SYNTHESIS OF AN UNNATURAL P-N BOND CATALYZED WITH *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

The formation of peptide bonds catalyzed by ribosomes has been studied using a number of 2′(3′)-*O*-peptidyladenosine-5′-phosphates or analogs of tRNA [1]. The present work describes the peptide donor properties of the organophosphorus analog pA-(AcMetGly^P) of one of the model donors of ribosomal PTC–pA-(AcMetGly) with an activity close to that of pA-(fMet) under fragment reaction conditions [1]. The compound pA-(AcMetGly^P) reacts with [^{14}C]Phe-tRNA in the presence of ribosomes giving rise to 'tripeptidyl'-tRNA (compound I in scheme 1) with an unnatural phosphinoamide bond.

2. Materials and methods

2.1. *Chemical compounds*

Synthesis of pA-(fMet) was performed as in [2], of pA-(AcMetGly^P) and the tripeptide analog (compound II) as in [3].

2.2. *Ribosomes and [^{14}C]Phe-tRNA*

Ribosomes were obtained from *Escherichia coli* MRE-600 as in [4]. Enzymatic aminoacylation of total tRNA from *E. coli* by L-[^{14}C]phenylalanine (Amersham, specific radioactivity 522 Ci mol^{-1}) was done as in [5].

Abbreviations: pA-(AcMetGly^P), 2′(3′)-*O*-(*N*-acetylmethionylaminomethyl-methylphosphinyl)-adenosine-5′-phosphate; pA-(AcMetGly), 2′(3′)-*O*-(*N*-acetylmethionylglycyl)-adenosine-5′-phosphate; pA-(fMet), 2′(3′)-*O*-(*N*-formylmethionyl)-adenosine-5′-phosphate; pC, cytidine-5′-phosphate; PTC, peptidyl transferase center

2.3. *Assay in the system with ribosomes*

The reaction of pA-(fMet) with [^{14}C]Phe-tRNA was carried out as described in [6]. The model reaction of pA-(AcMetGly^P) with [^{14}C]Phe-tRNA was performed in 0.1 ml incubation mixture containing 0.06 M Tris-buffer (pH 7.5), 0.02 $MgCl_2$ and 0.4 M KCl, 80 pmol of ribosomes, 20 pmol of [^{14}C]Phe-tRNA and pA-(AcMetGly^P) in the quantities indicated in the legend to fig.1. The reaction was initiated by adding 0.1 ml MeOH. After incubation at 30°C for 1 h 50 μl of NaOH was added and then after 30 min at 37°C 200 μl of H_2SO_4. The mixture was immediately extracted with ethylacetate, the organic layer was dried with Na_2SO_4, and radioactivity counted as in [6]. The reaction was inhibited with 1 mM of lincomycin and chloramphenicol. This reaction was carried out also in the presence of pC (1 mM). The blank was the incubation mixture without pA-(AcMetGly^P).

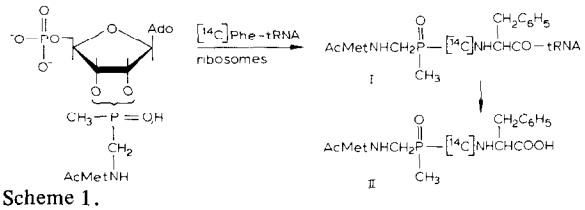
2.4. *Identification of the reaction product*

The reaction was performed as in section 2.3 at 1 mM concentration of pA-(AcMetGly^P). After addition of 50 μl of NaOH the mixture was neutralyzed with Dowex 50w × 8 (Py, 200 mesh), the supernatant was applied to a Kieselgel 607/254 plate (Merck), which was developed first in the system tert.-AmOH-Py-H_2O (5:2:3 v/v) and then in iPrOH-25% NH_4OH–H_2O (7:1:2 v/v) in the second direction. The plate was cut into squares and each section (0.5 × 0.5 cm) was counted in 5 ml of toluene scintillator. The ethylacetate extract obtained from the procedure in 2.3 was analyzed in the same way.

3. Results and discussion

We studied the possibility of the formation of a

peptidyl-tRNA analog (compound I in scheme 1) with a P–N bond resulting from the reaction of pA-(AcMetGly^P) with Phe-tRNA catalyzed by ribosomes (see scheme 1).



Scheme 1.

In order to identify the reaction product we hydrolyzed the reaction mixture and isolated the phosphinoamide peptide (compound II) by extraction with ethylacetate following a standard technique [6]. Two-dimensional TLC analysis showed that radioactive spots corresponded to phenylalanine and the compound which was identical to the chemically synthesized tripeptide (compound II). Therefore these products were formed on the hydrolysis of [^{14}C]-Phe-tRNA and (compound I), respectively. We had to isolate substance (compound II) immediately after the addition of acid on account of its instability in acid media. When the extraction was made 1 h later, compound II was practically absent from the organic solution.

The antibiotics lincomycin and chloramphenicol inhibited the reaction of pA-(AcMetGly^P) with Phe-tRNA. That proved the ribosomal nature of this reaction [7].

Earlier it had been found that tetrahedral phosphonates [8] and trigonal carboamide analogs [compound I] of model peptide donors are inhibitors of the donor site of ribosomes, the latter type of substances being more effective. Here we present evidence that the phosphinoester analog of the donor can react with Phe-tRNA. This means that this substance has an almost correct orientation in the ribosomal donor site towards the acceptor of the peptide. The structure of the transition state of the reaction between ribosome substrates (tetrahedral geometry) is significantly different from that in the reaction of the phosphinoester compound (trigonal bypyramide). The ability of the ribosomal PTC to catalyze the synthesis of peptide as well as phosphinoamide type bonds points to the absence of an exact complementarity of PTC to any definite transition-state structure. The data do not favour the nucleophilic catalysis in the transpeptidation process [1].

The yield of the ribosomal reaction of PA-(AcMetGly^P) with Phe-tRNA in the presence of pC is very low, whereas the stimulatory action of pC in the ribosomal system with pA-(fMet) is obvious (fig.1). As has been reported recently [9], pC increases the productivity of the complex (ribosomes + minimal peptide donor + Phe-tRNA), including some rearrangements in PTC with the resulting precise orientation of nucleophilic and electrophilic groups of substrates favorable for the reaction. Therefore the situation in PTC at this level is specific exclusively for peptide bond formation and is not suitable for the reaction of phosphonylation with some other geometry of the reactive group.

These results, together with those reported earlier on aminoacyl-tRNA-ligases [10], demonstrate the significance of organophosphorus analogs for investigation of the enzymatic acyl transfer.

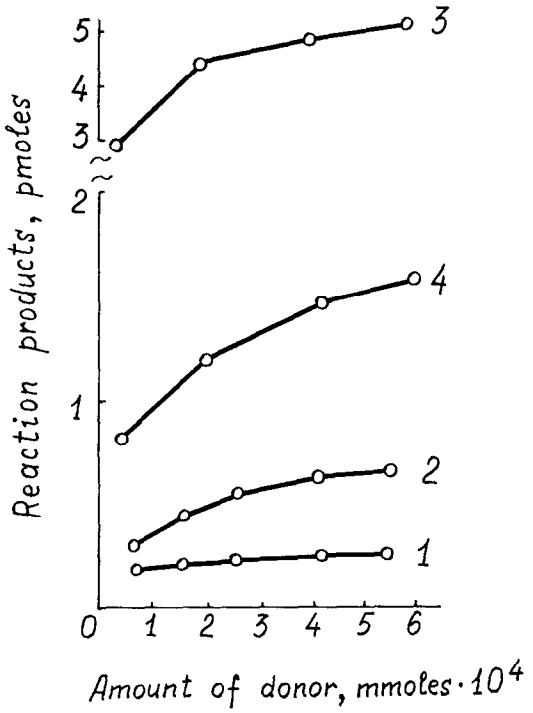


Fig.1. The dependence of peptide donor activity at different concentrations of pA-(AcMetGly^P) or pA-(fMet) on the presence of pC. pA-(AcMetGly^P) + pC (1 mM) (1); without pC (2); pA-(fMet) + pC (1 mM) (3) and without pC (4).

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