STEREOCHEMICAL CONTROL OF THE RIBOSOMAL PEPTIDYLTRANSFERASE REACTION

The role of acceptor substrate amino acid side chain orientation

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1. Introduction

The aminoacyl residue of aa-tRNA is bound by an ester linkage to the 2′- or 3′-hydroxyl groups of the ribose residue of the 3′-terminal adenosine [1] and is able to migrate rapidly between these two positions [2]. Thus, the determination of an 'active' aa-tRNA isomer in various subreactions of protein synthesis has been a topic of considerable interest. Modified aa-tRNAs, or their 3′-terminal fragments, in which the 3′⇄2′ transacylation cannot occur, have been recently used for studies of isomer-specificity of the acceptor site of peptidyltransferase [3].

Most of the studies have concluded that 3′-Phe-tRNA, is the only acceptor in the peptide bond-forming step [3], although a significant acceptor activity was also observed [4] with 2′-Phe-tRNA. The comparison of reaction rates of both isomers was not performed, but it was thought that the 3′-Phe-tRNA is probably the preferred substrate [5].

From these experiments important questions have evolved:

1. Is the peptidyltransferase really specific for 3′-aa-tRNA as acceptor substrate and is this specificity a general feature for all amino acids? To our knowledge all studies to date have been performed with nonisomerizable analogs of Phe-tRNA or their 3′-terminal fragments only [3].
2. How is the stereospecificity of peptidyltransferase achieved and what functional purpose does it serve?

In order to address these questions, we have used several 2′(3′)-aminoacyl oligonucleotides as analogs of the 2′- and 3′-aa-tRNA acceptor termini in the peptidyltransferase reaction. We have studied the properties of C-2′-dA-Phe, C-3′-dA-Phe, C-2′-dA-Leu, C-3′-dA-Leu, C-2′-dA-Gly, C-3′-dA-Gly, C-3′-NH_2A-Gly and C-2′-NH_2-A-Gly as acceptors of the fMet residue in the fMet-tRNA·A–U–G·70 S ribosome system and report here several novel findings which indicate that the peptidyltransferase A site is most probably specific for 3′-aminoacyl acceptors derived from optically active amino acids. On the other hand, there is virtually no specificity for 2′- and 3′-glycylderivatives.

2. Materials and methods

Three times NH_4Cl-washed ribosomes from *E. coli* MRE-600 cells were prepared as in [6]. *N*-formyl-[^{35}S]Met-tRNA was a gift from Drs C. M. Ganoza and B. Glick, University of Toronto, and was prepared and purified as in [7,8]. Specific activity of fMet-tRNA was 26.0 and 21.6 picomoles methionine/mg tRNA. The aminoacyl dinucleoside phosphates used here were prepared as in [6,9].

Abbreviations: C-2′-dA-Phe, cytidylyl (3′–5′)-2′-deoxy-3′-*O*-L-phenyl-alanyladenosine; C-3′-NH_2A-Gly, cytidylyl (3′–5′)3′-deoxy-3′-L-phenyl-alanylamidoadenosine; analogous abbreviations for other dinucleotide derivatives; tRNA-C-C-2′-dA, tRNA with incorporated 2′-deoxyadenosine on the 3′-terminus; analogous abbreviations for the other tRNA derivative

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The assay conditions for the peptidyltransferase-catalyzed transfer of fMet to acceptor are given in the figure legends.

3. Results and discussions

The acceptor activity in the peptidyltransferase reaction of all compounds was measured in the fMet-tRNA·A–U–G·70 S ribosome system. Figure 1a shows the activity of 2′- and 3′-phenylalanylderivatives C-3′-dA-Phe and C-2′-dA-Phe in this reaction. It is clear that both compounds can function as acceptors of the fMet residue. It is further seen that the 3′-isomer C-2′-dA-Phe is more active than 2′-isomer C-3′-dA-Phe with the app. K_m ratio* being >2 orders of magnitude. For comparison, the app. K_m of C-2′-dA-Phe in the Ac-Phe-tRNA·poly(U)·70 S ribosome system was estimated to be $\sim 7.5 \times 10^{-7}$ M and the 2′-isomer C-3′-dA-Phe was found inactive [6]. Thus, our results are in complete agreement with those in [4] where the acceptor activity of Phe-tRNA-C-C-3′dA was observed in the peptidyltransferase reaction using Ac-Phe-tRNA·poly(U)·70 S ribosomes system; however, this activity appeared to be lower than that of natural isomerizable Phe-tRNA. Thus, it follows that fragment assays of peptidyltransferase used herein compare well with assays using intact aa-tRNAs. Therefore, we can assume that the affinity of 2′-Phe-tRNA for the peptidyltransferase A site is about 200–400 lower than that of 3′-Phe-tRNA.

Similar results were obtained with the pair of leucine compounds, C-2′-dA-Leu and C-3′-dA-Leu (fig.1b). Again, the 3′-ester C-2′-dA-Leu proved to be a far more preferable acceptor of fMet residue (app. $K_m = 7.4 \times 10^{-8}$ M), relative to practically inactive 2′-ester C-3′-dA-Leu.

Figure 2 shows the results obtained with isomeric pairs of glycine derivatives C-2′-dA-Gly, C-3′-dA-Gly, C-3′-NH_2A-Gly and C-2′-NH_2A-Gly as acceptors of fMet residue. In contrast to results obtained with phenylalanyl and leucyl derivatives, both 3′- and 2′-glycyl derivatives C-2′-dA-Gly, C-3′-NH_2A-Gly, C-3′-dA-Gly and C-2′-NH_2A-Gly display comparable activity, with the 3′-derivatives being only slightly preferred (app. K_m 2′/3′ $\simeq 2$). The results thus show that there

* Apparent K_m is defined as the substrate concentration at one-half of the maximal activity

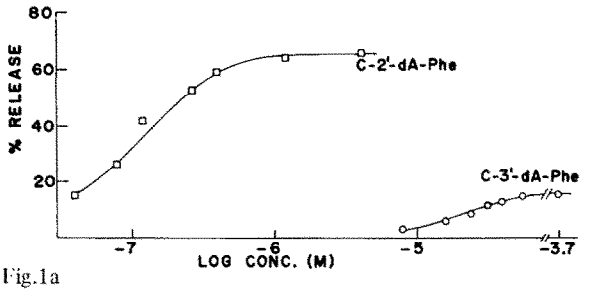

Fig.1a

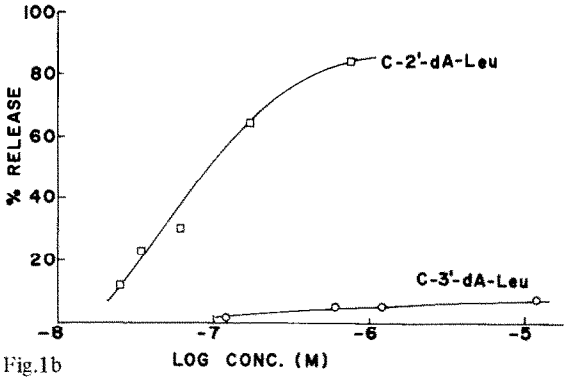

Fig.1b

Fig.1a. 2′- and 3′-*O*-phenylalanyldinucleoside phosphate-dependent release of *N*-formyl-[^{35}S]Met residue from fMet-tRNA in the peptidyltransferase reaction. Each reaction mixture contained in 0.05 ml: 100 mM Tris–HCl (pH 7.4), 50 mM KCl, 10 mM $MgCl_2$, 3.0 A_{260} units of ribosomes, 5 μg A–U–G and 0.025 A_{260} units of *N*-formyl-[^{35}S]Met-tRNA (15 000 cpm). The compounds C-2′-dA-Phe (□) and C-3′-dA-Phe (○) were added at the concentration indicated, and the reaction mixtures were incubated at 37°C for 10 min. The reaction was terminated by the addition of 2.0 ml 10% CCl_3COOH (w/v) at 4°C. After 15 min at 4°C mixtures were filtered through a Gelman-GN6 Metricel 45 μm pore size membrane which was then washed 3 times with 2.0 ml portions of cold 10% CCl_3COOH. After the membranes were dried, the radioactivity was determined in 4.5 g PPO + 100 mg POPOP/l toluene-base scintillation mixture. The amount of *N*-formyl-[^{35}S]Met residue transferred from *N*-formyl-[^{35}S]Met-tRNA to acceptors was determined as the difference between radioactivity retained on the filter after incubation without acceptor and that retained after incubation with an acceptor. It was expressed as percentage of radioactivity of *N*-formyl-[^{35}S]Met-tRNA retained in the filter after incubation without acceptor. Fig.1b. The same for 2′- and 3′-*O*-leucyldinucleoside phosphates C-2′-dA-Leu (□) and C-3′-dA-Leu (○) except that the incubation time was 45 min at 37°C.

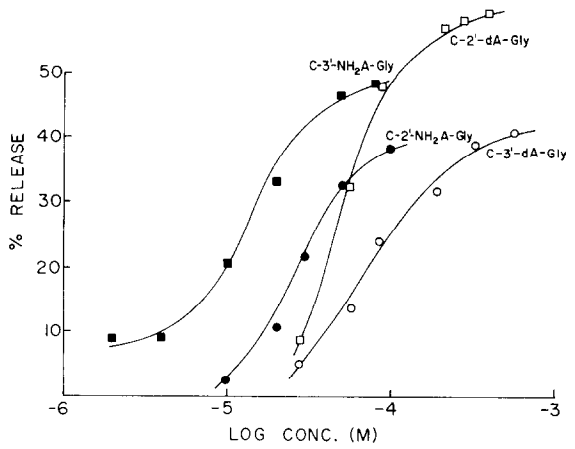

Fig.2. 2'- and 3'-glycyldinucleoside phosphate-dependent release of *N*-formyl-[^{35}S]Met residue from *N*-formyl-[^{35}S]Met-tRNA in the peptidyltransferase reaction. Reactions were carried out as in fig.1 with compounds C-2'-dA-Gly (□), C-3'-dA-Gly (○), C-3'-NH_2A-Gly (■) and C-2'-NH_2A-Gly (●), except that reaction mixtures were incubated at 37°C for 30 min.

is a phenomenal difference in affinities of 2'- and 3'-Phe or Leu derivatives, which definitely accounts for high degree of stereospecificity. Small differences in affinities of glycine derivatives indicate that glycine can probably be incorporated into proteins efficiently from both 2'- and 3'-positions of the terminus of tRNA.

Assuming a definite position for the α-amino group on the peptidyltransferase catalytic center, it is apparent that the amino acid side chains of 2'- and 3'-aminoacyl derivatives are oriented differently [4,10], indicating their stereochemical control function in the peptidyltransferase reaction. This should be true for all optically-active amino acids, although this suggestion needs to be supported by experiments with other aminoacyl derivatives. Evidently, both 2'- and 3'-glycine derivatives, due to the absence of side chain, must have enough flexibility to fit the peptidyltransferase A site and thus react efficiently as acceptors.

The rationale for the specificity of the peptidyltransferase A site is yet unknown. We have speculated that specificity of peptidyltransferase A site may have an important role in the control of the translation process [11]. The obvious feature of extremely easy transacylation within 2', 3'-*cis*-hydroxyl groups of the terminal adenosine [2] together with assertions that 2'-aa-tRNA may enter the ribosomal A site in the EF-T_u-mediated process [12,13] suggest this possibility.

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