THE STUDY OF THE STIMULATING MECHANISM OF THE PEPTIDYL DONOR ACTIVITY OF 2′(3′)-*O*-(*N*-FORMYLMETHIONYL)-ADENOSINE-5′-PHOSPHATE IN PEPTIDYL TRANSFERASE OF *E. COLI* RIBOSOME

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1. Introduction

Recently it has been shown that pA-(fMet)* is a rather good donor of the *N*-formylmethionyl residue in the ribosomal peptidyl transferase catalyzed reaction [1,2] and pC is a strong stimulant of the peptidyl donor activity of pA-(fMet) [3]. However, the mechanism of this stimulation is unknown. One can expect a direct binding of pC at free places on the D-site and the stimulation of the pA-(fMet) peptidyl donor activity as a result of structural reorganization of the D-site. The other possibility is an allosteric effect of pC on peptidyl transferase. We have studied the peptidyl donor activity of CpA-(fMet) and its stimulation with a series of dinucleoside phosphates to clear up the mechanism of stimulation. It has been shown that peptide donor activity of pA-(fMet) with pC as stimulant is close to that of CpA-(fMet). At the same time pC does not stimulate the reaction of CpA-(fMet) with Phe-tRNA.

2. Materials and methods

2.1. *Preparation of ribosomes*

Ribosomes were prepared from *E. coli* MRE-600 as described earlier [4].

**Abbreviations*: A- and D-sites, the acceptor and the donor sites of the peptidyltransferase accordingly; pA-(fMet), 2′(3′)-(*N*-formylmethionyl)-adenosine-5′-phosphate; CpA-(fMet), 2′(3′)-*O*-*N*-formylmethionine ester of cytidylyl-(3′→5′)-adenosine; pC, 5′-cytidylic acid; Cp, 2′(3′)-cytidylic acid; U*pC and A*pC, 3′-(N¹-uracyl)-propyloxy-pC and 3′-(N⁹-adenine)-propyloxy-pC accordingly.

2.2. *Preparation of substrates and stimulants*

pA-(fMet) was synthesized according to [5], CpA-(fMet) to [6], U*pC and A*pC were prepared as described earlier [7]. tRNA from yeast was aminoacylated with [^{3}H]phenylalanine (spec. act. 40 Ci/mmol).

2.3. *Preparation of highly purified [^{3}H]Phe-tRNA^{Phe}*

Yeast tRNA was enriched 10 times by chromatography on BD-cellulose. After that tRNA was aminoacylated with [^{3}H]phenylalanine; this preparation contained 6–8% of Phe-tRNA (sample 1). A portion of this Phe-tRNA was chromatographed once more on BD-cellulose. The eluting gradient was at first 0.3–1.0 M NaCl with 0.05 M AcONa, pH 5.0 and then 0.6% of dioxan in the presence of 1 M NaCl with 0.05 M AcONa pH 5.0. [^{3}H]Phe-tRNA^{Phe} was eluted from the column at a 4% concentration of dioxan. The final degree of purity of tRNA^{Phe} was 95–100%. One A_{260} unit of [^{3}H]Phe-tRNA^{Phe} corresponded to 1×10^8 cpm, 2.2×10^3 pmol.

2.4. *Transfer assay*

The assay was essentially as described in [1]. The reaction mixture contained (prior to methanol addition): 0.06 M Tris–HCl pH 7.5; 0.02 M $MgCl_2$ 0.4 M KCl; ribosomes 110 pmol and [^{3}H]Phe-tRNA $(3–4) \times 10^4$ cpm (0.7–0.9 pmol); donors and stimulants at concentrations shown for the individual experiments. The reaction was initiated by addition of methanol (50% of the total volume). The incubation was carried out at 0°C for 90 min. The reaction was terminated by addition of 50 μl of 3 N NaOH. After incubation for 40 min at 37°C to hydrolyze

the ester bond 200 μl of 5 N H_2SO_4 was added. The reaction products were extracted with 3 ml of ethyl acetate, the organic layer was washed with 0.5 ml 1% H_2SO_4 and then with 0.5 ml water, dried with Na_2SO_4 and the radioactivity of 1.5 ml of the organic phase was determined in 15 ml of a solution containing PPO–POPOP toluene scintillator and methylcellosolve 2:1 v/v and measured in the SL-30 counter (Intertechnique). The peptide acceptor activity of puromycin was assayed according to [8] with addition of stimulants. Ac-[^{3}H]Phe-tRNA prepared from 95–100% [^{3}H]Phe-tRNA [9]; one A_{260} unit corresponds to 1×10^8 cpm, 2.2×10^3 pmol.

3. Results

3.1. *The stimulation of the donor activity of pA(fMet)*

We have tested the donor activity of pA-(fMet) in the presence of pC, Cp, A*pC, CpC, U*pC and ApA. As can be seen from fig.1 pC has an extraordinary ability to promote the transfer of fMet residue from pA-(fMet) to [^{3}H]Phe-tRNA. U*pC and CpC have a significant effect, whereas A*pC acts relatively weakly (the same results were obtained with pApC), and Cp, ApA are inert. The stimulant concentration was equal to a maximal donor concentration (about 1×10^{-3} M).

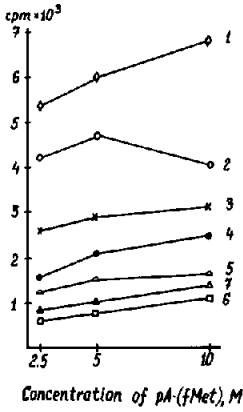

Fig.1. The stimulation of the reaction of pA-(fMet) with [^{3}H]Phe-tRNA (sample No. 1); pC (1), U*pC (2), CpC (3), A*pC (4), Cp (5), ApA (6); without stimulant (7). Conditions of assay are described in Materials and methods, section 2.4.

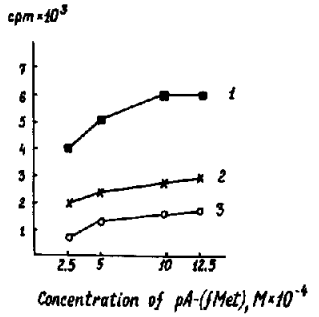

Fig.2. The stimulation of the reaction of pA-(fMet) with [^{3}H]Phe-tRNA (sample No. 2); + pC (1×10^{-3} M) (1), + CpC (1×10^{-3} M) (2); without stimulant (3).

Fig.2 shows the stimulation of the reaction of pA-(fMet) with 95–100% [^{3}H]Phe-tRNA by addition of pC and CpC. The stimulation was up to 350–650%.

3.2. *Peptide donor activity of CpA-(fMet) in comparison with pA-(fMet)*

CpA-(fMet) has a high peptide donor activity (fig.3). The activity of CpA-(fMet) is 20–60 times higher than that for pA-(fMet) at a low concentration; the difference is sharply reduced upon an increase of the pC concentration. Peptide donor activity of pA-(fMet) at pC concentration 1×10^{-3}–3×10^{-3} M

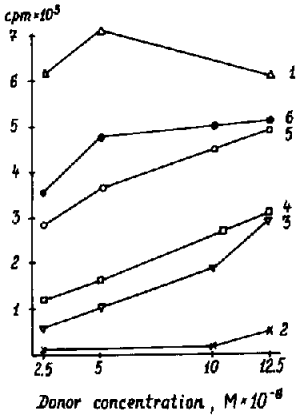

Fig.3. The peptide donor activity of CpA-(fMet) and pA-(fMet) at the different concentration of donors and stimulant pC. CpA-(fMet) (1), pA-(fMet) (2), pA-(fMet) + pC (1.5×10^{-4} M) (3), pA-(fMet) + pC (3×10^{-4} M) (4), pA-(fMet) + pC (1×10^{-3} M) (5), pA-(fMet) + pC (3×10^{-3} M) (6). ([^{3}H]Phe-tRNA - sample No. 1).

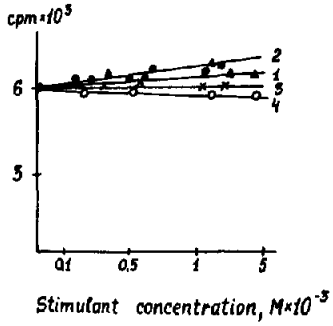

Fig.4. The stimulation of the peptide donor activity of CpA-(fMet); + pC (1.25×10^{-3} M) (1), + A*pC (1.25×10^{-3} M) (2), without stimulant (3). ([^{3}H]Phe-tRNA - sample No. 2).

approaches to that of CpA-(fMet), curves 5 and 6.

Fig.4 shows a typical set of results of the peptide donor activity of CpA-(fMet) in the presence of pC and A*pC. Labelled [^{3}H]Phe-tRNA containing 95–100% of the phenylalanine accepting fraction was used as the acceptor substrate. One can see the absence of visible effect of substances tested.

3.3. *Peptide donor activity of Ac-[^{3}H]Phe-tRNA*

Fig.5 shows the effect of pC, A*pC, CpC and Cp on the reaction of AcPhe-tRNA with puromycin.

As shown in fig.5 a slight stimulation of the process may be observed, no more than 15%.

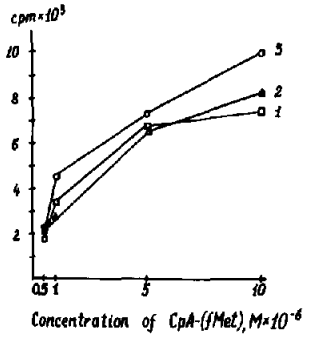

Fig.5. The stimulation of the transfer of Ac-[^{3}H]Phe-residue from Ac-[^{3}H]Phe-tRNA to puromycin (3×10^{-5} M); + pC (1), + CpC (2), + Cp (3), A*pC (4). Ac-[^{3}H]Phe-tRNA (sample No. 2) – 1.5×10^4 cpm in each tube.

4. Discussion

Data summarized in figs.1 and 2 support the results of Černa [3] on an active stimulation of the peptide donor activity of pA-(fMet) by pC. In the present work it has been shown that U*pC and CpC stimulate the process less strongly than does pC. This result is rather unexpected because it appears reasonable to assume that CpC has to stimulate the reaction more than U*pC. It is also interesting to note a relatively low stimulating effect of A*pC on the donor reaction since the fourth 3′-end nucleotide in tRNA is pA. If one supposes that pC binds to the place on ribosome that is usually occupied by the third 3′-terminal nucleotide in tRNA, A*pC must be an effective stimulant. One can think that the conformation of the 3′-terminal cytidine in A*pC, U*pC, CpC and Cp is different from the pC conformation, and therefore these substances possess a relatively low stimulating effect.

It is necessary to note that the circular dichroism spectra for the dinucleoside phosphate U*pC and A*pC are close to those of the natural dinucleoside phosphates UpC and ApC. This fact suggests that their conformations are closely related to each other in aqueous solution [10].

The peptide donor activity of CpA-(fMet) is much greater than that of pA-(fMet). While the stimulant concentration is increased the peptide donor activity for pA-(fMet) approaches to the activity for CpA-(fMet) (fig.3). At the same time there is almost no stimulation of the activity for CpA-(fMet) by pC and A*pC (fig.4). These data indicate that pC binds to the D-site but it does not stimulate the reaction allosterically by binding outside peptidyltransferase.

Stimulation of the reaction of pA-(fMet) with 6–8% [^{3}H]Phe-tRNA (figs.1 and 3) can be explained by the competition of pC with nonacylated tRNA, which can bind to D-site. It is shown (fig.2) that stimulation of the reaction of pA-(fMet) with 95–100% [^{3}H]Phe-tRNA by addition of pC and CpC takes place. These data exclude the possibility of competition.

It is interesting to compare the action of pC and other substances tested on D- and A-sites. As can be seen from fig.5 the stimulation of puromycin reaction is very low, not more than 15%. This fact

combined with the finding that peptide acceptor activity of A-Phe is close to that of CpA-Phe and puromycin indicates the lack of effective binding of cytidylic acid to the A-site.

Analysis of our data leads to the conclusion that the mechanism of the stimulating effect of pC is actually a spatial reorganization of the D-site of peptidyltransferase induced by binding of a stimulant. We have no good explanation at the moment for the increase of peptide donor activity of pA-(fMet) when pC is bound to the D-site of the ribosomes. The rate of the reaction of pA-(fMet) with Phe-tRNA is nearly the same in the presence of pC or without is, as was shown by Černa [3]. Therefore there are two possibilities: the stimulant may increase the quantity of active ribosomes in ribosomal preparations. On the other hand pC may increase the binding constants of donor or acceptor or both of them with the peptidyltransferase.

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