ON THE MECHANISM OF PEPTIDE BOND SYNTHESIS IN THE RIBOSOME. 3′-*O*-PHENYLALANYL-2′-*O*-METHYLADENOSINE AS A PEPTIDE ACCEPTOR

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1. Introduction

It is shown that puromycin (fig. 1A) reacts on the ribosome with peptidyl-tRNA as the acceptor of the peptidyl residue (i.e., as aminoacyl-tRNA) [1]. Along with puromycin some 2′(3′)-*O*-aminoacyl adenosines [2] and 2′,3′-*O*-bis-aminoacyl adenosines (fig. 1B) [3] can act as acceptors. It is also known that 3′-*O*-phenylalanyl-2′-deoxyadenosine has almost no such activity [4].

In the present paper 3′-*O*-phenylalanyl-2′-*O*-methyladenosine (fig. 1C), synthesized by us, was used as an acceptor in the peptidyl-transferase reaction, and it is shown that this compound possesses a significant acceptor activity close to that of puromycin.

2. Materials and methods

2.1. *3′-O-phenylalanyl-2′-O-methyladenosine*

2′-*O*-Methyladenosine was prepared by methylating

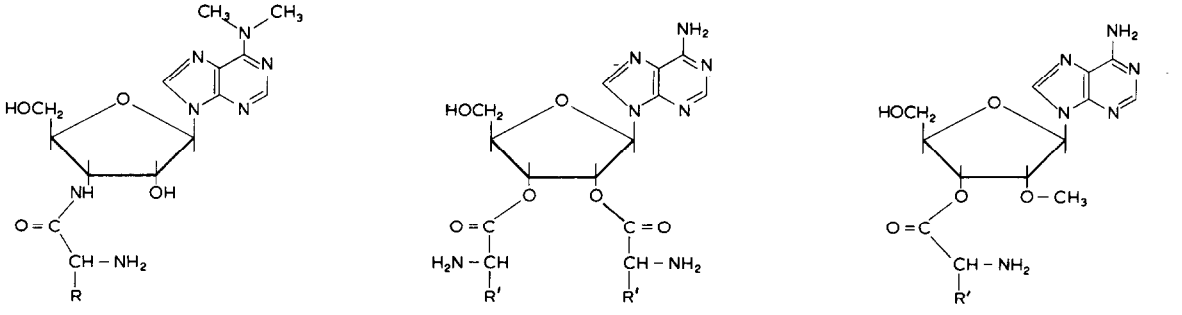

Fig. 1. A: Puromycin; B: 2′,3′-*O*-bis-aminoacyl adenosine; C: 3′-*O*-phenylalanyl-2′-*O*-methyladenosine.

Table 1
Transfer of AcPhe from AcPhe-tRNA to 3′-*O*-phenylalanyl-2′-*O*-methyladenosine in ribosomes with polyU.

Conditions of experiment	Binding of AcPhe-tRNA with ribosomes (cpm)	PoluU-stimulated binding of AcPhe-tRNA with ribosomes (cpm)	Transfer of AcPhe from AcPhe-tRNA to puromycin or 3′-*O*-phenylalanyl-2′-*O*-methyladenosine		Effectiveness of 3′-*O*-phenylalanyl-2′-*O*-methyladenosine relative to puromycin (%)
			(cpm)	(%)	
AcPhe-tRNA + ribosomes + PolyU	4160	3300	–	–	–
AcPhe-tRNA + ribosomes	860	–	–	–	–
AcPhe-tRNA + ribosomes + polyU + puromycin (10^{-3} M)	1920	1060	2240	68	–
AcPhe-tRNA + ribosomes + polyU + puromycin (5×10^{-4} M)	2160	1300	2000	61	–
AcPhe-tRNA + ribosomes + polyU + 3′-*O*-phenylalanyl-2′-*O*-methyladenosine (10^{-3} M)	2120	1260	2040	62	91
AcPhe-tRNA + ribosomes + polyU + 3′-*O*-phenylalanyl-2′-*O*-methyladenosine (5×10^{-4} M)	2600	1740	1560	47.5	78

Conditions of experiment:
Incubation mixture: AcPhe-tRNA, 10 100 cpm; 2.8 A_{260} units of ribosomes, 0.8 A_{260} units of polyU; buffer: 5×10^{-2} M triethanolamine, 0.015 M $MgCl_2$, 0.16 M NH_4Cl, pH 7.2. Total volume of mixture, 0.1 ml. Incubation time, 20 min, temperature, 30°. Incubation was stopped by adding 2 ml of cold buffer, filtering through nitrocellulose filters HUFS or VUFS (Chemapol, Czechoslovakia) and washing with 25 ml of the same buffer.

Table 2
Transfer of AcPhe from AcPhe-tRNA to 3′-*O*-phenylalanine-2′-*O*-methyladenosine in ribosomes not containing templates.

Conditions of experiment	Radioactivity in ethyl acetate (cpm)	Transfer of AcPhe from AcPhe-tRNA to puromycin or 3′-*O*-phenylalanyl-2′-*O*-methyladenosine		Effectiveness of 3′-*O*-phenylalanyl-2′-*O*-methyladenosine relative to puromycin (%)
		(cpm)	(%)	
AcPhe-tRNA + ribosomes	830	–	–	–
AcPhe-tRNA + ribosomes + puromycin (10^{-3} M)	2240	1410	31.0	–
AcPhe-tRNA + ribosomes + puromycin (10^{-4} M)	1720	890	19.5	–
AcPhe-tRNA + ribosomes + 3′-*O*-phenylalanyl-2′-*O*-methyladenosine (10^{-3} M)	1340	510	11.2	36
AcPhe-tRNA + ribosomes + 3′-*O*-phenylalanyl-2′-*O*-methyladenosine (10^{-4} M)	1200	370	8.1	41.5

Conditions of experiment:
Incubation mixture: AcPhe-tRNA, 5400 cpm; 3 A_{260} units of ribosomes; buffer: 0.4 M KCl, 0.05 M Tris-HCl, 0.01 M $MgCl_2$, pH 7.5; ethyl alcohol, 33%. Total volume of mixture, 0.3 ml. Incubation time, 4 hr, temperature, 24°. Incubation was stopped by adding 0.7 ml of buffer containing 0.01 M Tris-HCl, pH 7.0 and 3 ml of ethylacetate. After mixing, 2 ml of the organic layer was taken and its radioactivity measured in a scintillating liquid described by Prockop [12].

adenosine with diazomethane in the presence of stannic chloride [5]. 3′-*O*-Phenylalanyl-2′-*O*-methyladenosine was obtained in the same way as in the synthesis of 2′(3′)-*O*-phenylalanyladenosine [6] proceeding from 5′-*O*-trityl-2′-*O*-methyladenosine and tert-butyl-oxycarbonylphenylalanine anhydride. Protecting groups were removed by trifluoroacetic acid.

2.2. *Testing of peptide-acceptor activity*

E. coli B tRNA enriched with the phenylalanyl-acceptor fraction [7] and containing 18% tRNA was used in the study. [^{14}C] Phe-tRNA was prepared by the known method [8] (the labelled [^{14}C] phenylalanine used had a specific radioactivity of 220 μCi/mmole and was supplied by ÚVVVR, Czechoslovakia). Ac-[^{14}C] Phe-tRNA was synthesized from [^{14}C] Phe-tRNA and acetyloxysuccinimide by the method of Lapidot et al. [9]. Radioactivity of the preparations was measured in the SL-40 Liquid Scintillation Spectrometer (Intertechnique, France).

Testing of the 3′-*O*-phenylalanyl-2′-*O*-methyladenosine peptide-acceptor activity was performed in a system containing "charged" Ac-[^{14}C] Phe-tRNA *E. coli* B ribosomes both in the presence of the poly U [4] and in a template-free system in the presence of alcohol (according to [10]). In both cases the amount of acetyl-phenylalanine carried over from the Ac-[^{14}C] Phe-tRNA to the 3′-*O*-phenylalanyl-2′-*O*-methyladenosine served as the measure of activity (puromycin-like effect).

3. Results and discussion

Data is presented in table 1 and 2 showing that 3′-*O*-phenylalanyl-2′-*O*-methyladenosine possesses a noticeable peptide-acceptor activity which in a number of cases is comparable with puromycin activity. Needless to say a more precise and complete comparison can be made by investigating the kinetics of the process. Such studies are being carried out and the results will shortly be published.

In order directly to confirm the mechanism of action of 3′-*O*-phenylalanyl-2′-*O*-methyladenosine, a comparison of the reaction product formed on the ribosomes with the synthetically obtained material was made. Judging by electrophoretic mobility, both substances proved to be identical.

The fact that 3′-*O*-phenylalanyl-2′-*O*-methyladenosine appeared to be a good peptide acceptor permits one critically to evaluate the hypothesis of H. Neuman et al. [11] on the two-step mechanism of peptide bond synthesis in the ribosome. According to this hypothesis the peptide residue with the peptidyl-tRNA is carried over to the free hydroxyl of the aminoacyl-tRNA terminal adenosine at the first step of reaction. The second step, the formation of the peptide bond proper is, in essence, already an intramolecular reaction.

Data reported in the literature on the activity of different model compounds utilized as a peptide acceptor in ribosomes do not allow one to come to any unambiguous conclusion. For example it is known that 3′-*O*-phenylalanyl-2′-deoxyadenosine has almost no acceptor activity [4] which is evidence in favor of the hypothesis. On the other hand 2′,3′-*O*-L-bis-phenylalanyladenosine is an effective enough peptide acceptor [3] and this can be considered as a contradiction of the hypothesis. In this sense 3′-*O*-phenylalanyl-2′-*O*-methyladenosine is a convenient model for testing the hypothesis.

The results obtained by us provide evidence that during peptide bond synthesis in the ribosome, the 2′-oxygroup of the peptide acceptor molecule does not play the very essential role that is assigned to it in H. Neuman's hypothesis [11]. Of course, this fact does not mean that the 2′-oxygroup in the peptide donor molecule does not also have some functional meaning in the catalysis of the peptidyl-transferase reaction. The role of this oxygroup remains to be elucidated by further experiments.

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