EFFECTS OF TΨCG ON THE ENZYMATIC BINDING OF EUKARYOTIC AND PROKARYOTIC INITIATOR tRNAs TO RAT LIVER RIBOSOMES

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1. Introduction

In 1964 Rosset et al. discovered ribosomal 5 S RNA [1], which was subsequently sequenced from a number of prokaryotic [2–4] and eukaryotic organisms [5–7]. Comparison of the sequence of KB cell 5 S RNA [5] with that of *E. coli* [2] lead Forget and Weissman [5] to propose that the common 5 S RNA sequence CGAAC would interact with the common GTΨCPu (loop IV) sequence of tRNA [8,9]. More recent data has shown that all 5 S RNAs contain the oligonucleotide or one similar to CGAAC [2–7, 10]. In contrast recent tRNA sequencing experiments have shown that not all tRNAs contain the GTΨCPu oligonucleotide. The exceptions being: 1) eukaryotic initiator tRNA [11–13] and 2) $tRNA^{Gly}_{IA+IB}$ of *Staphylococcus epidermidis*, which are used exclusively for cell wall synthesis [14,15].

The possible importance of GTΨCPu for ribosomal tRNA binding was implicated by the observation that this tRNA fragment inhibits nonenzymatic binding of aminoacyl-tRNA to ribosomes [16,17]. More recently it could be shown that *E. coli* 50 S ribosomes incubated with TΨCG loose their ability to bind enzymatically aminoacyl-tRNA [18]. Further it could be shown that on the *E. coli* ribosome two adenines of 5 S RNA are exposed and readily modified by monoperphthalic acid [19,20]. 50 S ribosomal subunits reconstituted with such modified 5 S RNA exhibited a marked decrease in protein synthesis activity. In support of this finding oligonucleotide binding studies revealed that TΨCG is able to bind to specific 5 S RNA–protein complexes and that chemical modification of two adenines in the 5 S RNA abolishes the binding ability [19,20]. In addition it was shown that TΨCG inhibits MS synthesis [18], which is connected to the binding of uncharged tRNA to the ribosomal A-site [21].

The experiments cited above support the idea that tRNA interacts via its GTΨCPu (loop IV) sequence with CGAAC of 5 S RNA on the ribosomal A-site. The question whether or not a similar interaction takes also place at the ribosomal P-site is still open. The observation that eukaryotic initiation tRNAs do not contain the GTΨCPu oligonucleotide may be interpreted that these tRNAs and perhaps peptidyl tRNAs do not bind with the aid of such sequence to the ribosomal P-site. Experiments reported here show that the oligonucleotide TΨCG inhibits the binding of aminoacyl-tRNA but not that of initiator-tRNAs to rat liver ribosomes.

2. Materials and methods

2.1. *Isolation of TpΨpCpGp*

The tetranucleotide TpΨpCpGp was isolated essentially as previously described [17].

2.2. *Preparation of ribosomes and enzyme fraction*

60 S and 40 S rat liver ribosomal subunits were prepared as reported [23]. The enzyme fraction (S-300) from 50 g rat liver was prepared was described [23] and then bound to DEAE cellulose (Whatman, DE 52), which had been equilibrated with 0.02 M Tris–HCl, pH 7.1, 0.005 M $MgCl_2$, 0.012 M β-mercaptoethanol and 5% glycerol. After thorough washing of the column the enzyme was eluted with the same buffer except that it contained additional 0.3 M KCl. The peak fractions (less than 10% transmission at 280 nm) were pooled and used as enzyme source (DE 52-S300).

2.3. *tRNA binding assay*

The tRNA binding assays contained in 100 μl 0.02 M Tris–HCl, pH 7.1, 0.006 M $MgCl_2$, 0.006 M β-mercaptoethanol, 0.12 M KCl, 0.004 M GTP, 0.7 A_{260} units ApUpG, 1.65 A_{260} units 60 S and 1.1 A_{260} units 40 S ribosomes, 10 μl DE 52-S300 enzyme and various concentrations of TpΨpCpGp (0 to 3 A_{260} units). Blanks contained all components except ApUpG. The reaction mixtures were incubated for 5 min at 32°C and then placed on ice. After addition of 50 to 100 pmoles of the tRNA to be tested [^{3}H] Met-tRNA^{Met} (rabbit) specific activity 181 Ci/Mol; [^{3}H] Met-tRNA$_F^{Met}$ (rabbit) specific activity 181 Ci/mol; [^{14}C] Met-tRNA$_F^{Met}$ (*E. coli*) specictic activity 230 Ci/mol) the samples were again incubated at 32°C (15 min) and cooled to 0°C. The extent of tRNA binding was measured by the Millipore filter technique [24]. Wash buffer: 0.02 M Tris–HCl, pH 7.1, 0.006 M $MgCl_2$, 0.006 M β-mercaptoethanol and 0.12 M KCl. 100% binding corresponds for rabbit Met-tRNA^{Met} to 5 pmoles (blank of 0.7 pmoles subtracted) and for Met-tRNA$_F^{Met}$ 7 pmoles (blank 0.6 pmoles). For *E. coli* Met-tRNA$_F^{Met}$ 100% binding equaled 4 pmoles (blank 0.4 pmoles).

3. Results and discussions

Rat liver 80 S ribosomes were incubated with TΨCG and the AUG dependent enzymatic binding of rabbit Met-tRNA^{Met} and Met-tRNA$_F^{Met}$ was measured. Fig. 1 shows that the binding of Met-tRNA^{Met} is significantly inhibited by TψCG, a result which is in full accord with previous findings in the *E. coli* system [18]. In contrast the binding of initiation tRNAs from rabbit and E. coli was not influenced by prior binding of the tetranucleotide to the ribosomes (fig. 1). From these experiments we conclude that the initiation tRNAs do not bind at the same site on the ribosome as the aminoacyl-tRNAs, an observation which is in agreement with other previous findings (see recent review article ref. [22]). Further it is shown that eukaryotic initiation tRNAs do not need TΨCG or most likely a similar sequence for ribosome binding. Another interesting observation is that the bacterial Met-tRNA$_F^{Met}$ is not inhibited by TΨCG (fig. 1), although it does contain this sequence, which is interpreted, that it binds at the same site as the eukaryotic initiation tRNA. Recent evidence by Dube suggests that *E. coli* F-Met-tRNA$_F^{Met}$ interacts with its TΨCG sequence with the *E. coli* ribosome [25]. This observation and the data presented here would suggest that in prokaryotes the mechanism of initiator tRNA binding differs from that in eukaryotes [25]. The other result, that Met-tRNA^{Met} binding is inhibited by TΨCG, establishes the universal nature in which aminoacyl-tRNAs are bound to ribosomes.

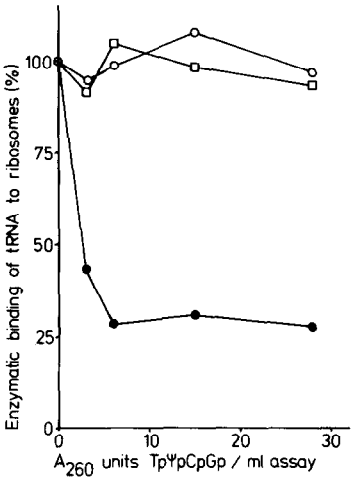

Fig. 1. Effect of TpΨpCpGp on the enzymatic binding of: ○——○ [^{3}H] Met-tRNA$_F^{Met}$ (rabbit); □——□ [^{14}C] Met-tRNA$_F^{Met}$ *(E. coli)* and ●——● [^{3}H] Met-tRNA^{Met} (rabbit) to rat liver ribosomes. For details see Materials and methods.

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