CODON-DEPENDENT REARRANGEMENT OF THE TERTIARY STRUCTURE OF $tRNA^{Phe}$ FROM YEAST

Ulrich SCHWARZ and Hans Günter GASSEN

Fachgebiet Biochemie, Institut für Organische Chemie und Biochemie, Technische Hochschule Darmstadt, Petersenstr. 22, D-6100 Darmstadt, FRG

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1. Introduction

We have recently shown that codon-dependent binding of aminoacylated $tRNA^{Phe}$ from *Escherichia coli* to 70 S ribosomes occurs in two consecutive steps [1]. The initial reaction is the recognition of the appropriate AA-tRNA by the codon bound to the 30 S ribosomal subunit, which triggers a rearrangement of the three-dimensional structure of the tRNA. This results in the exposure of the T–ψ-C–G sequence for double strand formation with the C–G–A–A sequence of the 5 S rRNA within the 50 S subunit. Thus, a selective type binding, codon–anticodon complex formation, is transformed via a structural change of the tRNA into a non-discriminating uniform binding reaction: T–ψ-C–G · C–G–A–A double strand formation, with a high binding constant ($K_{ass} = 7 \times 10^5\ M^{-1}$).

Additional evidence for the codon-dependent rearrangement of $tRNA^{Phe}$ as measured by equilibrium dialysis was obtained by fluorescence measurements using $tRNA^{Phe}$ containing a probe in the dihydrouridine loop [2]. From theoretical calculations additional changes in the tertiary structure of tRNA have been postulated for defined steps in protein biosynthesis [3].

In order to compare the static structure of $tRNA^{Phe}$ from yeast [4,5] and the conformational transitions initiated by codon–anticodon complex formation, we examined the rearrangement of the tertiary structure of $tRNA^{Phe}$ from yeast occurring during the coded binding to ribosomes. The data presented are discussed with respect to the conformation transfer over 60 Å from the anticodon loop to the T–ψ-C–G region.

2. Experimental

2.1. *Materials*

Pyruvate kinase (EC 2.7.1.40) and yeast $tRNA^{Phe}$ were purchased from Boehringer, Mannheim. Baker's yeast was a gift from Pleser, Darmstadt. C–G and all other nucleoside 5′-diphosphates were supplied by Pharma Waldhof, Düsseldorf. [^{3}H]Adenosine-5′-diphosphate (18 000 Ci/mol) and [^{3}H]phenylalanine (5000 Ci/mol) were obtained from Amersham-Buchler, Braunschweig. *Escherichia coli* MRE 600 was recieved from Merck, Darmstadt. Dialysis membranes were procured from Iris 3069, Rhône-Poulenc C., Paris.

2.2. *Preparation of the components*

70 S ribosomal subunits were isolated from mid log-phase *E. coli* MRE 600 according to the methods of Leifer and Kreuzer [6] and Noll et al. [7]. Storage and reactivation of ribosomes prior to use were as described earlier [1]. *E. coli* polypeptide elongation factor Tu(EF-Tu) was prepared according to Arai et al. [8] and was purified by three-fold crystallisation. Yeast $tRNA^{Phe}$ was charged with [^{3}H]phenylalanine (100 Ci/mol) or phenylalanine using a partially purified synthetase preparation. On an analytical scale the charging of $tRNA^{Phe}$ was 60%, and 25% in the preparative procedure. C–G–[^{3}H]A–[^{3}H]A (320 Ci/mol), $(U)_8$, and poly(U) were prepared with polynucleotide phosphorylase [1].

2.3. *Experimental conditions*

The oligouridylate-directed enzymatic and non-enzymatic binding of Phe-$tRNA^{Phe}_{yeast}$ to 70 S and

30 S ribosomes was measured by the method of Nirenberg and Leder [9]. The tests were performed at optimal ribosomal activity as described by Kaufmann and Zamir [10].

Equilibrium dialysis was done as previously described [11], using the following concentrations: 50 mM Tris–HCl, pH 7.5, 50 mM NH_4Cl, 50 mM KCl, 1 mM 1.4 dithioerythritol and $Mg(OAc)_2$ as indicated.

3. Results

The Phe-tRNA^{Phe} was fully active in the heterologous system when assayed in poly(U)-dependent poly(Phe) synthesis or in Phe–tRNA binding experiments [9]. The rearrangement of the tRNA structure and thus exposure of the T–ψ-C–G region was followed by the binding of C–G–[^{3}H]A–[^{3}H]A using the equilibrium dialysis [12]. Equilibrium was reached within 4 h with the type of membrane described under materials. The specificity of the C–G–A–A binding was tested in 'nonenzymatic' (–EF-Tu) ribosomal system.

Figure 1 shows that 25 pmol C–G–A–A were bound to the 30 S × U_8 × tRNA^{Phe} complex using a high excess of C–G–A–A. The data for the 'nonenzymatic' binding were analyzed according to Scatchard (insert to fig.1) [13]. The association constant and the number of binding sites were calculated by linear regression from the slope and the intercept: $K_{ass} = 5.4 \times 10^5\ M^{-1}$, $n = 1.16$. These data demonstrate the existence of one binding site with an association constant as expected for the T–ψ-C–G · C–G–A–A complex. The influence of the Mg^{2+} concentration on C–G–A–A binding was examined (fig.2A), since it had been shown in the *E. coli* system that the rearrangement of the tRNA is Mg^{2+}-dependent. C–G–A–A binding to the tRNA^{Phe} in the presence of 30 S ribosomes shows a sigmoidal Mg^{2+}dependence with a point of inflection at 5 mM and saturation at 10–12 mM Mg^{2+}.

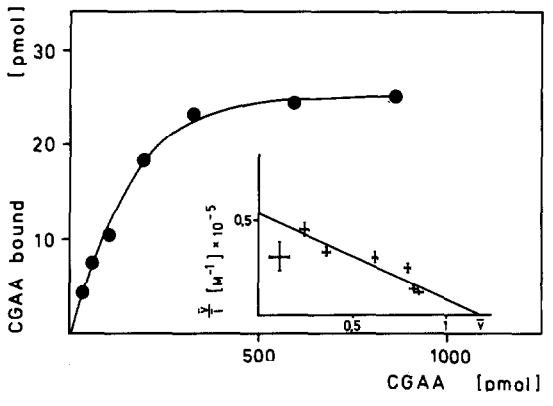

Fig.1. Binding of C–G–A–A to Phe-tRNA^{Phe} within the nonenzymatic ribosomal system as measured by equilibrium dialysis. Both chambers contained, in a total volume of 100 µl buffer, 100 pmol 30 S subunits, 400 pmol Phe-tRNA^{Phe} (charged with phenylalanine to 25%), and a final Mg^{2+} concentration of 10 mM. One compartment contained in addition 30 nmol $(U)_8$ and radioactive labelled C–G–A–A as indicated. The insert gives the Scatchard plot. The straight line was calculated by linear regression from assorted data points with $f_i = V(V/L)$.

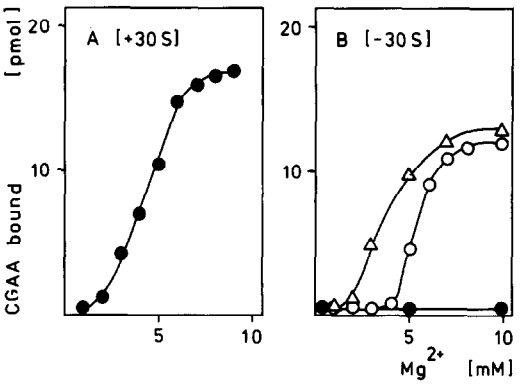

Fig.2. Mg^{2+}-Dependence of the binding of C–G–A–A to Phe-tRNA^{Phe} in the presence (A) or absence (B) of 30 S ribosomes. The compartments contained, in a total volume of 100 µl buffer, 400 pmol Phe-tRNA^{Phe}, and Mg^{2+} concentration as indicated. One of the compartments was supplemented with 300 pmol of C–G–A–A and $(U)_8$ as described. For the ribosomal system both chambers contained in addition 100 pmol 30 S subunits. For equilibrium dialysis within the enzymatic system both compartments contained additionally 800 pmol EF-Tu × GTP. Symbols used: closed circles, 0.3 mM $(U)_8$; open circles, 0.7 mM $(U)_8$ and triangles, 0.3 mM $(U)_8$ × EF-Tu × GTP.

This type of Mg^{2+}-dependence indicates that the binding of a AA–tRNA to the mRNA × 70 S complex is stabilized by an additional tRNA–50 S ribosomal subunit interaction (fig.3B). In the case of 30 S ribosomes where the exposure of the T–ψ-C–G sequence does not result in an additional binding of the tRNA to the 5 S rRNA a hyperbolic Mg^{2+}-dependence is

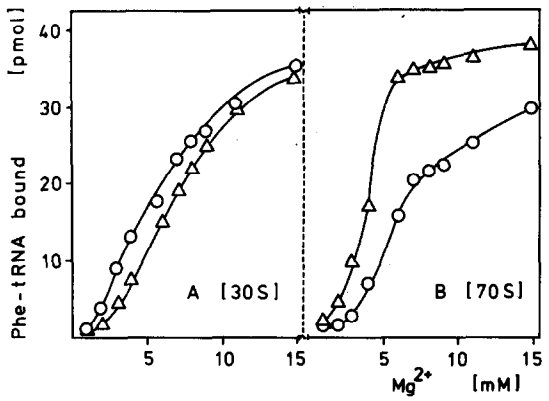

Fig.3. Mg^{2+}-Dependence of the nonenzymatic and enzymatic poly(U)-coded binding of [^{3}H]Phe-tRNA^{Phe} to 30 S and 70 S ribosomes. The incubation mixtures contained in total vol. 100 μl buffer: 100 pmol 70 S or 30 S ribosomes, 15 μg poly(U), 600 pmol Phe-tRNA^{Phe} (charged with [^{3}H]phenylalanine to 25%), 800 pmol of EF-Tu × GTP, and Mg^{2+} concentrations as indicated. For the nonenzymatic binding assays, EF-Tu × GTP was omitted. The samples were incubated for 40 min at 0°C. The amount of [^{3}H]Phe-tRNA^{Phe} bound was measured by the nitrocellulose filter assay. Blanks (without ribosomes) were subtracted. Symbols: circles, nonenzymatic binding; triangles, enzymatic binding.

found (fig.3A). The codon-dependent rearrangement is an intrinsic feature of the tRNA tertiary structure. When the C–G–A–A binding was examined in the absence of 30 S ribosomes at 0.3 mM $(U)_8$ which was saturating in the presence of ribosomes no binding could be detected. The $(U)_8$ or $(U)_{11}$ concentration respectively had to be raised to 3 mM to achieve a C–G–A–A binding in the non-ribosomal system (data not shown). Again, the Mg^{2+}-dependence showed a sigmoid type curve with a point of inflection at 5–7 mM (fig.2B). Using the ternary complex Phe-tRNA × GTP × EF-Tu the $(U)_8$ concentration could be lowered to 0.3 mM again (fig.2B).

4. Discussion

The data presented for the codon-dependent rearrangement of tRNA^{Phe} from yeast are in agreement with the findings obtained for the bacterial tRNA. The structural change of the tRNA is solely dependent on the presence of the appropriate codon and 10–12 mM Mg^{2+}. 30 S ribosomes and EF-Tu × GTP are not absolutely necessary but reduce the amount of oligonucleotide required.

If one examines the tertiary structure of tRNA^{Phe} from yeast the structural similarities in the anticodon and the T–ψ-C–G region are evident. The peculiarities in both regions have been summarized in a recent article by Rich and RajBhandary [14].

The anticodon loop of the tRNA^{Phe} represents, in part, a helix [15] comparable to that proposed by Saenger et al. [16] for oligo(A). The major difference between these two helices is a result of the more complete base overlap within the anticodon stack. The compact anticodon structure is stabilized by the so called 'U-turn' in the anticodon loop.

If double strand formation between the codon and anticodon occurs, the characteristic anticodon helix may be extended in a manner that the preformed anticodon stem double helix will be lengthened via the residues Y37 and A38. In this conformation the codon-anticodon helix may be stabilized on the one hand by stacking of the Y-base onto the codon–anticodon complex and on the other hand by the stacking of the residue U33 onto the following codon. This would result in a Fuller Hodgson type conformation [17]. The enlargement of the anticodon helix requires the abolition of the 'U-turn'.

This structural change may be transferred to the T–ψ-C loop by converting the relative orientation of the anticodon stem and the D-helix to a colinearity of the double helix axes. The tertiary interaction between residue G22 and m^{7}G46 may have a key function in the unmasking of the T–ψ-C loop.

The abolition of the interaction between T–ψ-C and D loop and the resulting T–ψ-C–G 5 S rRNA complex formation implies similar type conformational changes within the T–ψ-C loop as discussed for the anticodon loop. Thus, the T–ψ-C stem helix is continued by the T–ψ-C–G 5 S rRNA helix. Similar long distance interactions as described for tRNA^{Phe} from yeast are well known for regulating enzymes and postulating for DNA [18].

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