SYNTHESIS OF tRNA UREIDO DERIVATIVES AS SUBSTRATES FOR THE INVESTIGATION OF THE RIBOSOME PEPTIDYL TRANSFERASE CENTER

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1. Introduction

Acylaminoacyl or peptidyl-tRNAs chemically prepared from aminoacyl tRNAs are widely used in studies of the ribosomal peptidyl transferase center [1–3]. At present the preparation of these compounds is based on the condensation of carboxylic acids or *N*-protected amino acids and peptides with aminoacyl tRNA. Carboxyl groups of these compounds can be activated by a number of ways such as by formation of *p*-nitrophenyl [4] or *N*-hydroxysuccinimide esters [5–8], synthesis of symmetric anhydrides [9], or by using *N'*-cyclohexyl-*N''*-[β-(*N*-methylmorpholinium)-ethyl]-carbodiimide [10, 11].

In this communication we describe the synthesis of peptidyl-tRNA with modified peptide fragments. Our method opens a new possibility for the elongation of peptide residues in peptidyl-tRNA. It makes use of the condensation of *p*-nitrophenyloxycarbonyl-aminoacyl-tRNA with amino group-containing compounds [12].

2. Materials and methods

tRNA from *E. coli* Q 13 enriched with phenylalanine accepting fraction [13] has been used, the preparation contained 18% of $tRNA^{Phe}$. [^{14}C] Phe-tRNA (I) was prepared by the conventional procedure [14] using [^{14}C] phenylalanine with specific activity 220 mCi/mmole (UVVVR, Czechoslovakia). Radioactive material after the synthesis was precipitated by 5% trichloroacetic acid and filtered through nitrocellulose membrane filters. Radioactivity was measured in an SL-40 liquid scintillation counter (Intertechnique, France). *p*-Nitrophenyl chlorocarbonate (II) was synthesized from sodium *p*-nitrophenolate and phosgene [15]. Chromatographic separations were performed on Filtrak N 16 paper in the following systems: 1) isopropanol–ammonia–water (7:1:2); 2) ethanol–1 M ammonium acetate (7:3), and 3) n-butanol–pyridine–water (1 : 1 : 1). Reference substances for chromatographic separations ($HOOCCH_2NHCO$-Phe-OH, $C_2H_5OOCCH_2NHCO$-Phe-OH and $HOOCCH_2NHCOCH_2NHCO$-Phe-OH) were prepared by condensation of phenylalanine with *p*-nitrophenyl chlorocarbonate followed by addition of NH_2CH_2COOH, $NH_2CH_2COOC_2H_5$ or $NH_2CH_2CONHCH_2COOH$ to the reaction mixture, according to the protocol employed for the synthesis of ureido derivatives of Phe-tRNA (see below).

Ureido derivatives of phenylalanyl-tRNA (IV): 10 A_{260} units of tRNA containing 0.96×10^{-3} μmoles of [^{14}C] Phe-tRNA are dissolved in 0.1 ml of 0.1 N $NaHCO_3$ at 0°. Then 5 μmoles (1 mg) of *p*-nitrophenyl chlorocarbonate is added, the solution is mixed at 0° for 1 min, acidified with 6% acetic acid to pH 5 and precipitation of the product effected with 2 vol of ethanol. The precipitate is separated by centrifugation, washed with 70% ethanol, dried in vacuo at 4° for 20–30 min and dissolved in 0.5 ml of 0.05 M phosphate buffer, pH 7.5. This is followed by the addition of 3.0 μmoles of glycine (glycine ethyl ester or diglycine, respectively) dissolved in 0.1 ml of water. The solution is agitated overnight at 4° and treated with 1.2 ml of ethanol. The precipitate is separated by centrifugation, washed with 70% ethanol and dried in vacuo. The dried substance is dissolved in 0.1 ml water and applied to a column of Sephadex

G-25 (5 × 0.4 cm). The column is eluted with 0.01 M sodium acetate at 2 ml/hr. The eluate is then treated by 2 vol of ethanol and the precipitate washed with 70% ethanol and dried.

To characterize the product it was hydrolyzed for 1.5 hr in 0.7 N NaOH at 37° and the reaction mixture was chromatographed in system 3 where R_f of phenylalanine is 0.5 and for the three ureido derivatives (R = –OH, $-OC_2H_5$ and $-NHCH_2COOH$) 0.11, 0.3 and 0.2, respectively. No phenylalanine spots were found on these chromatograms.

To show the absence of side reactions involving other tRNA regions, non-acylated tRNA was processed in control experiments in a similar way but using [^{14}C] glycine with specific activity 14.3 mCi/mmole. In this case the eluate after gel filtration did not contain any radioactivity. Similar results were obtained when *p*-nitrophenyl chlorocarbonate and glycine were mixed with 5′-phosphates of adenosine, guanosine, uridine and cytidine (pA, pG, pU and pC, respectively). The treatment leaves these nucleotides unmodified.

3. Results and discussion

The synthesis of ureido derivatives of phenylalanyl-tRNA was achieved by condensation of Phe-tRNA with *p*-nitrophenyl chlorocarbonate followed by treatment of the resulting *p*-nitrophenyloxycarbonyl-phenylalanyl-tRNA with amino compounds which lead to the formation of the corresponding ureido derivatives.

$$\underset{\text{(I)}}{[^{14}C]\,\text{Phe-tRNA}} \xrightarrow[\text{(II)}]{p\text{-}NO_2C_6H_4COCl}$$

$$\underset{\text{(III)}}{p\text{-}NO_2C_6H_4OCO\text{-}[^{14}C]\,\text{Phe-tRNA}} \xrightarrow{RCOCH_2NH_2}$$

$$\underset{\text{(IV)}}{RCOCH_2NHCO\text{-}[^{14}C]\,\text{Phe-tRNA}}$$

where R is HO–, C_2H_5O–, $HOOCCH_2NH$–.

Hydrolysis of the resulting ureides and subsequent chromatographic separation of the hydrolysis products (see table and Materials) has demonstrated the complete absence of the initial Phe-tRNA in the final product; moreover the loss of Phe-radioactivity calculated per 1 A_{260} unit of tRNA was only 9–27% in experiment 1 and 21–37% in experiment 2. The overall recovery of ureides with respect to the initial Phe-tRNA is 53–72% in experiment 1 and 31–48% in experiment 2.

The condensation of *p*-nitrophenyl chlorocarbonate with Phe-tRNA under our experimental conditions is selective with respect to the α-amino group of

Table 1
Synthesis of ureido derivatives of phenylalanyl-tRNA.

No. of experiment	Initial components of reaction: Phe-tRNA tRNA (A_{260} units)	Phe-tRNA Amount (μmole Phe-tRNA × 10^3)	Phe-tRNA Total radioactivity (cpm × 10^3)	Amino acid or its derivatives: Formula	Amino acid or its derivatives: Amount (μmole)	R in ureido derivatives of Phe-tRNA	Derivatives of phenylalanyl-tRNA: Amount of material obtained, (A_{260} units)	Loss of specific radioactivity, (% per A_{260} unit)	Total radioactivity (cpm × 10^3)	Total yield, (%)
	10	0.96	550	H(Gly)OH	0.3	HO	8	9.1	400	72
1	10	0.96	550	H(Gly)OEt	0.3	C_2H_5O	7.8	12.7	374.4	68
	10	0.96	550	$H(Gly)_2OH$	0.3	$HOOCCH_2NH$	7.3	27.2	292	53.3
	5	0.70	400	H(Gly)OH	0.15	HO	3	31.0	189	47.5
2	5	0.7	400	H(Gly)OEt	0.15	C_2H_5O	3.5	40	203	50.7
	5	0.7	400	$H(Gly)_2OH$	0.15	$HOOCCH_2NH$	2.5	54.5	125	31.2

the phenylalanine residue. This was confirmed by control experiments where non-acylated tRNAs were mixed with *p*-nitrophenyl chlorocarbonate and then with [^{14}C] glycine. The tRNA obtained in these experiments contained essentially no radioactivity. Moreover, similar treatment of four usual nucleotides pA, pG, pC and pU also left them unmodified without any traces of radioactive labeling.

Summarizing one can say that the reaction, first described by Bruton and Hartley [12], for the covalent attachment of Met-tRNA to methionyl-tRNA-synthetase permitted the development of an efficient procedure for the synthesis of peptidyl-tRNA with reversed direction of the peptide chain. Compounds synthesized in this way extend our set of chemical tools (analogs and derivatives of peptidyl-tRNA) which might be used for studies of ribosomal function. The protection of carboxy- and hydroxy-groups is not necessary for the condensation of *p*-nitrophenyloxycarbonyl-Phe-tRNA with amino acids and peptides, which is an essential advantage of the method described. Moreover, the reversed peptidyl-tRNAs obtained, due to the presence of a reacting carboxy-group, can be directly used without any additional treatment for subsequent elongation of the chain (for example, ureides with R = OH and $HOOCCH_2NH$).

The compounds synthesized were tested as peptide donors using poly U charged ribosomes from *E. coli* MRE 600. All of them were active in the puromycin reaction. The results of these experiments will be published elsewhere.

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