U–U	4×10^3
U–U–U–U	1×10^4
U–U–U–U–U	3×10^4
A–A–A	3×10^4
A–A–A–A	6×10^4
A–A–A–A–A	1×10^5

The experimental conditions were the same as in table 1. 30 S ribosome concentration was 3.7 μM; oligonucleotides varied from 0.1–15 μM

sured association constant was related to the biochemical function of a codon and the tRNA. From a model of the tertiary structure of the tRNA anticodon loop and the increased binding constant for U–U–C–A, it was concluded that the anticodon loop is flexible and changes conformation on binding to the codon [3,12].

Our data prove that there is no straight-forward relationship between the effective binding of a complementary oligonucleotide to the tRNA and the coding activity of the respective oligonucleotide. The reduced codon activity of A_4 and U–U–C–A is not a result of the ribosome oligonucleotide association (table 2).

We have presented evidence in the past that codon–anticodon interaction triggers a conformational change in the tRNA tertiary structure [13]. From theoretical calculations, additional changes in the tertiary structure of tRNA have been postulated for defined steps in protein biosynthesis [14].

We would like to conclude from the data presented here, that U–U–C–A and A_4 stabilize a non-binding type conformation of their cognate aa-tRNA. This inactive conformation could be due to a base pairing of the terminal adenosine of the codon with U33 (tRNA) thereby disrupting the hydrogen bond HN^3 (U33) : O–P(A_{36}), which otherwise keeps the uridine$_{33}$ inside the anticodon loop [6]. Preliminary evidence shows that EF-Tu on the other hand aids in selecting the active aa-tRNA conformation, i.e., the one which is bound to the 30 S ribosome. The difference in codon activity between U_4 and U–U–C–A or A_3 and A_4 is less pronounced in the 70 S ribosomal system (data not shown). This may be due to the fact that, in 70 S ribosomes, positioning of uncharged tRNA to the peptidyl-site is required to produce effective aa-tRNA binding to the aminoacyl-site, and numerous interactions between aa-tRNA and the ribosome occur [16].

A non-linear arrangement of mRNA as well as defined peptidyl-tRNA–aa-tRNA interactions in biological systems may serve to avoid out of frame type reading of the mRNA.

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