The role of the anticodon in the interaction between methionyl-tRNA synthetase and bacterial initiator tRNA?

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ABSTRACT

Complementary and antiparallel oligonucleotides bind to exposed regions of the tRNA molecule. Aminoacylation in the presence of triplets has been used to determine the role of the anticodon in the interaction between methionyl-tRNA synthetase and initiator tRNA. ApUpG has no effect on the charging even when 70% of the tRNA is bound to the triplet, whereas in the presence of GpGpU which binds to the A-C-C sequence adjacent to the 3' terminal adenosine that fraction of the tRNA which is bound to the triplet is completely unavailable for charging. Hence the anticodon is probably not involved in a primary interaction while the A-C-C-A-OH clearly is. This conclusion is supported by the failure of the isolated anticodon loop and stem oligonucleotides to inhibit the aminoacylation reaction.

INTRODUCTION

Many techniques have been used to assign features of the tRNA molecule which are specifically recognised by its correct aminoacyl-tRNA synthetase^{1,2}. It has been reported for tRNA_f^{Met} from *Escherichia coli* that the conversion of the C residue in the anticodon to U by treatment with sodium bisulphite abolishes the acceptor activity³ and Dube⁴ has recently suggested a three point attachment of tRNA_f^{Met} to methionyl-tRNA synthetase from *E. coli* involving the anticodon loop, the extra arm and the 3' stem. We report here two separate lines of evidence that the binding of the anticodon loop to the synthetase can at best be very weak and it is probably insignificant.

MATERIALS AND METHODS

Methionyl-tRNA synthetase was purified to homogeneity as described previously from *E. coli* EM 20031⁵. tRNA_f^{Met} from *E. coli* CA 265 was purified as described by Doctor et al.⁶ Using the pure enzyme the tRNA had an acceptance of 1590 μ moles per A₂₆₀ unit. The hexadecanucleotide containing most of the anticodon stem and all

* Abbreviations: tRNA_f^{Met} - that species of methionine accepting tRNA which can be formylated when charged; tRNA_m^{Met} - that species which cannot be formylated.

of the loop of tRNA^{Met}_m - residues 26 to 41 - and the nonadecanucleotide containing all of the anticodon stem and loop of tRNA^{Met}_f - residues 28 to 46 - were gifts from Dr. B. P. Doctor.

Trinucleoside diphosphates were gifts from Mr. R. S. Brown and Mr. P. W. Piper. Their concentrations were estimated spectrophotometrically assuming that 31 A₂₆₀ units are equivalent to 1 mg. [³H] L-methionine was purchased from the Radiochemical Centre, Amersham, England.

Aminoacylation assays were performed at pH 7.0 at 0° in a volume of 200 µl. The reaction mixture contained 100 mM HEPES, pH 7.0, 7 mM MgCl₂, 200 mM NaCl, 5 mM ATP, 0.1 mM [³H] L-methionine and 10 mM 2-mercaptoethanol. The reaction was started by the addition of 1 µg of pure enzyme. Aliquots were withdrawn into cold 5% trichloroacetic acid and the precipitated tRNA collected on a Millipore filter. Radioactivity was measured in a Unilux II Liquid Scintillation Spectrometer using BBOT in toluene/2-methoxyethanol as scintillant⁷.

RESULTS AND DISCUSSION

Doctor *et al.*⁶ showed that the hexadecanucleotide containing the anticodon from tRNA^{Met}_m did not reduce the extent of charging of either tRNA^{Met} species when incubated in six to eight fold excess over the tRNA at 37°. Under these conditions the oligonucleotide would behave as a linear sequence and furthermore the experiment made no attempt to measure the initial rate of aminoacylation which is, of course, the most sensitive test of inhibition. Table 1 summarises the result of experiments with the oligonucleotides containing the anticodon loops from both tRNA^{Met} species at 0°.

Even when the oligonucleotides are present in one hundred fold excess over the tRNA which is itself present at a sub-optimal concentration, there is no detectable inhibition of the initial rate of aminoacylation. Assuming that a 10% reduction in rate would have been detected, this indicates that the oligonucleotides have a K_i for binding to the synthetase greater than 5 x 10⁻⁴ M compared with a K_s for the intact tRNA of about 10⁻⁷ M^{7,8}. Although the oligonucleotides can fold into more than one loop we would expect that at 0° an appreciable fraction would be in the conformation of the anticodon loop. These data indicate that the isolated loops and stems have a very low affinity for the synthetase.

Table 1

Effect of anticodon loop oligonucleotides on the aminoacylation of tRNA _f ^{Met}							
tRNA (conc.)	Enzyme (1 µg)	HDN (10 ⁻⁵ M)	NDN (10 ⁻⁵ M)	c. p. m. recorded after			
				1	2	3	5 min.
10 ⁻⁶ <u>M</u>	-	-	-	427	365	449	397
10 ⁻⁶ <u>M</u>	+	-	-	1156	1809	2559	3901
10 ⁻⁶ <u>M</u>	+	+	-	1136	1847	2468	3988
10 ⁻⁶ <u>M</u>	+	-	+	1161	1894	2501	3864
10 ⁻⁷ <u>M</u>	-	-	-	364	401	379	324
10 ⁻⁷ <u>M</u>	+	-	-	604	822	1056	1454
10 ⁻⁷ <u>M</u>	+	+	-	581	813	1079	1526
10 ⁻⁷ <u>M</u>	+	-	+	594	761	1127	1492

The reactions were performed as described in the Methods except that NaCl was omitted from the assay medium. The [³H] L-methionine had a specific activity of 1.2×10^5 c. p. m. /µmole. All figures are the average of two incubations. HDN, Hexadecanucleotide from tRNA_m^{Met}. NDN, Nonadecanucleotide from tRNA_f^{Met}.

Complementary and antiparallel oligonucleotides will bind to single-stranded regions of the tRNA molecule^{9, 10}. The association constant of the triplet ApUpG for tRNA_f^{Met} from *E. coli* has been estimated at 6×10^4 M⁻¹ at pH 7.0 and in the presence of 0.2 M NaCl and 10 mM MgCl₂ at 2°¹¹. It is thus possible to attempt to aminoacylate the tRNA molecule in the presence of large concentrations of triplet under conditions where one would expect a large fraction of the tRNA to be bound to the triplet. The exact magnitude of this fraction in these experiments was governed by the availability of the triplets. If the region of the tRNA where the triplet is bound is involved in an important interaction with the synthetase, we would expect the introduction of the bulky triplet on the exterior of the tRNA to reduce the initial rate of aminoacylation.

Table 2 shows the results obtained with tRNA_f^{Met} at a concentration of 10⁻⁷ M and ApUpG or ApApG at concentrations of 4×10^{-4} M. Under these conditions 70% of the tRNA should be bound to the triplet. ApApG was chosen as a non-binding triplet control as it was readily available. The data clearly indicate that there is no specific inhibition. Both triplets cause a reduction in the initial rate of aminoacylation of about 5% probably due to their binding Mg⁺⁺ and reducing its effective concentration.

Table 2 Effect of triplets on the aminoacylation of tRNA_f^{Met}

Additions	c. p. m. recorded after			
	1	2	4	30 min.
None	464	522	440	451
Enzyme	1226	1915	3236	8137
Enzyme + ApUpG	1171	1823	2984	8216
Enzyme + ApApG	1198	1846	2976	8146

[tRNA] = 10^{-7} M, [triplet] = 4×10^{-4} M. The [³H] L-methionine had a specific activity of 1.55×10^6 c. p. m. / μ mole. All figures are the average of two incubations.

This was confirmed by repeating the experiments at 10 mM MgCl₂ where there was no detectable inhibition with either triplet. Before one can assess the significance of these data it is essential to show that a triplet bound at a site which does interact with the synthetase would be detected by this technique.

The association constant of GpGpU for tRNA_f^{Met} in the presence of 1.0 M NaCl, 10 mM MgCl₂ and 10 mM sodium phosphate pH 7.0 at 0° has been estimated at 4×10^5 M⁻¹ ¹². This high salt concentration is unsuitable for the aminoacylation experiments, but the data of Hogenauer *et al* ¹¹ show that for the anticodon loop triplets the affinity is higher at 0.2 M NaCl; hence this was used in these experiments. The triplet binds to the tRNA molecule at the A-C-C sequence adjacent to the 3' terminal adenosine. Table 3 shows the results obtained with tRNA_f^{Met} at a concentration of 10^{-7} M and GpGpU at a concentration of 10^{-4} M.

Table 3 Effect of GpGpU on the aminoacylation of tRNA_f^{Met}

Additions	c. p. m. recorded after			
	1	2	4	30 min.
None	412	475	471	474
Enzyme	1236	1916	3134	8214
Enzyme + GpGpU	625	870	1231	4149
Enzyme (5 μ g) + GpGpU	1174	1907	3181	8199

[tRNA] = 10^{-7} M, [GpGpU] = 10^{-4} M. The [³H] L-methionine had a specific activity of 1.55×10^6 c. p. m. / μ mole. All figures are the average of two incubations.

Under these conditions about 80% of the tRNA should be bound to the triplet. This is precisely what the data indicate. The initial rate of aminoacylation is reduced to about 20% of the control and it can be restored by adding five times as much enzyme. In the presence of 1 mM spermine which stabilizes the tRNA/triplet interaction the rate is reduced to about 15% of the control and ten times the amount of enzyme is required to restore it. Clearly when a triplet binds at a critical site on the tRNA molecule, that fraction of the tRNA which is complexed is not available for aminoacylation.

This positive demonstration of inhibition by GpGpU binding to the tRNA molecule adjacent to the 3' terminal adenosine strongly suggests that the complete lack of inhibition by the codon is due to the fact that the anticodon loop does not have a primary interaction with the synthetase. This view is reinforced by experiments with the isolated loops themselves. The loss of acceptor activity following the bisulphite reaction³ could thus be due to a change in conformation of other parts of the molecule coincident with the C to U conversion.

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