THE PEPTIDYL-SUBSTITUENT WITHIN PEPTIDYL-tRNA INCREASES THE STABILITY OF tRNA–mRNA ASSOCIATION

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1. Introduction

We have recently become interested in the mechanism of the translocation reaction, a GTP-consuming step in the translational cycle of ribosomal protein synthesis [1,2]. A crucial question is how the mRNA and peptidyl-tRNA are translocated. They may be either translocated from the aminoacyl-tRNA binding site (A site) to the peptidyl-tRNA binding site (P site) of the 70 S ribosome as a unit or as separate components. The experimental evidence available, in particular [3,4], support the idea of simultaneous translocation of these components. We present evidence that the peptidyl substituent within the peptidyl-tRNA, when in the presence of ribosomes, serves to stabilize codon–anticodon interaction in the binary complex. These data further support the view that the peptidyl-tRNA–mRNA complex as a whole is translocated from the A to the P site.

2. Experimental

Ribosomes were isolated from *E. coli* MRE 600 and characterized for their biochemical functions as in [5]. Oligonucleotides with spec. act. 135–300 Ci/mol [6] were prepared with polynucleotide phosphorylase (EC 2.7.7.8). tRNAs were aminoacylated, and formylated or acetylated by known procedures, with the modification that low-M_r components were removed by dialysis (500 μl cell compartments) and not by gel permeation chromatography [7,8]. Acylation of the purified tRNAs was as follows: Phe-tRNA > 95%; AcPhe-tRNA 90%; fMet-tRNA 65%; AcMet-tRNA$_m^{Met}$ 50%. In the following text purified aminoacylated tRNAs are denoted by Phe-tRNA for example, and not by Phe-tRNA^{Phe}.

Equilibrium dialysis was performed as in [2]. Reaction mixture (100 μl) contained: 50 mM Tris–HCl (pH 7.5), 150 mM NH_4Cl, 20 mM $MgCl_2$, 10 mM mercaptoethanol, 20 pmol 70 S ribosomes, 150 pmol tRNA and 10–500 pmol ^{3}H-labelled oligonucleotide. Equilibrium was reached after 6–8 h at 0–4°C. The apparent association constants (K'_{ass}) of the binary complexes were determined using 250 pmol tRNA and 5 nmol oligonucleotide [9].

Velocity sedimentation experiments using a 'Spinco model E' analytical ultracentrifuge have been described in [10]. The optical cells contained in 450 μl: 50 mM Tris–HCl (pH 7.5), 150 mM NH_4Cl, 10 mM mercaptoethanol, 8–35 mM $MgCl_2$, 90 pmol 70 S ribosomes, 450 pmol tRNA, and 30 nmol AUG. The concentration of free tRNA was determined at 285 nm. Centrifugation was for 100 min at 10°C.

3. Results

Table 1 summarizes the association constants for the binary complexes oligonucleotide–tRNA, for the initiator and two elongator tRNAs. In all cases examined the peptidyl-tRNA binds the codon 3–10-fold more tightly than the corresponding deacylated tRNA. Phe-tRNA shows the same affinity for UUCA as tRNA^{Phe}. Whilst this confirms our earlier results that aminoacylation of the tRNA does not influence codon–anticodon interaction [11], it contradicts

Abbreviations: tRNA$_f^{Met}$ and tRNA$_m^{Met}$, the methionine tRNAs which can and cannot be formylated, respectively; AcMet-tRNA$_m^{Met}$ and AcPhe-tRNA^{Phe}, N^{α}-acetylmethionyl-tRNA and N^{α}-acetylphenylalanyl-tRNA

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Table 1
Effect of the peptidyl moiety on the stability of codon–anticodon interaction

Oligo-nucleotide	tRNA	Ribosomes, 70-S	K'_{ass} [M^{-1}]
AUG	$tRNA_f^{Met}$	–	4.4×10^3
	fMet-$tRNA_f^{Met}$	–	1.2×10^4
AUGA	$tRNA_f^{Met}$	–	4.0×10^4
	fMet-$tRNA_f^{Met}$	–	1.2×10^5
$AUGU_3$	$tRNA_f^{Met}$	+	4.6×10^7
	fMet-$tRNA_f^{Met}$	+	1.1×10^8
	$tRNA_m^{Met}$	+	3.9×10^6
	AcMet-$tRNA_f^{Met}$	+	5.3×10^7
UUC	$tRNA^{Phe}$	–	1.7×10^3
	AcPhe-tRNA	–	1.1×10^4
	$tRNA^{Phe}$	+	6.3×10^5
	AcPhe-$tRNA^{Phe}$	+	9.5×10^6
UUCA	$tRNA^{Phe}$		4.0×10^4
	Phe-$tRNA^{Phe}$	–	3.8×10^4
	AcPhe-tRNA	–	1.9×10^5
	$tRNA^{Phe}$	+	2.3×10^6
	AcPhe-tRNA	+	1.8×10^7

The association constant was calculated for 10 different oligonucleotide concentrations using a Scatchard plot. With fMet-$tRNA_f^{Met}$ and AcMet-$tRNA_m^{Met}$ there should be an even greater difference in the association constants between the tRNA and peptidyl-tRNA, since acylation was only 65% and 50%, respectively

other reports [12]. To avoid deacylation of the Phe-tRNA during the 8 h incubation period we lowered the pH to 6.0. The pH dependence of tRNA–oligonucleotide association was examined over pH 5.5–7.5 with UUCA and $tRNA^{Phe}$. No pH effect was found on complex formation between oligonucleotide and tRNA within these limits.

The differences between peptidyl-tRNA and tRNA became even more pronounced when the binding of the oligonucleotide to ribosome–tRNA complexes was examined (table 1). Depending on their sequence cognate oligonucleotides are bound 5–20-fold more effectively to ribosomes containing a peptidyl-tRNA, than to those with a deacylated tRNA. For these experiments we used the hexanucleotide $AUGU_3$ in addition to the tetranucleotides, since this compound represents an excellent mRNA analogue for functional studies with the ribosome [2].

Since the [Mg^{2+}] employed in these experiments (20 mM) were inhibitory for poly(U)-dependent poly(Phe) synthesis, we illustrate in fig.1 the influence of [Mg^{2+}] on the association constants for the ribosome–oligonucleotide, tRNA–oligonucleotide

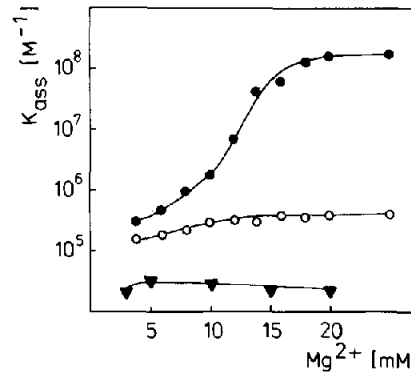

Fig.1. Association constants for the ribosome–oligonucleotide, tRNA–oligonucleotide and ribosome–oligonucleotide–tRNA complexes with varying [Mg^{2+}]. The reaction mixture contained in 100 µl, 50 mM Tris–HCl (pH 7.5), 150 mM NH_4Cl, 3–25 mM Mg^{2+}, 10 mM mercaptoethanol, and: (a) 20 pmol 70 S ribosome and 500 pmol [^{3}H]$AUGU_3$ (spec. act. 300 Ci/mol) (▾); (b) 1.1 nmol $tRNA^{Phe}$ and 2.3 nmol [^{3}H]UUCA (spec. act. 75 Ci/mol) (○); (c) 20 pmol 70 S ribosomes, 500 pmol [^{3}H]$AUGU_3$ (spec. act. 300 Ci/mol), 100 pmol $tRNA_f^{Met}$ and 100 pmol $tRNA^{Phe}$ (●).

and ribosome–oligonucleotide–tRNA complexes. The association constants for the first two complexes are virtually independent of [Mg^{2+}]. The stability of the tRNA–ribosome interaction, however, is strongly influenced by [Mg^{2+}] up to 20 mM. This further supports the finding that an increased [Mg^{2+}] only inhibits the removal of the deacylated tRNA from the P site [13].

The differences in biochemical behaviour of a peptidyl-tRNA and of a deacylated tRNA are shown by the characteristics of their binding to the ribosome. Here, we used velocity sedimentation in the analytical ultracentrifuge and altered the affinity of the tRNA towards the programmed ribosome by varying the [Mg^{2+}] [10]. Free $tRNA_f^{Met}$ does not discriminate between the A and the P site of the ribosome and two tRNA molecules are bound with equal affinity to both sites. However, with AUG programmed ribosomes one site, presumably the P site, is preferentially occupied up to 20 mM Mg^{2+}. Only at higher [Mg^{2+}] does a second tRNA associate with the ribosome. fMet-tRNA, however, clearly discriminates between the A and the P site. Even at 30 mM Mg^{2+}, only one fMet-tRNA molecule associates with the ribosome. In this case, the presence of AUG results in an increased association constant (fig.2). Similar results were obtained when we compared AcPhe-tRNA and $tRNA^{Phe}$ [14]. We would conclude from these data

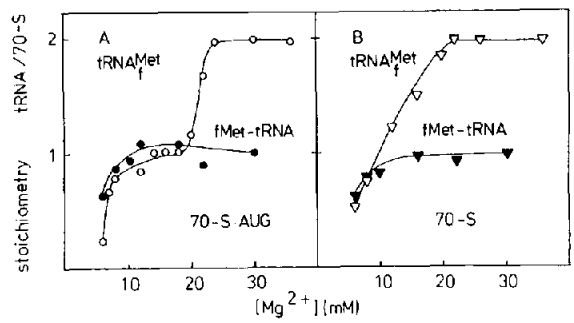

Fig.2. Binding of $tRNA_f^{Met}$ or fMet-tRNA to free and AUG-programmed 70 S ribosomes. The apparent binding constants were determined for one site-occupation at 8 mM Mg^{2+}:

$tRNA_f^{Met}$–(70-S–AUG)	$K'_{ass} = 2.1 \times 10^5\ M^{-1}$
fMet-$tRNA_f^{Met}$–(70-S–AUG)	$K'_{ass} = 9.8 \times 10^6\ M^{-1}$
fMet-$tRNA_f^{Met}$–70-S	$K'_{ass} = 3.4 \times 10^6\ M^{-1}$

(A) (○) 70-S–AUG–$tRNA_f^{Met}$, (●) 70-S–AUG–fMet-tRNA
(B) (▽) 70-S–$tRNA_f^{Met}$, (▼) 70-S–fMet-tRNA.

that the formylmethionyl moiety alters the conformation of the $tRNA_f^{Met}$ such that it becomes specific for the P site. Since a partial discrimination of $tRNA_f^{Met}$ is already achieved by the mere presence of AUG it would appear that both the formylmethionyl group and the codon cooperate in altering the conformation of the $tRNA_f^{Met}$.

4. Discussion

Transfer RNA, like certain proteins, may undergo ligand-induced structural transitions which result in altered biochemical properties [12]. The carrier protein elongation factor Tu–GTP, the codon, both the aminoacyl- and the peptidyl-substituents and Mg^{2+}, as well as the oligo-cations, spermine and spermidine, may function as ligands. The biochemical consequences of structural transitions, e.g., the type of interaction between the invariant TψC (G,A) sequence of the tRNA and the ribosomal RNA, are under analysis [13]. We show here that the peptidyl moiety of the peptidyl-tRNA tightens the codon–anticodon interaction, both in the binary complex and in the presence of ribosomes. This finding may be explained by a long range structural transition in the tRNA, caused by the peptidyl group. The dominant effect of the formylmethionyl group on the binding characteristics of fMet-tRNA to the ribosome supports the postulated effect of the 3′-substituent on the conformation of the initiator tRNA. With regard to the translocation mechanism this phenomenon has the following consequences:

(i) Removal of the peptidyl moiety from the peptidyl-tRNA in the P site by transpeptidation weakens the mRNA–tRNA interaction and thus facilitates the dissociation of the tRNA from the P site;

(ii) Transfer of the nascent peptide to the aminoacyl-tRNA located in the A site strengthens the complex formed between the mRNA and the new peptidyl-tRNA, and thus supports the idea of a translocation of a peptidyl-tRNA–mRNA complex.

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