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COVALENT ATTACHMENT OF POLY (U) TEMPLATE TO 40S - MAMMALIAN RIBOSOMAL SUBUNITS

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Summary

When 40S subunits are irradiated at 254nm in presence of [^{3}H] poly (U), formation of a 40S subunit-poly (U) complex can be demonstrated either by filtration technique at low Mg^{++} concentration or by polyacrylamide gel electrophoresis. No stable complex was detected using unirradiated samples under the same conditions. Electrophoresis of this complex in the presence of dodecyl sulfate showed that part of the poly (U) directly associates with 18S RNA. This association is not through proteins, since it is not disrupted by pronase treatment.

Introduction

Information on protein - RNA interactions within ribosomes and subunits from rat liver have been obtained using UV irradiation (1. 2).

The same methodology should also be suitable to identify the ribosomal components which interact with messenger RNA, aminoacyl tRNA and peptidyl tRNA during protein synthesis. As a first step we have attempted in this report to identify the ribosomal components interacting with poly (U). As previously mentioned, only the 40S subunits are active in this binding (3).

Material and methods

1) Material

Poly [5-^{3}H]uridylic acid (1.63 mCi/mg) was obtained from the radiochemical centre, Amersham. It was diluted with unlabelled poly (U) (from Miles laboratories) as indicated in the legend of the figures.

2) Methods

Active 40S ribosomal subunits were prepared from rat liver free polysomes as described by Madjar et al (4) using a method adapted from that of Blobel and Sabatini (5). We assumed that 1 A_{260} unit equals

Abbreviations used : poly (U) : polyuridylic acid ; SDS : sodium dodecyl sulfate ; 1 D - PAGE : one dimensional polyacrylamide gel electrophoresis.

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51 pmoles of 40S subunits. 40S subunits (28 A_{260} units/ml) were incubated in presence of diluted [^{3}H] poly (U) (84 moles uracil per mole of subunits), 10 mM Tris-HCL,pH 7.5, 50 mM KCL, 10 mM $MgCl_2$ for 5 min at 4°, and then divided into 2 identical samples (140 µl each, 2mm solution depth). One aliquot was kept at 4° as a control and the other irradiated at this temperature with a low-pressure mercury lamp having a maximum output at 253.7 nm and producing 2.9 x 10^2 erg/mm^2/s at the distance of the sample (10 cm). Incident radiation doses were determined by ferrioxalate actinometry (6). At given time intervals 30 µg of 40S subunits were removed for sedimentation and biological activity analysis, and the remainder was shaken manually before further irradiation. At the end of the irradiation, the reaction mixtures were immediately adjusted to 0.1mM Mg^{++} and filtered through a nitrocellulose filter (0.45µ). In other experiments, samples were analyzed by one-dimensional polyacrylamide gel electrophoresis (1 D - PAGE) as described in the legend of figure 2.

Results and discussion

The binding of [^{3}H] poly (U) to 40S subunits as measured by filtration is characterized by a marked dependence on $MgCl_2$ concentration. The bound poly (U) is released from subunits when at the end of the incubation, the Mg^{++} concentration is lowered from 10 mM to 0.1 mM. In contrast, increasing amounts of [^{3}H]poly (U) are recovered in association with subunits when [^{3}H]poly (U) - 40S subunit complexes have been subjected to increasing doses of radiation before being filtered with 0.1 mM $MgCl_2$ buffer (fig. 1). A plateau was reached for 10^{18} quanta, at a level which represents 15 - 20 % of the [^{3}H] poly (U) originally bound to control subunits at 10 mM $MgCl_2$. Subunits irradiated alone under these conditions kept their non-covalent binding affinity for the [^{3}H] poly (U) template, 62 - 85 % of their biological activity in polyphenylalanine synthesis and their sedimentation characteristics. Irradiation of [^{3}H] poly (U) with the same dose of 10^{18} quanta had no detectable effect on its affinity for subunits. The binding increase observed for higher doses (3 - 4 x 10^{18} quanta) is most likely due to the modification of the subunit structure as seen from the sedimentation analysis (results not shown). All the following experiments were therefore carried out using 10^{18} quanta.

Much the same results were obtained as with the filtration technique on isolating the irradiated [^{3}H] poly (U) - 40S complex by 1 D - PAGE in the low Mg^{++} buffer (fig. 2). We observed that part of the [^{3}H] poly (U)

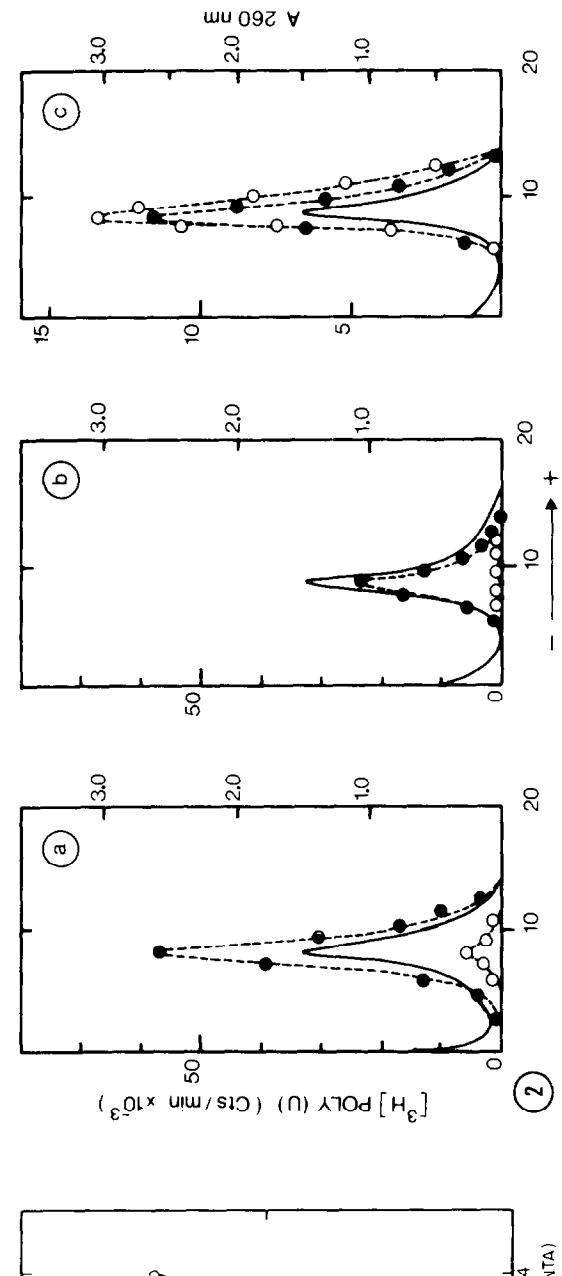

Figure 1 - Dependence of [^{3}H] poly (U) - 40S subunits crosslinking upon the irradiation dose. The binding mixtures as described under "Methods" contained 1.4 A260 units of 40S subunits and 1.94 µg of [^{3}H] poly (U) (0.09 µCi/µg). They were irradiated with increasing doses of quanta, diluted to 0.1 mM $MgCl_2$ and then isolated by filtration through nitrocellulose filters treated with 0.5N KOH (7). The tube and filter were thoroughly rinsed (6 times) with a low-Mg++ buffer (10mM Tris-HCL, pH 7.5, 50 mM KCL, 0.1 mM $MgCl_2$). In each case, the radioactivity of identical non-irradiated samples (about 200 counts / min) has been subtracted.

Figure 2 - [^{3}H] poly (U) - 18S RNA cross-linking after irradiation of 40S subunits in presence of [^{3}H] poly (U). a - Samples containing 4 A260 units of 40S subunits were incubated with 5.53 µg of [^{3}H] poly (U) (0.45 µCi/µg), irradiated or not as described in "Methods" and electrophoresed on 1 D - PAGE (100 V for 3.75 h) using 3% acrylamide (8) and a low Mg++ buffer: 36 mM Tris-acetate, pH 7.8, 30 mM KH_2PO_4 and 0.1 mM Mg acetate. All the gels were scanned at 260 nm (continuous line). Two of them corresponding respectively to unirradiated (○) and irradiated material (○) were cut into slices approximately 3 mm thick. These were incubated with 1 ml of NCS solubilizer (2 h at 50°) and counted in 10 ml of scintillation mixture prepared in toluene. Radioactivity of each vial was corrected for counting efficiency. b - Segments containing the radioactive complex were cut out from the other gels and examined for [^{3}H] poly (U) - 18S RNA complexes by 1 D - PAGE (100 V for 2.33 h) using 3% acrylamide and a buffer containing 0.2% SDS, 40 mM Tris-HCl, pH 7.3, 20 mM sodium acetate and 1 mM EDTA. Gels were scanned and radioactivity was counted as in (a). c - Covalent [^{3}H] poly (U) - 18S RNA complex extracted from two gels identical to that represented in (b), using 1% SDS (16 h at 20°) was dialyzed against water, lyophilized, dissolved in 20 mM Tris-HCl, pH 7.5, 10 mM EDTA, 0.1% SDS and divided into 2 identical samples. 20 - 200 µg of ribonuclease-free pronase was added to one, and both were incubated at 37° for 30 min (8). At the end of the incubation, the mixtures were electrophoresed under the same conditions as in (b). Radioactivity corresponding to pronase-treated (●) or untreated (○) samples are represented in the same figure. When subjected alone to 1 D - PAGE under the conditions used in (b) and (c), [^{3}H] poly (U) escaped completely from the gel. In all the gels, the same amount of A260 absorbing material was used. Continuous lines represent A260 profiles.

originally bound to 40S subunits was recovered as a 40S - [^{3}H] poly (U) complex after irradiation with 10^{18} quanta ; only a very low amount of complex was observed without irradiation (fig. 2a). Furthermore, part of the [^{3}H] poly (U) remained associated with 18S RNA when electrophoresis was performed in the presence of 0.2 % SDS, and this was observed only in the case of irradiated material (fig. 2b). After two or three successive electrophoreses in SDS, there remained a constant percentage of radioactivity associated with 18S RNA (20 - 27 % of the total poly (U) which co-migrated with the 40S ribosomal subunits). This association might result from cross-links of the type [^{3}H] poly (U) - ribosomal proteins - 18S RNA, in view of the proteins (15 % of total mass) which were found to be cross-linked to 18S RNA under these conditions (1). To check this possibility, complexes isolated from irradiated subunits were tentatively disrupted by extensive pronase treatment and then analyzed again on 1 D - PAGE in 0.2 % SDS (fig. 2c). After such treatment 15 - 22 % of the radioactivity originally cross-linked to the 40S subunits consistently migrated with 18S RNA. We conclude from these experiments that part of the [^{3}H] poly (U) cross-linked to 40S subunits was directly complexed to 18S RNA, the remaining (78 - 85 %) being cross-linked to proteins. The fact that poly (U) can combine with specific ribosomal proteins in 40S subunits was recently reported by Terao and Ogata (10). These authors were not able to show a cross-link of poly (U) to 18S RNA, but they used quite different irradiation conditions from ours. It is interesting to note that it has been shown, using E. Coli 30S ribosomes, that poly (U) can be cross-linked to both 16S RNA and proteins (11).

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