EFFECT OF CYTIDINE-5'-MONOPHOSPHATE ON PEPTIDYL TRANSFERASE ACTIVITY

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1. Introduction

First studies on the structural specificity of the donor site of peptidyl transferase indicated that the 3'-terminal sequence of peptidyl-tRNA, CpCpA-acylaminoacyl, was necessary for effective interaction with this site [1,2]. In our previous paper [3] we demonstrated that pA-fMet had also donor activity provided that it was used at very high concentrations (1 mM). The donor activity of pA-fMet was observed at about 100 000 times the concentration in comparison with the more complete donor substrate CpCpA-fMet. The striking difference in donor activity between both compounds has indicated that pA-fMet fulfils the basic requirements for a donor substrate, but that the presence of other nucleotides of the terminal sequence may also be important for proper alignment of the donor substrate to its binding site.

In this paper evidence is presented that free cytidine-5'-monophosphate, the neighbour of pA in the CpCpA terminal sequence of tRNA, markedly increases the donor activity of pA-fMet.

2. Materials and methods

2.1. *Materials*

AMP, CMP, GMP and UMP were products of Calbiochem, USA. D-threochloramphenicol (Spofa, Czechoslovakia), erythromycin lactobionate (laurylin, Pierrel, Italy), carbomycin (magnamycin, Pfizer and Co., USA) and gougerotin (Calbiochem, USA) were obtained from the sources indicated.

Radioactive L-[^{3}H] phenylalanine (labelled at C-4, 21 Ci/mmol) was a product of the Radiochemical Centre (Amersham, UK). L-[U-^{14}C] leucine (83 mCi/mmol) was prepared at the Institute for Research, Production and Application of Radioisotopes, Czechoslovakia.

2.2 *Preparation of ribosomes*

Ribosomes were prepared from Escherichia coli B either by washing with 0.5 M NH_4Cl as described elsewhere [4] or by washing with 1 M NH_4Cl and incubation with puromycin [5]. We did not find any significant difference between the activities of both ribosomal preparations under our experimental conditions.

2.3. *Preparation of substrates*

The preparation of [^{3}H] Phe-tRNA and of the terminal fragments CpApCpCpA-[^{3}H] Phe and CpApCpCpA-ac [^{14}C] Leu were described earlier [1]. The 2'(3')-*O*-(*N*-formylmethionyl)-adenosine-5'-phosphate was a gift from Dr Krayevsky of the Institute of Molecular Biology, Moscow, prepared by the method for synthesis of *N*-acyl-aminoacyl-nucleoside-5'-phosphates developed by Gottikh et al. [6].

2.4. *Transfer assay*

Assay of the reaction of ac[^{14}C] Leu-pentanucleotide with puromycin was carried out under the conditions of fragment reaction described by Monro et al. [1]. The transfer reaction with pA-fMet and [^{3}H] Phe-tRNA was assayed as described previously [3].

2.5. *Binding of donor substrate*

The binding of CpApCpCpA-ac[^{14}C] Leu to ribosomes was examined according to Celma et al.

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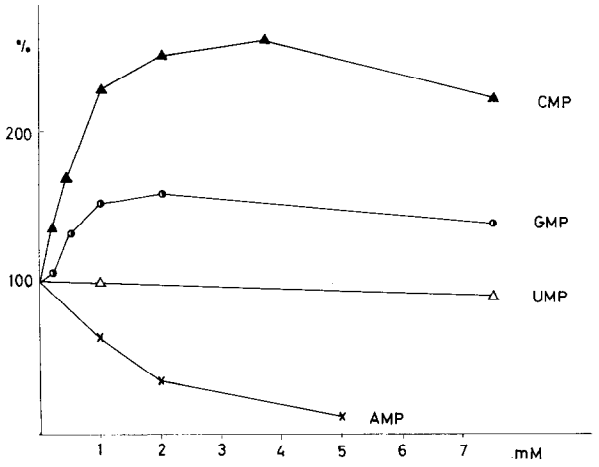

Fig.1. The effect of mononucleotides on the reaction of pA-fMet with CpApCpCpA-[^{3}H]Phe. Concentration of pA-fMet was 1 mM. % = fMet [^{3}H]Phe formation (100% transfer corresponded to 2200 counts/min); mM = concentration of the tested mononucleotides.

[7], using 70S ribosomes instead of 50S ribosomal subunits.

3. Results

3.1. *The effect of mononucleotides on the transfer reaction with pA-fMet as donor substrate*

As shown in fig.1, the transfer reaction examined with pA-fMet as donor substrate and CpApCpCpA-[^{3}H]Phe as acceptor substrate is strongly stimulated by CMP (the stimulation varies between 250% and 450% according to the various preparations of [^{3}H]Phe pentanucleotide). Of the other nucleotides tested, GMP also stimulated the transfer reaction (about 150%), UMP was without any effect and AMP showed a rather high inhibitory effect. The stimulatory effect of CMP (1 mM) on transfer reaction does not change at an increasing concentration of pA-fMet (fig.2).

With Phe-tRNA, a more complex acceptor substrate, similar effects of mononucleotides on the transfer reaction were observed, with the exception of GMP, which inhibited this transfer reaction (fig.3). These results indicated that CMP was acting at the donor site and not at the acceptor site, which was occupied in both cases by the acceptor substrate.

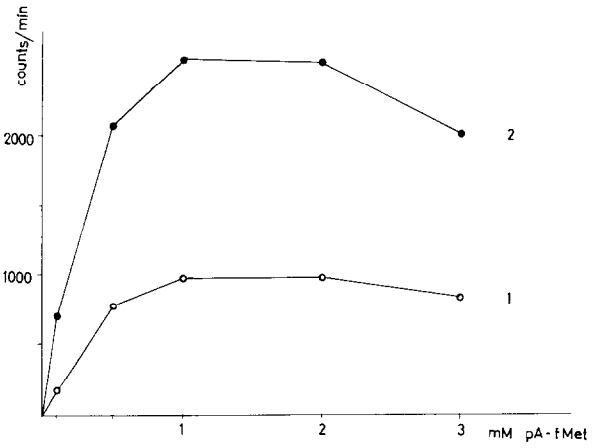

Fig.2. The effect of CMP on the reaction of pA-fMet with CpApCpCpA-[^{3}H]Phe. Concentration of CMP was 1 mM. (1) pA-fMet; (2) pA-fMet + CMP.

The effect of CMP on the time course of the transfer reaction (fig.4) indicates that CMP markedly increases the rate of the transfer reaction.

3.2. *The effect of mononucleotides on the transfer reaction with CpApCpCpA-ac[^{14}C]Leu as donor substrate*

If CMP binds to the donor site to the position

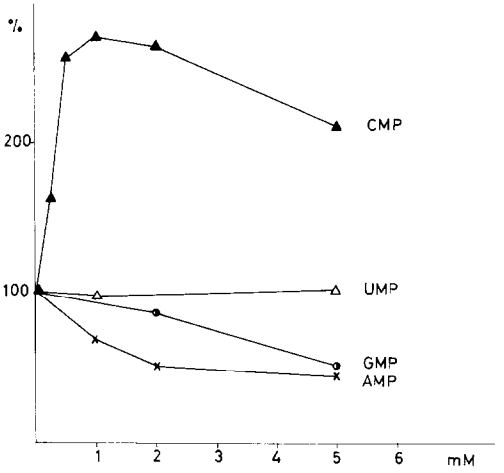

Fig.3. The effect of mononucleotides on the reaction of pA-fMet with [^{3}H]Phe-tRNA. Concentration of pA-fMet was 1 mM. % = fMet[^{3}H]Phe formation (100% transfer corresponded to 2000 counts/min). mM = concentration of tested mononucleotides.

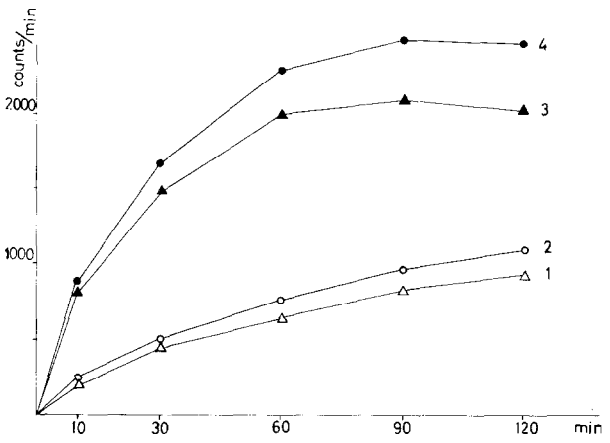

Fig.4. The effect of CMP on the time course of the transfer reaction of pA-fMet with CpApCpCpA-[^{3}H]Phe and [^{3}H]Phe-tRNA Concentration of pA-fMet and CMP was 1 mM. (1) [^{3}H]Phe-tRNA; (2) [^{3}H]Phe-pentanucleotide; (3) [^{3}H]Phe-tRNA + CMP; (4) [^{3}H]Phe-pentanucleotide + CMP.

which is used under natural conditions for binding the cytidine residues of the terminal sequence CpCpA-acylaminoacyl, it might be expected that CMP should compete with the binding of terminal acylaminoacyl-pentanucleotide and with the donor activity of this substrate. For the same reason AMP should inhibit the binding of both pA-fMet and more complete substrates and inhibit also the transfer from these substrates.

Table 1
The effect of mononucleotides on binding of CpApCpCpA-ac[^{14}C] Leu to ribosomal donor site

	Concn. (mM)	counts/min	%
–	–	2320	100
CMP	1	1950	84
	5	1210	52
AMP	1	1530	66
	5	1410	60
GMP	1	2318	100
	5	2300	99
UMP	1	2180	94
	5	2435	105

Results reported in table 1 show the effects of mononucleotides on binding of CpApCpCpA-ac[^{14}C]-Leu to ribosomes: both CMP and AMP, which are contained in the terminal sequence, interfere with the binding of donor substrate, whereas GMP and UMP produce no evident inhibition of binding. No significant effect of mononucleotides on binding of acceptor substrate was observed.

The effect of mononucleotides on the transfer reaction from CpApCpCpA-ac[^{14}C] Leu to puromycin is shown in fig.5. AMP and CMP inhibit the transfer reaction which may be viewed as interference with the binding of CpApCpCpA-ac[^{14}C] Leu to the donor binding site.

3.3. *Effect of antibiotics on the CMP-stimulated reaction*

We tested the effect of antibiotics, which are known to inhibit peptidyl transferase, on the CMP-stimulated transfer reaction. It is evident from the data of table 2 chloramphenicol, sparsomycin and erythromycin affect both the stimulated and non-stimulated transfer to the same extent, only carbomycin and gougerotin produce stronger inhibition of the stimulated transfer reaction.

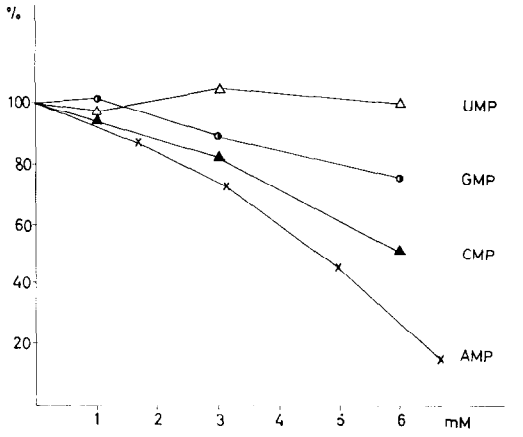

Fig.5. The effect of mononucleotides on the fragment reaction of CpApCpCpA-ac[^{14}C] Leu with puromycin. Concentration of ac[^{14}C] Leu-pentanucleotide was about 100 mM; % = ac-[^{14}C] Leu-puromycin formation (1100 counts/min. transferred); mM = concentration of the tested mononucleotide.

Table 2
The effect of antibiotics on the reaction of pA-fMet (1 mM) with CpApCpCpA-[^{3}H] Phe resp. [^{3}H] Phe-tRNA in the presence of CMP

Antibiotic	Conc. mM	CpApCpCpA-[^{3}H]Phe	CMP 1 mM	[^{3}H]Phe-tRNA	CMP 1 mM
Chloramphenicol	0.1	34%	31%	28%	43%
Erythromycin	0.1	113%	106%	110%	106%
Carbomycin	0.01	27%	10%	30%	11%
Sparsomycin	0.5	41%	35%	50%	37%
Gougerotin	1	48%	22%	69%	46%

The results are expressed as % of the transfer activity without antibiotic. 100% transfer with CpApCpCpA-[^{3}H] Phe corresponded to 1500 counts/min and in the presence of CMP to 5600 counts/min 100% transfer with [^{3}H] Phe-tRNA corresponded to 1100 counts/min and in the presence of CMP to 3230 counts/min.

4. Discussion

We have found that CMP markedly stimulates ribosomal peptidyl transferase activity with pA-fMet as a donor substrate (fig.1–4). This stimulation may be due to the simultaneous binding of CMP and pA-fMet to the respective sites which are occupied under natural conditions with the cytidine and adenosine residues of the terminal CpCpA-acylaminoacyl sequence. The binding of CMP may induce a conformational change in the ribosomes and hence activation of the catalytic centre of peptidyl transferase.

The CMP-stimulation is in agreement with the induced-fit theory [8] which assumes that the substrate or a part of it induces a change in the conformation of the enzyme and that this change is capable of affecting enzyme activity. The CMP-stimulation corresponds to the idea that a molecule which is itself not involved directly in the enzyme action can induce a conformational change favorable for the reaction. A similar effect was reported for a trypsin catalyzed reaction [9].

On the other hand, the inhibition of binding of the donor substrate containing the CpApCpCpA terminal sequence (table 1) and the inhibition of the transfer reaction with this substrate (fig.5) by AMP and CMP may be viewed as a competition for the donor binding site between the terminal sequence CpCpA-acylaminoacyl and AMP or CMP.

The CMP-stimulated transfer reaction is inhibited by chloramphenicol, carbomycin, sparsomycin and gougerotin which indicates that the reaction is catalysed by ribosomal peptidyl transferase.

At present, we are not able to explain the discrepancy between the data concerning the effect of GMP on the transfer reaction from pA-fMet of Phe-pentanucleotide (fig.1) and from acLeu-pentanucleotide to puromycin (fig.5).

5. Conclusions

The transfer reaction with pA-fMet as a donor substrate is strongly stimulated by CMP, whereas the transfer reaction with CpApCpCpA-acLeu as a donor substrate is inhibited by CMP. These results indicate that the donor site of peptidyl transferase contains specific binding sites for the terminal adenosine and for the cytidylic acid residue in the terminal sequence CpCpA of tRNA and that an attachment of proper nucleotides to the donor site induces a conformational change in peptidyl transferase.

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