SYNTHESIS OF THIOAMIDE BOND CATALYZED BY *E. COLI* RIBOSOMES

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1. Introduction

Previous studies have indicated that bacterial ribosomes besides amide bonds catalyzed the formation of ester [1] and thioester bonds [2]. In both cases puromycin analogues – hydroxy- and thiolpuromycin, respectively – were used as acceptor substrates. However, a modified donor of the peptide group is more important for the study of the mechanism of ribosomal catalysis than an acceptor because the peptide donor is an active component of protein synthesis. To investigate the ability of ribosomes to synthesize thioamide bonds we prepared pA-(Ac-thioLeu)*. The present communication shows that in the reaction of pA-(Ac-thioLeu) with [^{3}H]Phe-tRNA or CACCA-[^{3}H]Phe, the ribosomes catalyze the synthesis of thioamide bonds with acyldipeptide Ac-thioLeu-Phe formation.

2. Materials and methods

2.1. *Preparation of ribosomes and [^{3}H]Phe-tRNA*

The ribosomes were isolated from *E. coli* MRE-600 according to the known procedure [3]. The preparation of total tRNA from *E. coli* B was aminoacylated by [^{3}H]phenylalanine with specific radioactivity 11 Ci/mmol, according to [4]. [^{3}H]Phe-tRNA contained 1.3×10^4 cpm in 1 pmol. CACCA-[^{3}H]Phe was obtained from [^{3}H]Phe-tRNA according to [5].

2.2. *Preparation of substrates*

pA-(fMet) was synthesized according to [6]. pA-(Ac-thioLeu) was obtained using the imidazole method (manuscript in preparation).

2.3. *Transfer assay*

The donor reaction was performed as described in [7] with modifications according to [8].

2.4. *Ac-thioLeu-Phe identification*

(a) Ac-thioLeu-Phe, identified by comparison with a chemically synthesized marker, was applied to Whatman paper I and chromatographed in *n*-BuOH-water-AcOH (78:17:5).

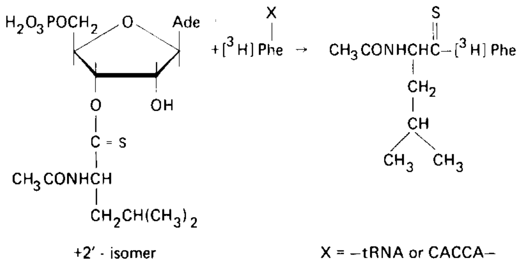
(b) The extracts of the products of pA-(Ac-Leu) or pA-(Ac-thioLeu) and [^{3}H]Phe-tRNA reaction in ethyl acetate were washed with water and dried with Na_2SO_4. They were then reduced by shaking for 30 min in the presence of portions of 20–30 mg of nickel (Reney). When the nickel was completely resolved by the addition of 1 ml of 20% HCl to the reaction mixtures, the organic and water phases separated. The organic phase was washed with 1 ml of water and dried with Na_2SO_4, and then 1 ml of the phase was assayed with a toluene-methylcellosolve-scintillator. The water phase was neutralized by 3N NaOH with the addition of 10 mg of EDTA and the aliquot of 0.2 ml was assayed with 10 ml of dioxyane scintillator.

3. Results

3.1. *The peptide donor activity of pA-(Ac-thioLeu)*

We have studied the reaction of pA-(Ac-thioLeu) with [^{3}H]Phe-tRNA or CACCA-[^{3}H]Phe (Scheme 1).

**Abbreviations*: pA-(fMet) – *N*-formylmethionine ester of 5′-adenylic acid; pA-(Ac-Leu) – *N*-acetylleucine ester of 5′-adenylic acid; pA(Ac-thioLeu) – *N*-acetylthioleucine ester of 5′-adenylic acid; pC – 5′-cytidylic acid.



Figures 1 and 2 show the dependence of peptide donor activity of pA-(Ac-thioLeu) on its concentration. [^{3}H]Phe-tRNA (fig.1) and CACCA-[^{3}H]Phe (fig.2) were used as peptide acceptors. As is obvious from the given data, pA-(Ac-thioLeu) in the presence of pC possesses relatively high peptide donor activity reaching 60% of the activity of pA-(Ac-Leu) and

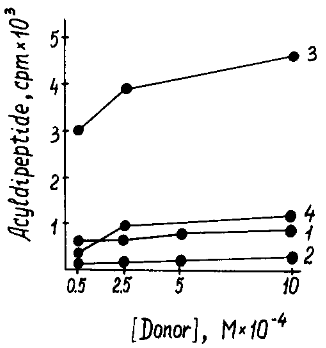


Fig.2. The dependence of peptide donor activity of pA-(Ac-thioLeu) on its concentration. pA-(Ac-thioLeu) + pC (1 × 10^{-3} M) (1) and without pC (2); pA-(fMet) + pC (1 × 10^{-3} M) (3) and without pC (4). CACCA-[^{3}H]Phe – (3–4) × 10^4 cpm (2.3–3 pmol).

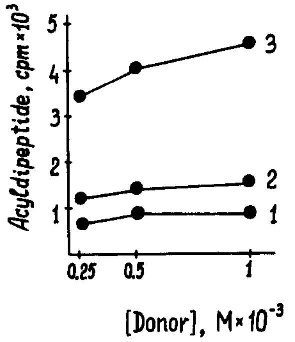


Fig.1. The peptide donor activity of pA-(Ac-thioLeu) at the different concentrations of donor in the presence of pC (1 × 10^{-3} M); pA-(Ac-thioLeu) (1), pA-(Ac-Leu) (2) and pA-(fMet) (3). [^{3}H]Phe-tRNA – (3–4) × 10^4 cpm (2.3 – 3 pmol).

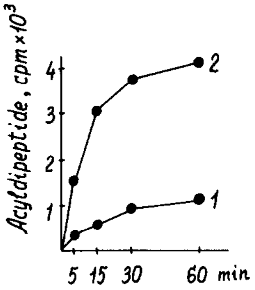


Fig.3. Time course of reaction of pA-(Ac-thioLeu) (5 × 10^{-4} M) (1) or pA-(fMet) (5 × 10^{-4} M) (2) with [^{3}H] Phe-tRNA in the presence of pC (1 × 10^{-3} M). [^{3}H]Phe-tRNA (3–4) × 10^4 cpm (2.3–3 pmol).

Table 1
Inhibition of peptide donor activity of pA-(Ac-thioLeu) by antibiotics

Antibiotics	Ac-thioLeu-Phe obtained			
	[^{3}H]Phe-tRNA		CACCA-[^{3}H]Phe	
	(cpm)	(%)	(cpm)	(%)
	1550	100	660	100
Chloramphenicol 1 × 10^{-3} M	50	3	40	6
Lincomycin 1 × 10^{-3} M	30	2	0	0
Eruthromycin 1 × 10^{-5} M	1470	95	920	140

20% of the activity of pA-(fMet). Similar to pA-(Ac-Leu) [9] pA-(Ac-thioLeu) interacting with Phe-tRNA required the presence of a stimulant to display peptide donor activity (fig.2, curve 2).

3.2. *Time course of the reaction*

pA-(Ac-thioLeu) and Phe-tRNA reaction was also dependent on the time of incubation. Figure 3 shows that pA-(Ac-thioLeu) and Phe-tRNA reaction appears to be completed within the same time as the reaction where pA-(fMet) is used as peptide donor.

3.3. *Antibiotics effect on the reaction*

The table indicates that antibiotics lincomycin and chloramphenicol almost completely inhibited the formation of Ac-thioLeu-Phe, whereas erythromycin did not.

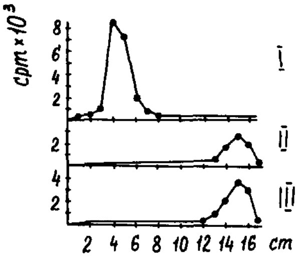
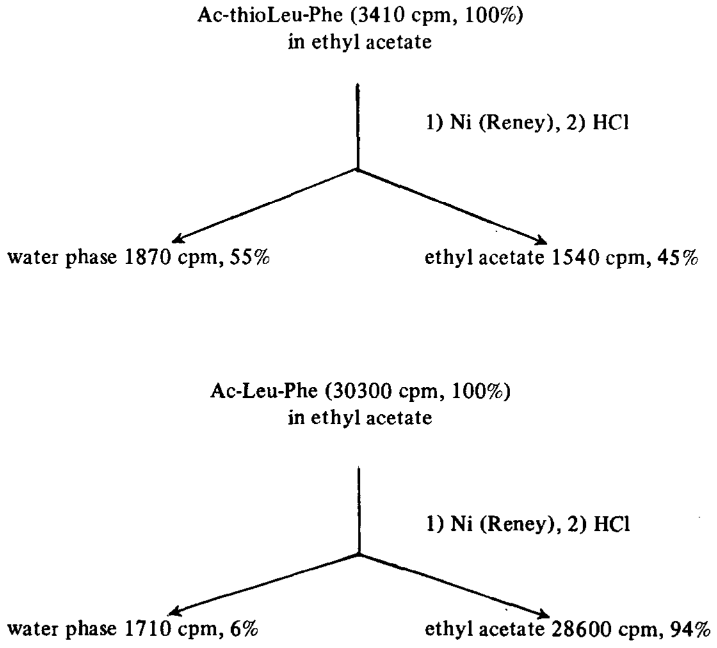


Fig.4. Paper chromatographic analysis of product formed by transfer of the Ac-thioLeu residue from pA-(Ac-thioLeu) to [^{3}H]Phe-tRNA with subsequent alkaline hydrolysis and extraction with ethyl acetate at pH 1–2. (I) Phenylalanine; (II) Ac-thioLeu-Phe, chemically synthesized marker: (III) Product of the reaction.

3.4. *Identification of the reaction product*

We have identified the product of pA-(Ac-thioLeu) and [^{3}H]Phe-tRNA by means of paper chromotography (fig.4). This figure shows that in the chromatograms the peak of the product's radioactivity corresponded to the position of the chemically synthesized Ac-thioLeu-Phe. To prove the nature of thioamide bond resulting from the reaction catalyzed by ribosomes we carried out the reduction of the incubation products pA-(Ac-thioLeu) and Phe-tRNA over nickel (Reney) (table 2). For control we also reduced acyldipeptide Ac-Leu-Phe obtained from AcLeu-tRNA and [^{3}H]Phe-

Table 2
Treatment of the reaction products and ribosomes with nickel (Reney)



tRNA reaction in the presence of ribosomes. The table shows that after treatment with nickel acyldipeptide Ac-Leu-Phe was almost completely extracted into ethyl acetate from acid media which suggested the absence of reduction of the CO-NH group and charge formation on the nitrogen. Parallel to this the product under investigation was 50% in the water phase. The extraction of the product could occur due to the reduction of the CS-NH group and consequently the appearance of a positively charged secondary amino group.

4. Discussion

The studies described here indicate that in the presence of pC pA-(Ac-thioLeu) develops rather high peptide donor activity provided that Phe-tRNA or its 3'-end fragment CACCA-Phe is used as acceptor. The antibiotics lincomycin and chloramphenicol inhibit the reaction which proves its ribosomal nature. Erythromycin does not influence the process to any extent. Direct identification of the product of transferase reaction was carried out by paper chromatography. The reduction of the reaction product with nickel supports the presence of thioamide bond, the synthesis of which is catalyzed by ribosomes.

Peptide donor activity of pA-(Ac-thioLeu) is approximately 60% of the activity of its nearest chemical analogue – pA-(Ac-Leu). pA-(Ac-thioLeu) just as pA-(Ac-Leu) were practically inactive without the stimulation.

In conclusion it should be mentioned that it is the first modification of the donor component that showed the ability of ribosomes to catalyse the synthesis of an unnatural type of bond. Such modifications of the donor substrates may be of interest for the studies of the mechanism of peptide bond catalysis in ribosomes.

Acknowledgements

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