THE SYNTHESIS OF N-ACETYLPHENYLALANYL-sRNA

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Received August 4, 1966

Recently the occurrence of N-formylmethionyl-sRNA in E. coli was reported (Marcker and Sanger, 1964). It has been suggested that N-formylmethionyl-sRNA and other N-acylaminoacyl-sRNA's may serve as initiators for protein synthesis in E. coli (Nakamoto and Kolakofsky, 1966). Therefore it is of great interest to synthesize chemically well defined acylated aminoacyl-sRNA compounds and to test their behaviour in biological systems.

Haenni and Chapeville (1966) reported the preparation of N-acetylphenylalanyl-sRNA. Their method involves acetylation of phenylalanyl-sRNA with acetic anhydride. When their [^{14}C] acetylphenylalanyl-sRNA was treated with Tris base at pH 9.5 and the products analyzed by paper electrophoresis (pH 2), four radioactive spots were found. Only about 50% of the radioactivity was found in the spot corresponding to acetylphenylalanine. From the results obtained it appears that acetic anhydride reacts not only with the amino group of the esterified phenylalanine but also with certain groups of the sRNA. In this connection it is worthwhile to mention the work of Stuart and Khorana (1964) who acetylated the hydroxyl groups of poly-deoxynucleotides with acetic anhydride in similar reaction conditions as used by Haenni and Chapeville (1966).

In this communication we report on the preparation of acetyl-phenylalanyl-sRNA using a highly specific acetylating agent.

Recently, Anderson et al. (1964) reported the synthesis of N-hydroxysuccinimide esters of acyl amino acids. These esters were highly reactive in peptide synthesis in organic solvents as well as in aqueous solutions. Similarly, N-hydroxysuccinimide ester of acetic acid (III) was prepared by the reaction of acetic acid (1 mmole) and N-hydroxysuccinimide (II) (1 mmole) in the presence of dicyclohexylcarbodiimide (1 mmole) using ethyl acetate as solvent. The reaction mixture was left at room temperature overnight. The dicyclohexyl-urea which precipitated, was removed by filtration, and the filtrate was concentrated in vacuo to yield white crystals. The product was recrystallized from water (yield 93%, m.p. 130° C. Elementary analysis: Cal.: C, 45.85%, H, 4.45%, N. 8.91%. Found: C, 46.11%, H, 4.24%, N, 8.56%) and it moved as a single spot on a thin layer chromatogram (Fig. 1). The same procedure was used for the preparation of compound III labeled in the acetyl group with [^{3}H].

The specificity of N-hydroxysuccinimide ester of acetic acid was tested by reacting it on adenosine-5'-phosphate and on cytidine-5'-phosphate. Both nucleotides remained unaffected (Lapidot et al.). In addition, the N-hydroxysuccinimide ester of [^{3}H] acetic acid was added to alkaline stripped and dialyzed sRNA, and kept under the same conditions as described in the preparation of N-acetyl [^{14}C] phenylalanyl-sRNA (see below). The sRNA was reisolated and its radioactivity was determined in a Packard liquid scintillation counter (Model 3003). According to the results obtained, not more than one out of every 5,000 molecules of sRNA could have reacted with the acetylating reagent. It seems to us most likely that no reaction

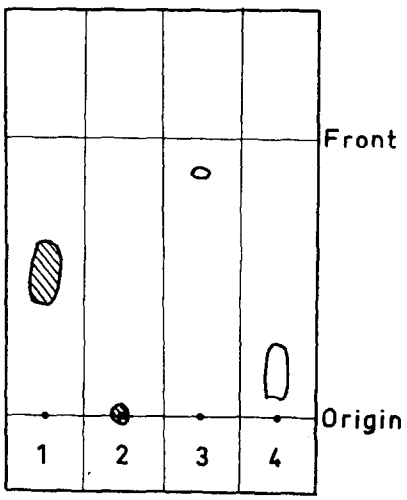


Fig. 1. Thin layer chromatography on microchromatoplates coated with silica gel by dipping (Peifer, 1962). Solvent: chloroform. Indicators: open spots were made visible by charring with aqueous sulfuric acid 1:1 (v/v). Solid spots were made visible (red color) by applying a spray consisting of a mixture of 14% hydroxylamine solution in water (20 ml) and 14% NaOH (8.5 ml), followed after 2 minutes, by spraying with 5% $FeCl_3$ in 1.2 N HCl. 1, N-hydroxysuccinimide ester of acetic acid; 2, N-hydroxysuccinimide; 3, dicyclohexylcarbodiimide; 4, dicyclohexyl-urea.

took place with the sRNA proper, and the radioactivity found in the product can be accounted for by some non-nucleotide material like protein or peptide accompanying the sRNA.

The high reactivity of hydroxysuccinimide esters enable their use in a solid phase reaction similar to the procedure used in peptide synthesis (Merrifield, 1963).

1.9 mg [^{14}C] phenylalanyl-sRNA* (200 $\mu\mu$mole phenylalanine, specific activity 220 μc/μmole) dissolved in 0.1 M acetate buffer pH 5.0 (0.4 ml) was added to a solution of III (70 mg, 440 μmoles) in dimethylformamide (1.6 ml). The suspension was shaken in a Vortex test tube mixer at room temperature for 15 hrs. The sRNA was isolated by centrifugation (18,000 x g, 20

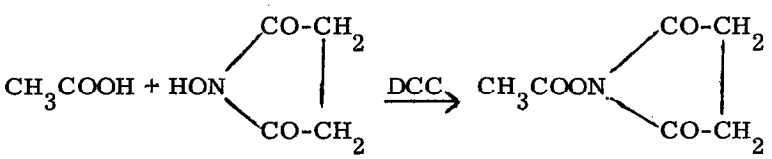
* Prepared from yeast sRNA purchased from Calbiochem, California.

min at 4° C). The precipitate was washed with dimethylformamide (3 x 3 ml) and with ethanol (3 ml). The sRNA was dissolved in 0.1 M acetate buffer pH 5 and an aliquot (0.01 ml) was treated with 0.5 N NaOH for 30 min at 37° C. The hydrolyzate was analyzed by paper chromatography (solvent system; n-butanol:acetic acid:water 78:5:17 v/v) together with a sample of acetylated [^{14}C] phenylalanyl sRNA (0.01 ml) not treated with alkali. The radioactivity was determined by cutting the chromatogram into small strips and counting them directly in a liquid scintillation counter. In the sample of acetylated [^{14}C] phenylalanyl-sRNA, all the radioactivity remained at the origin (the Rf value of sRNA in the solvent used is zero). In the sample after alkaline treatment all the radioactivity moved as N-acetylphenylalanine (Rf 0.8).*

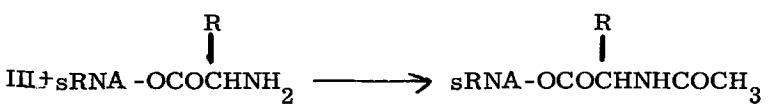
A relative high concentration of the acetylating reagent seems to be critical in order to obtain 100% acetylation of the phenylalanine bound to the sRNA. When the concentration of compound III in the reaction mixture was reduced to 50% of that described above, only 85% of the amino acid was acetylated.

[^{3}H] acetyl-[^{14}C] phenylalanyl-sRNA was prepared and analyzed by the same methods, and similar results were obtained.

The reaction can be summarized in the following scheme:



I II III



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DCC = dicyclohexylcarbodiimide.

* The Rf value of phenylalanine is 0.35.

The rate of hydrolysis of the ester linkage between N-acetyl [^{14}C] phenylalanine and the sRNA is given in Table 1.

Table 1

Rate of alkaline hydrolysis of acetyl-[^{14}C] phenylalanyl-sRNA

Incubation time (minutes)	Radioactivity remained at the origin (cpm)	Radioactivity moving as acetylphenyl-alanine Rf 0.8 (cpm)	% hydrolysis
0	750	0	0
20	568	185	24.6
50	453	296	39.5
70	301	413	55
0.5 N NaOH for 30 min	0	761	100

N-acetyl [^{14}C] phenylalanyl-sRNA was incubated in 0.1 M Tris buffer, pH 8.5, at 37° C. 0.01 ml aliquots containing 750 cpm were taken out, brought to pH 5 by adding 1 M acetate buffer and analyzed by paper chromatography as described above.

The half lifetime of N-acetylphenylalanyl-sRNA calculated from the above results is about 65 min, this being in agreement with the results obtained by Haenni and Chapeville (1966).

Glycyl-[^{14}C] phenylalanyl-sRNA was prepared according to the same general scheme (Lapidot et al., 1966).

The biological properties of the acylated phenylalanyl-sRNA compounds is at present under investigation in our laboratory.

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