RIBOSOMAL PEPTIDYL TRANSFERASE: RECOGNITION POINTS ON THE 3′-TERMINUS OF AA tRNA

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1. Introduction

Current models of the active center of ribosomal peptidyl transferase generally invoke a P-subsite which binds the CCA terminus of peptidyl-tRNA and an A-subsite which binds at least part of the CCA terminus of AA-tRNA [1]. Since the CCA sequence occurs at the 3′-terminus of all tRNAs, it is at least indirectly involved in peptide bond formation. It therefore follows that the elucidation of the role of these sequence in binding to peptidyl transferase sites is a necessary step toward understanding the nature of the peptide bond formation center.

One approach which has met with some success in investigating the involvement of the CCA termini of peptidyl- and AA-tRNAs in the peptidyl transferase reaction has involved the use of *N*-acylaminoacyl or aminoacyl terminal fragments of tRNA as substrate analogs of the parent molecules [2–16]. Compounds of this type (e.g., AA-oligonucleotides or AA-nucleosides) can be prepared by chemical synthesis or by enzymatic degradation of the corresponding tRNA derivatives. Despite its complexity, chemical synthesis of substrate analogs for peptidyl transferase offers the important advantage that unnatural compounds, i.e., non-CCA-AA sequences, can be synthesized. The study of such compounds in ribosomal systems can thus enable one to define several portions of the acceptor or donor substrate molecule which are important for interactions with the A- or P-subsites.

Four new AA-dinucleoside phosphates, CAPhe, CdAPhe, CdAGly and CUPhe, were synthesized and examined with respect to their ability to serve as acceptor substrate analogs of AA-tRNA in the peptidyl transferase reaction. CAPhe and CdAPhe were found to be far more potent acceptors than puromycin while CdAGly and CUPhe were found to be inactive. Results are discussed with respect to the role of the 2′-hydroxy group of the terminal adenosine unit, the amino acid side chain, and the base sequence in the binding of the CCa terminus of AA-tRNA to the A subsite.

2. Materials and methods

Bacterial ribosomes were prepared from late log *E. coli* MRE 600 (RNAase 1⁻) cells (General Biochemicals) as previously described [17]. The ribosomes were washed three times by centrifugation in the presence of high NH_4Cl concentrations [18] and showed no requirement for pre-activation [19] to give maximum activity in the assay systems used here.

Ac-[^{14}C]Phe-tRNA was prepared as before [17] and had a specific radioactivity of 220 000 cpm/mg tRNA which corresponds to a charging efficiency of 0.4 nmole [^{14}C]penylalanine/mg tRNA.

The chemical synthesis of the 2′(3′)-O-aminoacyldinucleoside phosphates used in this study is described elsewhere [20, 21].

Peptidyl transferase activity was measured essentially as described by Rychlík et al. [8]. Details of the reaction conditions and contents are given in the legend to fig. 1.

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** *Abbreviations*: CAPhe, cytidylyl (3′-5′)-2′(3′)-O-*L*-phenylalanyladenosine; CdAPhe, cytidylyl (3′-5′)-2′-deoxy-3′-O-*L*-phenylalanyladenosine; CdAGly, cytidylyl (3′-5′)-2′-deoxy-3′-glycyladenosine; CUPhe, cytidylyl (3′-5′)-2′(3′)-O-phenylalanyluridine; APhe, 2′(3′)-O-*L*-phenylalanyladenosine.

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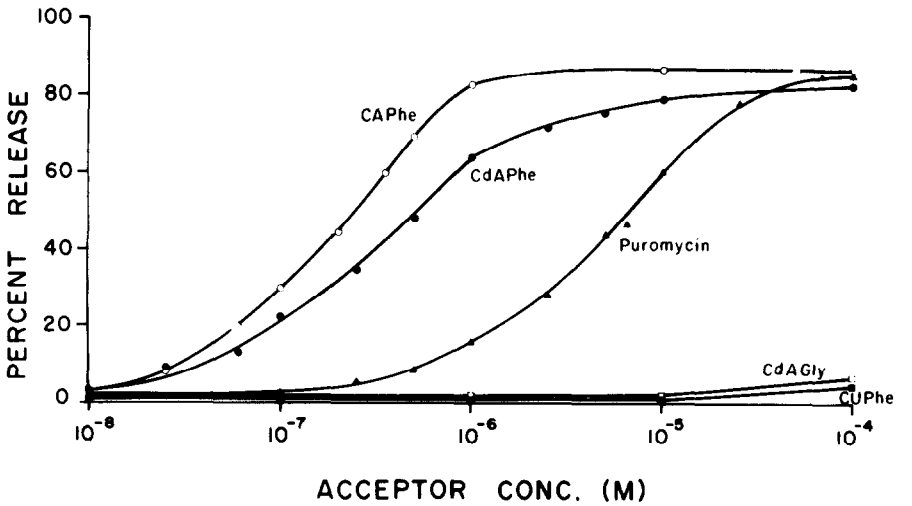

Fig. 1. 2′(3′)-O-Aminoacyldinucleoside phosphate dependent release of the *N*-acetyl-[^{14}C] phenylalanyl residue from *N*-acetyl -[^{14}C] phenylalanyl-tRNA in the peptidyl transferase reaction. Each reaction mixture contained in 0.15 ml: 50 mM Tris–HCl (pH 7.4); 100 mM NH_4Cl; 10 mM $MgCl_2$; 3.2 A_{260} units of ribosomes; 10 μg poly(U); 0.15 A_{260} units of Ac-[^{14}C] Phe-tRNA (1350 cpm). The reaction was initiated by the addition of the acceptor compounds at the concentrations indicated. Following incubation at 37°C for 30 min, the reaction was terminated by the addition of 2.0 ml of 2.5% CCl_3COOH (TCA) at 4°C. After 15 min at 4°C, the entire reaction mixture was filtered through a HAWP–Millipore membrane which was then washed with three 2.0 ml portions of cold 2.5% TCA. After the membranes were dried, the radioactivity was determined in a 4.5 g/100 mg dimethylPOPOP/1 liter toluene scintillation mixture. The amount of Ac-[^{14}C] Phe residue transferred from Ac-[^{14}C] Phe-tRNA to the acceptor was determined as the difference between radioactivity retained on the filter after incubation without acceptor and that retained after incubation which an acceptor. It was expressed as the percentage of the radioactivity of Ac-[^{14}C] Phe-tRNA added to the experimental mixture.

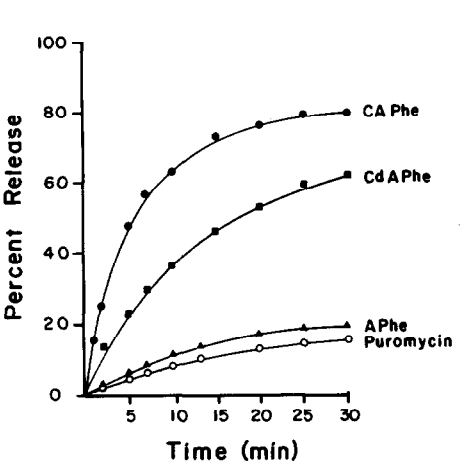

Fig. 2. Time course of *N*-acetyl-[^{14}C] phenylalanine transfer from *N*-acetyl-[^{14}C] phenylallnyl-tRNA to 2′(3′)-O-aminoacyldinucleoside phosphates. The percent of *N*-acetyl-[^{14}C] phenylalanine released during the peptidyl transferase reaction from the donor molecule was determines as described in fig. 1. Acceptor molecule concentration was the same for all reactions, 1.0×10^{-6} M.,

3. Results and discussion

Of the four 2′(3′)-O-aminoacyldinucleoside phosphates tested for acceptor activity (fig. 1), CAPhe, the 3′-terminus of Phe-tRNA, was the best acceptor of the Ac-Phe residue. Relative to puromycin, a standard acceptor substrate, CAPhe demonstrated very appreciable acceptor activity at low acceptor concentrations (10^{-8} to 10^{-6} M) giving 50% release at 2.5×10^{-7} M while puromycin showed acceptor activity comparable to CAPhe. As can be seen in fig. 2, CAPhe also has a higher rate of reaction than puromycin. The remarkable increase of acceptor activity of CAPhe compared to APhe clearly indicates that A-subsite possesses a locus for the penultimate Cp residue as we have previously suggested [4]. This is in agreement with the recent report [16] that the 5′-cytidylyl derivative of puromycin shows 7 times greater activity than puromyncin in the inhibition of de novo polypeptide synthesis in a cell-free system from rabbit reticulocytes.

Although dAPhe has been previously found to be

essentially inactive as an acceptor [8], its 5′-cytidylyl derivative, CdAPhe, displays both acceptor activity (50% release at 5.0×10^{-7} M) and rate of reaction approaching that of CAPhe (figs. 1 and 2). Also, as reported earlier, two modified derivatives of APhe having a substituent at the 2′-OH, namely 2′, 3′-O-bis-*L*-phenylalanyladenosine [10] and 2′-O-methyl-3′-O-*L*-phenylalanyldensine [14], are good acceptors. Therefore, it seems reasonable to assume that the 2′-oxygen plays an auxiliary role in the binding of the acceptor substrate to the A-subsite but the free hydroxyl group is not essential.

CdAPhe and CdAGly possess identical nucleotide sequences, yet they demonstrate vastly different activities (fig. 1). Whereas CdAPhe had activity between that of CAPhe and puromycin, CdAGly showed slight activity and only at high acceptor concentrations (< 10% release at 10^{-4} M). While CdAGly possesses the Cp residue, it lacks both an aminoacyl side chain and the 2′-OH group which cause the glycine residue to be less firmly attached to the A-subsite and thus less active as an acceptor.

In order to explain the high activity of AA-derivatives containing aromatic amino acids, we have postulated that the ribosomal A-subsite contains a hydrophobic locus whose interaction with the aromatic ring of the amino acid residue would be strongly enhanced by the π-electron system [12]. Alternatively, but not excluding the first hypothesis, the sandwhich-like structure resulting from ring stacking in CAPhe can be considered as an active form of the molecule which is recognized by the A-subsite. As shown from the crystallographic study of puromycin by Sundaralingam and Arora [22], purine and tyrosine rings can form alternating stacks. It is conceivable that this stacking interaction could act as an extension of the stacking of the A- and C-residues of the 3′-terminus of tRNA. This ordered structure, with the properly oriented α-amino group, would be far more probable with aromatic amino acids, thus possibly explaining the high affinity of substrates containing aromatic amino acids such as APhe and CAPhe for the A-subsite. Warshaw and Cantor [23] have pointed out that in mixed deoxy- and ribodinucleoside phosphates it is the conformation of the 3′-linked monomer that determines the overall geometry of the dimer. Thus, a sandwich-like structure as referred to above for CAPhe is also feasible for CdAPhe.

The last compound tested, CUPhe, which contains the benzyl residue of phenylalanine, 2′-OH and penultimate Cp residue, was inactive (fig. 1) indicating that replacement of adenosine by uridine in the ultimate position resulted in the loss of acceptor activity [8]. Apparently the uridine residue in CUPhe cannot bind to the adenosine locus of the A-subsite at all and CUPhe, although it contains other binding points, cannot be accommodated by the A-subsite.

From these results and the above discussion we conclude that at least one of two particular portions of the acceptor molecule (amino acid side chain or 2′-OH) appear to be necessary for acceptor activity. It is expected that the biochemical evaluation of other AA- and Ac-AA-oligonucleotide derivatives, currently of peptidyl transferase sites.

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