3′(2′)-*O*-AMINOACYLNUCLEOTIDES AS POLYPEPTIDE ACCEPTORS AT THE RIBOSOMAL PEPTIDYLTRANSFERASE CENTER

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Received 27 January 1970

1. Introduction

In recent investigations of the mechanism of peptide bond formation in ribosomes *O*-aminoacyl-nucleotides, *O*-glycyladenosine-5′-phosphate and *O*-glycylnucleosidediphosphate were used as peptide acceptors in parallel with puromycin [1–4].

These studies showed that the nature of the amino acid side chain greatly influences the acceptor activity of these compounds; of the amino acids tested it was maximal for the phenylalanyl residue whereas the glycyl residue was practically inactive [4]. These data are in good agreement with those obtained with aminoacyl analogs of puromycin [5, 6]. Adenosine derivatives were the most active among the five (A, I, C, G, U) heterocyclic bases tested [4].

Up to now in investigations of the acceptor capacity of various puromycin-like analogs, *O*-amino-acyl-nucleotides have not been tested, with one exception, namely pA-Gly which possesses no acceptor activity [3].

The aim of this work was to find out whether 3′(2′)-*O*-aminoacylnucleotides could serve as polypeptide acceptors in the formation of the peptide bond catalyzed by the polypeptidyl transferase center of the 50 S ribosomal subunit. It is of interest to investigate whether the phosphate group and/or its negative charges could influence the acceptor ability of analogs.

2. Methods

The synthesis of several 3′(2′)-*O*-aminoacyl derivatives of adenylic acid was performed by a new method developed in our laboratory for aminoacylation of nucleotides [7]. The principle of this method consists of imidazolic activation of the amino acid carboxylic group followed by condensation with a nucleotide. The NH_2-group of the amino acid was protected either by protonation or with a tert-butyloxicarbonylic residue. [^{14}C] Polyphenylalanyl-tRNA served as a polypeptide donor in the *E. coli* Q13 70 S ribosomal system. The preparation of loaded ribosomes as well as measurements of release of polyphenylalanine from ribosomes were done as described by Monro et al. [8].

3. Results and discussion

A comparison between the activity of various derivatives of adenosine-5′-phosphate and of puromycin is presented in the table. From these data it follows that the presence of the phosphate group does not prevent the manifestation of acceptor activity. Therefore it is highly probable that the absence of activity in pA-Gly [3] is attributable to the nature of the amino acid side chain. Esterification of one of the OH-groups of the phosphate residue which is equivalent to the removal of one negative charge, markedly stimulates the acceptor activity. Among the aminoacylnucleotides with various amino acid residues phenylalanyl-adenylic acid was found to be the most active. Activity decreases progressively for the corresponding esters with other amino acyl groups, in good agreement with the results obtained

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Table 1
Relative activity of 2′(3′)-*O*-aminoacylnucleotides as peptide acceptors.
The values are given as percentages relative to puromycin activity.

Peptide acceptor	Concentration (mM)			
	1.0	0.5	0.1	0.05
pA-L-Pro	24.5	9.4	6.9	–
pA-L-Val	62	32.5	19	–
pA-L-Phe	72	57	57	40
pA-D-Phe	10	–	–	–
*MepA-L-Phe	–	91	85	79
A-L-Phe	46	40	36	24

Figures are the means of several experiments.

The release of polyphenylalanine from polyphenylalanyl-tRNA by puromycin was taken as 100%. Incubation mixture contained tris-HCl pH 7.2, 12 mM; $MgCl_2$, 12 mM; NH_4Cl 180 mM; puromycin or analogs at concentrations shown and 1.5 absorbance units of 70 S ribosomes per tube: Total volume, 0.3 ml, incubation 15 min at 30°. After incubation, tubes were cooled in ice, 3 ml *m*-cresol was added per tube, the emulsion was mixed with a Vortex mixer for 10 sec and left to stand for 1 hr at + 4°. Mixing was repeated and the emulsion was filtered through nitrocellulose millipore discs (RUFS, Chemapol, Czechoslovakia). Each disc was washed with 1.5 ml of *m*-cresol and with toluene or ethanol (30 ml). Dry filters were counted in PPO-POPOP toluene scintillator with 60% efficiency for ^{14}C.

* 3′(2′)-*O*-L-Phenylalanyladenosine-5′-methylphosphate.

by others with nucleoside derivatives and analogs of puromycin [4, 5].

It seems to us that 3′(2′)-*O*-aminoacylnucleotides, which are now widely accessible due to the simple new method developed for their synthesis, will find further application in the investigation of peptide bond formation in ribosomes.

A full account of this work will be published elsewhere [9].

4. Conclusion

3′(2′)-*O*-Aminoacylnucleotides can serve as polypeptide acceptors when tested with 70 S ribosomes isolated from *E. coli* Q13 loaded with [^{14}C] polyphenylalanyl-tRNA. The acceptor activity depends both on the number of negative charges on the phosphate residue and on the nature of the amino acid side chain.

Acknowledgements

We thank Prof. W.Engelhardt and Prof. A.Spirin for interest and encouragement in the course of this work. Part of this study was done in collaboration with Dr. N.Belitsina (Institute for Protein Research, Puschino) and Dr. P.Purygin from our Laboratory to whom the authors wish to express their gratitude.

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