PHOTOAFFINITY REACTION BETWEEN POLYURIDYLIC ACID AND PROTEIN S1 ON THE *ESCHERICHIA COLI* RIBOSOME

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1. Introduction

A role for protein S1 of the 30 S ribosomal subunit in binding mRNA has been suggested both by functional and chemical assays [1–8]. We have shown recently that upon incubation with ribosomes poly s^4U* is intimately associated with protein S1 [9]. Poly s^4U resembles poly U in coding for the synthesis of polyphenylalanine in an in vitro protein synthesizing system. It can be photoactivated by irradiation at 335 nm to react with protein resulting in covalent bond formation. Irradiation of a mixture containing [^{3}H]poly s^4U, ribosomes and Phe-tRNA leads to incorporation of radioactivity into a ribonucleoprotein complex, which was shown to be resistant to ribonucleases A and T1. Hydrolysis of this complex by alkali revealed the presence of radioactively labelled S1 as the major protein component.

Previous work by Gorelic et al. [10] has shown that upon irradiation at 253 nm 1,3-dimethyluracil forms covalent bonds with 1-aminopropane and poly L-lysin. Schoemaker and Schimmel [11] have used irradiation at 253 nm to covalently link tRNA to the corresponding aminoacyl-tRNA synthetase. Recently Schenkman et al. [12] have given evidence for covalent attachment of poly U to *E. coli* ribosomes following UV irradiation. However, the ribosomal protein involved has not been identified. We have independently investigated the photoaffinity reaction of [^{3}H]poly U bound to ribosomes. The results indicate that [^{3}H]poly U becomes covalently attached to protein S1 upon UV irradiation.

*Abbreviations: poly s^4U, poly-4-thiouridylic acid; poly U, polyuridylic acid.

2. Materials and methods

2.1. *Materials*

E. coli D10 70S ribosomes were 'tight couples' prepared according to Noll [13]. [^{3}H]poly U had a spec. act. of 7×10^8 cpm per mg (kind gift of Dr P. Swetly, Arzneimittelforschung, Vienna).

2.2. *Size of [^{3}H]poly U*

The approx. mol. wt of [^{3}H]poly U was determined by chromatography using a Sephadex G25 column (1.5 × 22 cm). *E. coli* tRNA (mol. wt 25 000) and UMP (mol. wt 324) were employed as molecular weight markers. A mixture of [^{3}H]poly U (5600 cpm), 2 A_{260} units tRNA and 2 A_{260} units of 2',3'-uridylic acid in 100 µl buffer (0.1 M NaCl, 0.01 M Tris–HCl pH 7.1), and 0.001 M EDTA) was layered on the column and eluted with the same buffer.

2.3. *Irradiation of the ribosomal [^{3}H]poly U complex*

The incubation was carried out in a total volume of 0.6 ml: 5 mg ribosomes, 1 mg Phe-tRNA and 2×10^5 cpm of [^{3}H]poly U in a buffer containing 60 mM NH_4Cl, 100 mM Tris–HCl pH 7.4, 20 mM magnesium acetate, and 4 mM dithiothreitol were incubated for 20 min at 25°C. Irradiation was performed for 2 hr at 0°C with a low pressure mercury-lamp (Hanau, NN15/44 VK) at 253.7 nm. Subsequently, urea and EDTA were added to final concentrations of 3 M and 0.1 M respectively and the samples were digested for 2 hr at 37°C with 15 µg ribonuclease A and 2 µg ribonuclease T1 added per ml. Protein was precipitated by 10% trichloroacetic acid. It was dissolved in 2 ml 10 M urea, dialyzed against 10% and 1% acetic acid and lyophilized.

2.4. *Electrophoresis on polyacrylamide-SDS-gels*

200 μg of lyophilized protein was dissolved in 85 μl of a solution containing 1 M urea, 30 mM sodium phosphate pH 7.1, 10 mM dithiothreitol and 1% SDS and was heated for 90 sec to 100°C. It was then cooled, 30 μl of 10 M urea was added and the sample layered on a 7.5% polyacrylamide gel in SDS-buffer [14].

In a parallel experiment 200 μg of lyophilized protein was dissolved in 0.25 ml of a solution containing 0.5 M urea, 0.1% SDS and 10 mM dithiothreitol. NaOH was then added to a final concentration of 0.5 M and the mixture was incubated for 20 min at 37°C. Protein was then precipitated with 10% trichloroacetic acid. It was subsequently dissolved in 85 μl of a solution containing 1 M urea, 30 mM sodium phosphate pH 7.1, 10 mM dithiothreitol and 1% SDS, heated and layered on gels as described above.

Electrophoresis was carried out for 14 hr at 5 mA per tube. Gels were stained with Coomassie Brilliant Blue and the radioactivity determined as described previously [9].

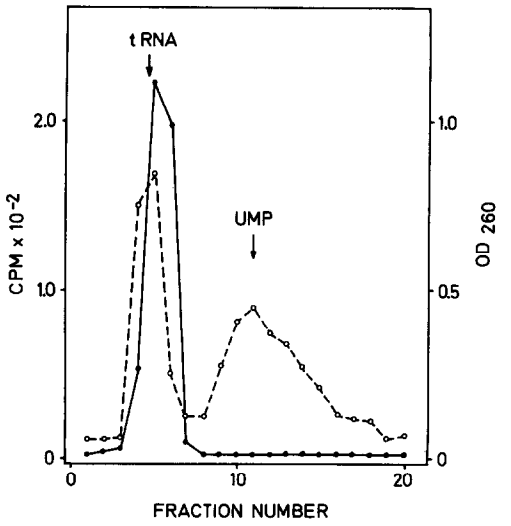

Fig.1. Gel-chromatography of [^{3}H]poly U. The position of the markers was determined by measuring the A_{260} (o- - -o) in the eluate. Radioactivity (•——•) was determined by counting samples in toluene-triton X-100 scintillator.

3. Results and discussion

For this experiment a [^{3}H]poly U preparation of short chain length was employed. The mol. wt was determined by chromatography on Sephadex G25. A comparison of the elution profiles in fig.1 indicates that the [^{3}H]poly U is somewhat smaller in size than the tRNA added as a marker.

A complex of [^{3}H]poly U, ribosomes and Phe-tRNA was formed by incubation at 25°C. It was subsequently cooled on ice and irradiated with a low pressure mercury lamp. In order to search for covalent bond formation between [^{3}H]poly U and ribosomal protein, the sample was digested extensively with a mixture of ribonucleases A and T1 in the presence of urea and EDTA. Proteins were precipitated with trichloroacetic acid to remove low mol. wt digestion products. The proteins were then dissolved in urea-SDS and subject to electrophoresis on polyacrylamide gels. The results are shown in fig.2. The radioactivity peak is found to correspond to the position of protein S1 in the gel. No radioactivity was found in protein when UV irradiation was omitted (data not shown).

In order to investigate whether this reflects

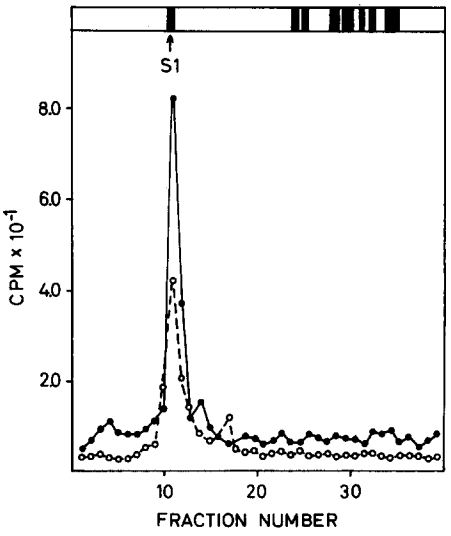

Fig.2. Identification of labelled protein by SDS gel electrophoresis. The conditions of the electrophoresis are described in Materials and methods. One sample (o- - -o) of ribosomal protein was treated with alkali prior to layering on the gel, the other sample was not treated with alkali (•——•).

radioactively labelled S1 or a ribonucleoprotein composed of several small proteins attached to a fragment of [^{3}H]poly U, the total protein obtained after ribonuclease digestion was subject to alkaline hydrolysis. The distribution of radioactivity following electrophoresis on a polyacrylamide gel is given in fig.2. The position of the radioactivity peak again coincides with the S1 band. The specific activity of the protein, however, is decreased indicating that part of the [^{3}H]poly U attached to S1 has been hydrolysed during the alkali treatment. The corresponding small loss in mol. wt obviously does not affect the electrophoretic mobility of the labelled protein.

In contrast, photoaffinity labelling of ribosomes with poly s^{4}U yields a large ribonucleoprotein complex, which in addition to S1 also contains other proteins (Fiser, Scheit, Stöffler and Kuechler, in preparation). There are several possible reasons for this discrepancy. The chemical reactions, which occur upon photoactivation, are quite different. Poly s^{4}U reacts at C-4 of the pyrimidine ring in a nucleophilic substitution with concomitant elimination of the S-atom [15]. In contrast, poly U reacts presumably by a nucloephilic addition to the double bond between C-5 and C-6 of the pyrimidine ring [10]. Furthermore, a marked effect of the size of poly U on the product of the photoreaction was noted. Long-chain poly U (mol. wt > 50 000) also yields large size ribonucleoprotein complexes (unpublished experiments). In addition, ribosomes are at least partly inactivated upon irradiation at 253 nm [16,17]. This is not too surprising, since light of this wavelength not only photoactivates uracil-moieties in poly U but also in ribosomal RNA. In contrast light of 300–400 nm wavelength used for affinity labelling with poly s^{4}U does not affect the activity of the ribosome.

Nevertheless the fact that protein S1 becomes labelled both in the poly s^{4}U and the poly U photoaffinity systems indicates that the architecture of the ribosome is not extensively altered by irradiation at 253 nm. Pongs and Lanka (personal communication) have recently obtained evidence for covalent cross-links between poly-5-bromouridylic acid and protein S1. These studies lend further support to the hypothesis that protein S1 is involved in the binding of mRNA on the ribosome.

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