SOME BIOLOGICAL PROPERTIES OF ACETYL- AND FORMYLPHENYLALANYL-sRNA

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Introduction

Evidence has been accumulated indicating that formylmethionine serves in E. coli as an initiator in protein synthesis (Clark and Marcker, 1966; Sundararajan and Thach, 1966). Its role in cells of other organisms is not known, but these observations arouse great interest in N substituted aminoacyl-sRNA. Recently we reported on the synthesis of acetylphenylalanyl-sRNA by using N-hydroxysuccinimide ester of acetic acid as acetylation agent (de Groot et al. 1966). We found that the reaction is a general one and can be used for preparing acylaminoacyl-sRNA of short and long chain fatty acids.

In this communication the synthesis of formyl [^{14}C] phenylalanyl-sRNA is described and results obtained in binding and incorporation experiments using acetyl (and formyl) [^{14}C] phenylalanyl-sRNA and wheat germ ribosomes, are reported.

Materials

[^{14}C] Phenylalanyl-sRNA was prepared from yeast-sRNA (Calbiochem.) as described elsewhere (Lapidot et al., in press). Acetyl [^{14}C] phenylalanyl-sRNA was prepared as reported previously (de Groot et al., 1966). The product obtained contained 98% acetyl [^{14}C] phenylalanyl-sRNA and 2% [^{14}C] phenylalanyl-sRNA. Formyl [^{14}C] phenylalanyl-sRNA was prepared in a similar way by reacting the N-hydroxysuccinimide ester of formic acid (Lapidot, Rappoport and Wolman, in press) with [^{14}C] phenylalanyl-sRNA.

[^{14}C] Phenylalanyl-sRNA (1.56 mg, 580 $\mu\mu$moles phenylalanine, specific activity 244 μc/μmole) dissolved in 0.1 M acetate buffer, pH 5.0 (1.2 ml) was added to a solution of N-hydroxysuccinimide ester of formic acid (286 mg, 2 mmole) in dimethylformamide (4.8 ml). The suspension was shaken in a Vortex test tube mixer at room temperature for 19 hr. The sRNA was isolated by centrifugation (18,000 X g, 20 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 5.0, and an aliquot 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 formylated [^{14}C] phenylalanyl-sRNA not treated with alkali. In the sample not treated with NaOH all the radioactivity remained at the origin. In the sample after alkaline treatment 98% of the radioactivity moved as formylphenylalanine (R_f 0.8) and 2% moved as phenylalanine (R_f 0.35). The formyl and the acetyl derivatives had the same specific activity as the [^{14}C] phenylalanyl-sRNA used in the acylation reactions.

Wheat germ ribosomes and "dialyzed supernatant" were prepared essentially as described by Marcus and Feeley (1964). The crude ribosomal pellet was washed by suspending the pellet in 0.01 M Tris acetate buffer, pH 7.5 containing 0.001 M magnesium acetate, 2% sucrose and 0.005 M mercaptoethanol. The suspension was centrifuged at 14,000 X X g for 20 min. and at 100,000 X g for 1 hr. The washing was repeated and the final pellet was taken up in the same buffer and the ribosomes were frozen in liquid air and stored at -20°C.

Results and Discussion

The binding of [^{14}C] phenylalanyl-sRNA and acetyl [^{14}C] phenylalanyl-sRNA to wheat germ ribosomes as a function of substrate concentration, in the presence and absence of poly U, is given in Fig. 1. At substrate concentration below 15 $\mu\mu$moles acetyl [^{14}C] phenylalanyl-sRNA binds considerably less than [^{14}C] phenylalanyl-sRNA. However, the maximum amount that can be bound per ribosome is approximately the same for both compounds.

The effect of Mg^{++} concentration upon the binding of acetyl [^{14}C] phenylalanyl-sRNA as compared to [^{14}C] phenylalanyl-sRNA binding

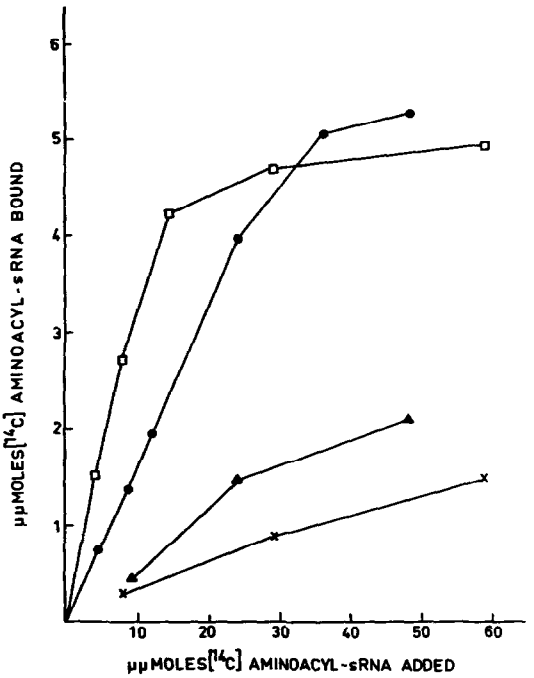

Fig. 1. Binding of aminoacyl-sRNA to wheat germ ribosomes as a function of aminoacyl-sRNA concentration.
Binding assays were performed by the method developed by Nirenberg and Leder (1964). Reaction mixtures in a final volume of 0.1 ml contained: Tris acetate, 5 μmoles, pH 7.3; KCl, 5 μmoles; magnesium acetate, 3 μmoles; poly U, 20 γ; ribosomes, 1.15 A^{260} units. Incubation at 30°C for 5 min.

▲—▲ acetyl [^{14}C] phenylalanyl-sRNA, — poly U
●—● acetyl [^{14}C] phenylalanyl-sRNA, + poly U
X—X [^{14}C] phenylalanyl-sRNA, — poly U
□—□ [^{14}C] phenylalanyl-sRNA, + poly U.

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is given in Fig. 2. At low magnesium concentration (0.01-0.02 M) acetyl [^{14}C] phenylalanyl-sRNA binds much less to ribosomes than [^{14}C] phenylalanyl-sRNA. However, at higher magnesium concentration the relative difference between the binding of the two compounds decreases. [^{14}C]-phenylalanyl-sRNA was treated with dimethylformamide under the conditions prevailing in the acylation reaction. Binding reactions were carried out with the dimethylformamide treated compound as described in Fig. 1 and 2. In the presence of poly U the results obtained with the dimethylformamide treated compound were identical to those obtained with untreated [^{14}C] phenylalanyl-sRNA. However in the absence of poly U the

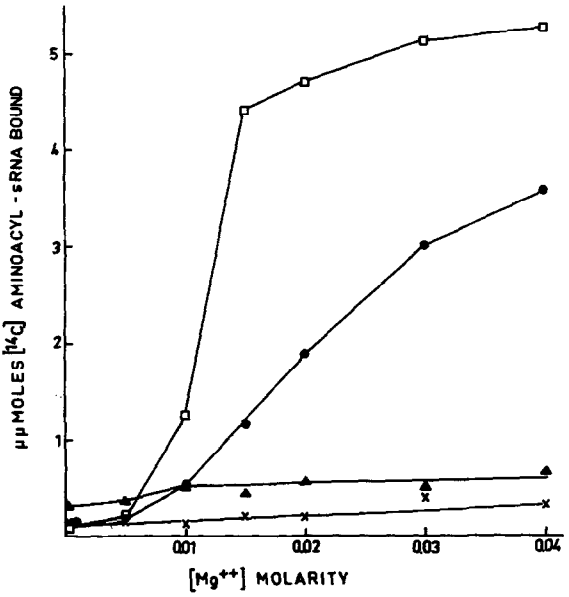

Fig. 2. Binding of aminoacyl-sRNA to wheat germ ribosomes as a function of Mg^{++} concentration.
Assay conditions as in Fig. 1.
Aminoacyl-sRNA, 8.2 μμmoles, 2,800 c.p.m.,
Ribosomes 4.5 A^{260} units.

▲—▲ acetyl [^{14}C] phenylalanyl-sRNA, — poly U
●—● acetyl [^{14}C] phenylalanyl-sRNA, + poly U
X—X [^{14}C] phenylalanyl-sRNA, — poly U
□—□ [^{14}C] phenylalanyl-sRNA, + poly U.

binding of the dimethylformamide treated compound was considerably higher than that of untreated [^{14}C] phenylalanyl-sRNA and was approximately the same as that of acetyl [^{14}C] phenylalanyl-sRNA (Fig. 1). It seems therefore that the differences between the binding of [^{14}C] phenylalanyl-sRNA and of acetyl [^{14}C] phenylalanyl-sRNA in the presence of poly U are due to the acetylation of the amino group of the phenylalanine attached to the sRNA and not to the dimethylformamide treatment.

We investigated the possibility of deacetylation of acetyl [^{14}C]-phenylalanyl-sRNA during the binding reaction. A binding reaction mixture with acetyl [^{14}C] phenylalanine-sRNA in the presence of poly U (for conditions see legend of Fig. 1) was poured on a Millipore filter and after washing the filter was soaked in 0.05 ml NaOH 0.5 M for 1 hr at room temperature. The filter was washed with 0.5 ml of water, the solution con-

Table 1

Incorporation of acylated and unacylated [^{14}C] phenylalanyl-sRNA into peptide material

Substrate	c.p.m. added	Preincubation	c.p.m. incorporated
[^{14}C] phenylalanyl-sRNA	5000	-	2505
[^{14}C] phenylalanyl-sRNA	5000	+	2478
[^{14}C] phenylalanyl-sRNA	5000	-	283 *
acetyl [^{14}C] phenylalanyl-sRNA	7500	-	44 **
acetyl [^{14}C] phenylalanyl-sRNA + 100 $\mu\mu$moles phenylalanine + 100 γ yeast-sRNA	7500	-	48 **
acetyl [^{14}C] phenylalanyl-sRNA	7500	+	625
acetyl [^{14}C] phenylalanyl-sRNA + 100 $\mu\mu$moles phenylalanine + 100 γ yeast-sRNA	7500	+	945
acetyl [^{14}C] phenylalanyl-sRNA	7500	-	25 *, **
acetyl [^{14}C] phenylalanyl-sRNA	7500	+ (0.01 M Mg^{++})	284
formyl [^{14}C] phenylalanyl-sRNA	4200	-	36 **
formyl [^{14}C] phenylalanyl-sRNA	4200	+	205

Incorporation mixture contained in a 1.0 ml volume: Tris acetate 50 μmoles, pH 7.5; KCl, 50 μmoles; magnesium acetate, 11 μmoles; poly U, 40 γ; ATP, 1 μmole; GTP, 0.5 μmole; PEP, 5 μmoles; pyruvate kinase, 25 γ; mercaptoethanol, 5 μmoles; wheat germ supernatant, 1 mg protein; substrate as indicated. The reaction was started by adding 6.0 A^{260} units of ribosomes. Incubation: 30 min. at 30°C. The hot 5% TCA insoluble material was filtered on Millipore filters and counted in a Packard liquid scintillation counter. Preincubation was conducted as described for binding assay (see Fig. 1) with 40 γ poly U and 6.0 A^{260} units of ribosomes. After 5 min. preincubation at 30°C, reagents were added in order to obtain an incorporation mixture as described above.

* Magnesium acetate, 21 μmoles, in incorporation mixture.
** This small incorporation can be explained by the presence of 2% [^{14}C]-phenylalanyl-sRNA in the acylated compounds.

centrated in vacuo, and the residue was run on a paper chromatogram. All the radioactivity moved as acetylphenylalanine.

When acetyl [^{14}C] phenylalanyl-sRNA was incubated with wheat

germ ribosomes in a complete incorporation mixture containing poly U, in the presence and absence of unlabeled phenylalanyl-sRNA, no radioactivity was found in the hot 5% TCA insoluble material. Under the same conditions, [^{14}C] phenylalanine from [^{14}C] phenylalanyl-sRNA was effectively incorporated into polypeptide material. Incorporation of radioactivity in hot 5% TCA insoluble material from acetyl [^{14}C] phenylalanyl-sRNA took place, however, when the substance was preincubated together with poly U and ribosomes in the presence of 0.02 M magnesium acetate. When the preincubation was carried out in 0.01 M magnesium acetate a much smaller but still significant incorporation took place. No incorporation was found when the incorporation (without preincubation) was carried out at 0.02 M magnesium acetate. It has to be noted that 0.02 M magnesium acetate is highly inhibitory in the phenylalanine incorporation reaction. The preincubation step had little or no effect on [^{14}C] phenylalanine incorporation with [^{14}C] phenylalanyl-sRNA as substrate (Table 1). Incorporation reactions conducted with dimethylformamide treated [^{14}C] phenylalanyl-sRNA gave identical results to those obtained with [^{14}C] phenylalanyl-sRNA untreated with dimethylformamide. From these results we conclude that preincubation is an absolute requirement for incorporation from acetyl [^{14}C]-phenylalanyl-sRNA. The extent of the incorporation depends on the Mg^{++} concentration during the preincubation.

Haenni and Chapeville (1966) got considerable incorporation from acetyl [^{14}C] phenylalanyl-sRNA with E. coli ribosomes without a preincubation step. We could confirm this observation with E. coli ribosomes and preincubation did not influence the extent of the incorporation.

The behavior of formyl [^{14}C] phenylalanyl-sRNA was very similar to that of acetyl [^{14}C] phenylalanyl-sRNA in respect to binding to ribosomes (Figs. 1 and 2) and incorporation into polypeptide material (Table 1).

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