ON THE mRNA INDUCED CONFORMATIONAL CHANGE OF AA-tRNA EXPOSING THE T-Ψ-C-G SEQUENCE FOR BINDING TO THE 50S RIBOSOMAL SUBUNIT

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Received November 5,1973

Summary: A model is proposed which assumes that the codon-anti-codon interaction induces a conformational change in the tertiary structure of AA-tRNA exposing the hidden T-Ψ-C-G sequence for binding to the C-G-A-A sequence in the 5S RNA of the 50S ribosomal subunit. The model is supported by binding experiments with C-G-(^{3}H)A-(^{3}H)A and by the inhibition of poly(Phe) synthesis by C-G-A-A. The process is dependent on the 30S subunit, the template, and the AA-tRNA x EF-T_u x GTP complex.

Introduction: Almost all tRNAs, regardless to their function and origin, contain the common tetranucleoside triphosphate T-Ψ-C-G (1,2). The location of the sequence is always in exactly the same position of the primary sequence of a tRNA, i. e. in the position 1 to 4 of the T-Ψ-C-G loop. One of the known exceptions is the $tRNA^{Gly}$ from Staph. epidermis lacking this tetranucleotide. This tRNA, however, is inactive in protein synthesis (3). It was speculated very early that this sequence represents the binding site of a tRNA to the 50S ribosomal subunit (4), and this interpretation was supported by the finding that the 5S RNA of the 50S ribosome contains the complementary sequence C-G-A-A (5). In 5S RNA from different procaryotic organisms this sequence has been found in a nearly identical position (No 43 - 46). The sequence should be non base paired, and should be located in an exposed region, as this sequence is most suitable for RNase T_1 hydrolysis and N-oxidation as well (6). It was stated by Ofengand and Henes that T-Ψ-C-G inhibits nonenzymatic binding of AA-tRNA to

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both the aminoacyl- and the peptidyl-site of the ribosome up to nearly 50% (4). As has been concluded from chemical modification experiments, however, this sequence is usually hidden within the tertiary structure of tRNA (7). This approach was supported by binding studies between tRNA and complementary oligonucleotides (8). Therefore it can be postulated that the tRNA undergoes a conformational change before a complex between T-Ψ-C-G and C-G-A-A has been formed. This conformational change has to be dependent upon codon-anticodon recognition as well as on the AA-tRNA x EF-T_U x GTP complex formation, otherwise all non fitting and uncharged tRNAs would compete for the same binding site on the 50S ribosome. This idea implies a model in which codon-anticodon recognition induces a conformational change in the tertiary structure of the tRNA, which exposes the T-Ψ-C-G sequence for binding. A model based on these assumptions is outlined in Fig. 1. The recent experiments deal with the elucidation of the model proposed.

Materials and methods: E. coli MRE 600 cells were obtained from Whatman, London, (^{3}H)Phe, (^{3}H)GTP, and (^{3}H)ADP from the "Radiochemical Centre", Amersham. All other materials and chemicals were obtained from commercial sources and were of the purest grade available. Phe-tRNA, Lys-tRNA, Pro-tRNA, and (^{3}H)Phe-tRNA were prepared as described previously (9). E. coli elongation factor T_U (EF-T_U) was

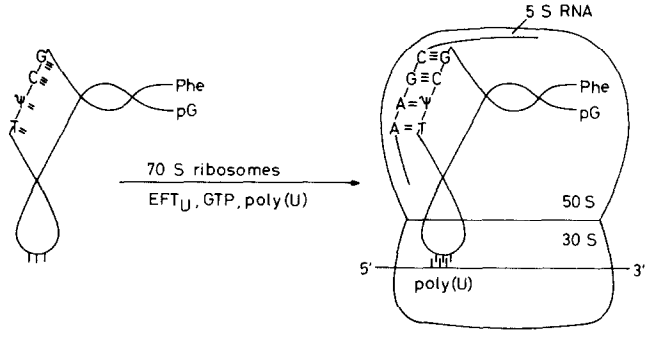
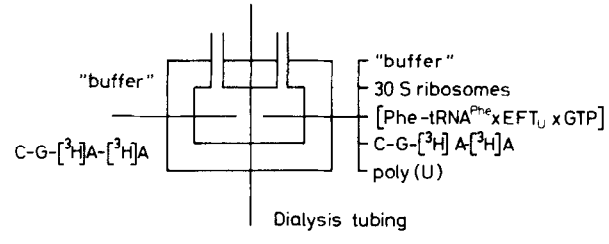


Fig. 1: Schematic drawing of the model proposed for the poly(U) dependent conformational change of the AA-tRNA x EF-T_U x GTP complex to expose the T-Ψ-C-G sequence for binding to the C-G-A-A sequence of the 5S RNA in the 50S ribosomal subunit.

purified from the E. coli 100 000 x g supernatant according to the procedure of Ravel (10). 30S ribosomal subunits were kindly supplied by Dr. H. Matthaei, Göttingen. The activity of the elongation factor T_u was determined by the method of Ravel and Shorey (11). Equilibrium dialysis was performed by the method of Uhlenbeck et al. (12). The labeled and unlabeled tetranucleoside triphosphate C-G-A-A was synthesized with primer dependent polynucleotide phosphorylase as described elsewhere (13). The tetranucleotides were purified by DEAE-cellulose chromatography, followed by paper electrophoresis and Sephadex G-25 chromatography. The oligonucleotides were analyzed and identified in terms of nucleosides and specific activity with the nucleoside analysator (14).

Results: The binding of C-G-(^{3}H)A-(^{3}H)A to the exposed region of T-Ψ-C-G in the AA-tRNA x EF-T_u x GTP complex was assayed by the equilibrium dialysis process as outlined in Fig. 2. The labeled tetranucleotide was added to both chambers in order to enhance the adjustment of the equilibrium maximally. Binding could be observed for only 24 h, because of the presence of nucleases, which as contaminants of the EF-T_u-preparation slowly degraded the poly(U)



30S + [Phe-tRNA^{Phe} x EFT$_U$ x GTP] + poly(U) + C-G-[^{3}H]A-[^{3}H]A
⟶ [30 S x Phe-tRNA^{Phe} x EFT$_U$ x GTP x poly(U) x C-G-[^{3}H]A-[^{3}H]A]

Fig. 2: Equilibrium dialysis to measure the binding of C-G-(^{3}H)A-(^{3}H)A to the complex listed above. The system contained 100 pmoles 30S ribosomes which were limiting and 300 units EF-T_u. These 300 units EF-T_u formed 130 pmoles AA-tRNA x EF-T_u x GTP complex from which 30 to 40 pmoles were bound to 100 pmoles of 30S subunits.

Table 1: Binding of C-G-(^{3}H)A-(^{3}H)A to the 30S x Phe-tRNA x EF-T_u x GTP x poly(U) complex as determined by equilibrium dialysis (0°C- 4°C). The complete system contained in a total volume of 100 µl: $MgCl_2$ (2 µmoles), NH_4Cl (10 µmoles), Tris-HCl, pH 7.5 (2.5 µmoles), DTT (0.1 µmoles), 30S ribosomes (100 pmoles), poly(U) (400 pmoles), GTP (1 nmole), AA-tRNA (40 nmoles), $Phe\text{-}tRNA^{Phe}$ (60 pmoles), and EF-T_u (300 units).

	C-G-(^{3}H)A-(^{3}H)A bound (pmoles)
complete system	37.6 ± 10
- poly(U)	9.7 ± 10
- Phe-tRNA	2.3 ± 10
- EF-T_u	8.7 ± 10
- GTP	12.8 ± 10
- 30S	0. ± 10

template. Nevertheless, the stability of the oligonucleotide C-G-(^{3}H)A-(^{3}H)A and of the ternary complex was being observed for at least 60 h. The results of these binding studies, measured by equilibrium dialysis, are shown in Table 1. The values represent averages calculated from five measurements. Nearly 40 pmoles C-G-(^{3}H)A-(^{3}H)A were bound to the 30S x Phe-tRNA x EF-T_u x GTP x poly(U) complex. This is equal roughly to the amounts of ternary complex, bound to 30S ribosomes as shown in the legend to Fig. 2. In the absence of poly(U) only 10 pmoles oligonucleotide are bound. This should indicate that the postulated change in the tertiary structure of the tRNA is dependent on codon-anticodon recognition. Since without EF-T_u or GTP no complex is formed, the conformational change requires the formation of the ternary complex AA-tRNA x EF-T_u x GTP

In order to prevent the degradation resulted from the long incubation

time factor during equilibrium dialysis, the binding of C-G-(^{3}H)A-(^{3}H)A to the tRNA was checked by the nitrocellulose filter assay (15). The data obtained are listed in Table 2. They are comparable with the values obtained from equilibrium dialysis. In this assay

Table 2: Poly(C) stimulated binding of C-G-(^{3}H)A-(^{3}H)A to the 30S x Pro-tRNA x EF-T_u x GTP x poly(C) complex as measured by absorption on nitrocellulose filters. The system contained the same components as listed in Table 1, except that poly(U) was replaced by poly(C) and Phe-tRNA by Pro-tRNA and was incubated at 0°C for 20 min.

	C-G-(^{3}H)A-(^{3}H)A bound (pmoles)
complete sytem	33.4 ± 5
- poly(C)	14.6 ± 5
- EF-T_u	5.7 ± 5
- 30S	0.5 ± 5

system poly(U) was replaced by poly(C) indicating that the binding observed was not restricted to the poly(U)-Phe-tRNA system. Experiments in order to show the binding of C-G-(^{3}H)A-(^{3}H)A to Lys-tRNA in the presence of poly(A) gave a negative result.

For the evidence that the binding of C-G-A-A to the T-Ψ-C-G loop effects protein synthesis, the inhibition of poly(Phe) synthesis by this tetranucleoside triphosphate was measured. As can be seen in Fig. 3, poly(Phe) synthesis was inhibited up to about 40%. Since C-G-A-A containing the anticodon sequence of $tRNA^{Phe}$ is able to bind to poly(U) and thus inhibits poly(Phe) synthesis, we used A-A-A-A as a control. This tetranucleotide shows no inhibition. It seems evident that C-G-A-A inhibits the poly(Phe) synthesis by binding to the exposed T-Ψ-C-G sequence.

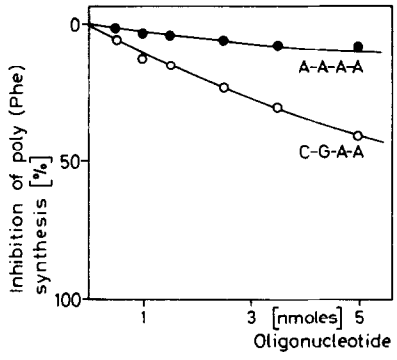


Fig. 3: Inhibition of poly(Phe) synthesis by the tetranucleoside triphosphates C-G-A-A and A-A-A-A. 0% inhibition corresponds to 17.6 pmoles poly(Phe) synthesized per 50 pmoles 70S ribosomes. The complete system contained in a total volume of 100 µl: $Mg(OAc)_2$ (1.5 µmoles), NH_4Cl (10 µmoles), Tris-HCl, pH 7.5 (2.5 µmoles) ATP (1.2 µmoles), GTP (0.5 nmoles), PEP (3.7 µmoles), DTT (0.5 µmoles), pyruvatkinase (2.5 ng), (^{3}H)Phe-tRNA (1.6 nmoles, charged with 24.0 pmoles (^{3}H)Phe (specific activity 1000 (Ci/Mol))), poly(U) (20 µg), 70S ribosomes (50 pmoles), EF-T (42.0 µg), and EF-G (50.0 µg). The reaction mixture was incubated at 37°C for 20 min.

Discussion: As stated by Ofengand and Henes high concentrations of T-Ψ-C-G inhibit the nonenzymatic poly(U)-dependent binding of Phe-tRNA to 70S ribosomes (4). These findings should indicate the formation of a complex between T-Ψ-C-G and C-G-A-A which on stereochemical basis prevents the interaction of the AA-tRNA with the 50S subunit of the ribosome. Excess Phe-tRNA did not compete with the binding of C-G-A-A, which is easily understandable, since during the nonenzymatic binding the T-Ψ-C-G sequence remains hidden in the tertiary structure of the AA-tRNA, and therefore is not available for complex formation. Furthermore, additional evidence for the model proposed is the work of Urbanke et al. (16), who showed that the structure of tRNA is subject to several thermal transitions. The first low temperature transition is due to a change in the tertiary structure resulting in the disruption of presumeably two A:U and one G:C base pairs. According to the model proposed in Fig. 1 this transition could be attributed to an unfolding of the T-Ψ-C-G loop or

to a loss of the interaction between the dihydrouridine and T-Ψ-C-G loop.

The proposed model of the binding of the AA-tRNA to the 70S ribosome requires a more dynamic role for the tRNA rather than its function as an aminoacyl carrier. In this model depending on codon-anticodon recognition the tRNA can undergo a conformational change which results in the binding of the T-Ψ-C-G sequence to the 50S subunit. Thus besides codon-anticodon interaction a second binding takes place which on one hand bridges the 30S and 50S subunits (17) and on the other binds the AA-tRNA to the 50S subunit near the peptidyltransferase center. This binding seems to be necessary, since the EF-T_u factor which mediates the binding of the aminoacyl-end of the AA-tRNA to the 50S particle is separated from the complex before the formation of peptide bond. Furthermore, the change in the tertiary structure of the tRNA may reduce the affinity of the tRNA for EF-T_u which is removed under GTP hydrolysis (18) and may open up a new binding site for the elongation factor G.

Acknowledgement: This work was supported by grants from the "Deutsche Forschungsgemeinschaft" and the "Fond der Chemischen Industrie".

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