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STRUCTURAL ALTERATION OF rRNA IN THE L7/L12 REGION OF 50S RIBOSOME ON REMOVAL OF L7/L12 PROTEINS

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Core particles of 50S ribosomes depleted of L7/L12 proteins are degraded by RNase I at a considerably slower rate than intact 50S ribosomes. The normal rate is restored on incorporating L7/L12 proteins into the core particles. The capacity of the core particles to inhibit the RNase I-catalyzed hydrolysis of poly A and to bind ethidium bromide is also greater with core particles than with intact 50S ribosomes. It appears from these results that the region(s) of rRNA in the vicinity of L7/L12 proteins has less ordered structure which, on removal of L7/L12 proteins, becomes more organized. Apparently, binding of L7/L12 proteins to the 50S core leads to the destabilization of double-stranded regions of rRNA.

INTRODUCTION

Although much work is in progress in various laboratories on the structure and function of ribosomes (review by Brimacombe et al., Ref. 1), the nature of interaction between ribosomal RNAs and proteins is not yet known. It is, however, believed that the secondary structure of free rRNA and rRNA present in ribosomes may not be widely different (2,3). Evidence will be presented here to indicate that the double-stranded regions of rRNA in the vicinity of L7/L12 proteins may be destabilized on association with L7/L12. This is in agreement with the earlier observation made in this laboratory that this region is the primary site of attack by RNase I (4) which specifically hydrolyzes single-stranded RNAs.

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MATERIALS AND METHODS

70S Ribosomes and 50S subunits were prepared from *E. coli* MRE600 (RNase I^-) according to the method of Datta and Burma (5). Core particles depleted of L7/L12 proteins were prepared by treatment of 50S ribosomes with 1M NH_4Cl and 50% ethanol, according to the procedure described by Hamel et al. (6). L7/L12 proteins were isolated from the extract by the procedure of the same authors. 50S Ribosomes were reconstituted from core particles and excess of L7/L12 proteins (1:2 ratio) according to the procedure described by Raziuddin et al. (4). RNase I was prepared from *S. typhimurium* as described by Datta and Burma (5). All commercial preparations were of analytical grade.

The kinetics of degradation of 50S ribosomes, core particles and reconstituted 50S ribosomes by RNase I was followed by measuring hyperchromicity at 260 nm in PMQ II Zeiss spectrophotometer, as described by Datta and Burma (5). The inhibition of RNase I catalyzed hydrolysis of poly A by 50S ribosomes and core particles was measured by spectrophotometric method as described by Datta and Burma (5).

The binding of ethidium bromide to 50S ribosomes and core particles was studied by measuring the decrease in absorbancy of ethidium bromide at 490 nm in Carl Zeiss PMQ II spectrophotometer (7).

RESULTS AND DISCUSSION

The kinetics of degradation of the 50S ribosomes and L7/L12-depleted core particles by RNase I show interesting differences (Fig. 1). The depleted ribosomes are significantly more resistant to the hydrolytic action of the enzyme. This was surprising as it was expected that, on removal of the proteins, some regions of rRNA would be exposed and become more susceptible to the action of RNase I. Similar resistance was not observed on treatment with ethanol alone, which did not lead to the release of L7/L12 or other proteins. The reconstituted 50S ribosomes from the core particles and L7/L12 proteins lose their resistance. Since RNase I hydrolyzes single-stranded RNAs (8,9) but has strong affinity for double-stranded RNAs (unpublished results from this laboratory), the kinetic data indicate that double-stranded regions of rRNA are generated on removal of L7/L12 proteins.

Previous studies in this laboratory have shown that, unlike 30S ribosomes, 50S ribosomes do not inhibit the hydrolysis of Poly A by RNase I when Mg^{++} is present at a concentration of 1 mM but, at higher concentrations, it is capable of inhibiting the enzyme partially (4,5). This is

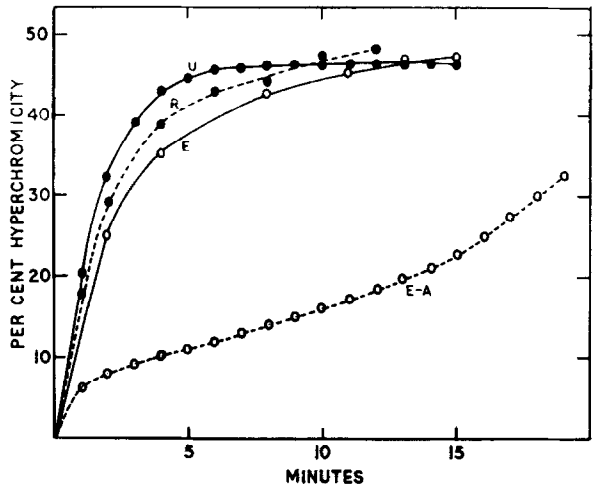

Fig. 1: Kinetics of degradation of 50S ribosomes and core particles. The reaction was carried out in 1 ml containing 0.1 M Tris, pH 7, 1 mM magnesium acetate, 1 A_{260} unit of 50S ribosome or core particle and 4 units of RNase I. The increase in absorbance at 260 nm was followed. U, untreated 50S ribosome; E, Ethanol-treated 50S ribosome; E-A, Ethanol-NH_4Cl treated 50S ribosome (depleted of L7/L12 proteins); R, Reconstituted 50S ribosome from 50S core particle and L7/L12 proteins.

also evident from the results presented in Fig. 2. Core particles, however, are more inhibitory than the intact 50S ribosomes. The difference is prominent at low Mg^{++} concentrations (1 mM) at which 50S ribosomes have no inhibitory capacity. From the studies with 30S ribosomes, it appears that the enzyme associates with the double-stranded regions of rRNA and becomes latent (unpublished observations from this laboratory). Similarly, RNase I becomes latent in contact with 50S ribosomes when the regions of 50S ribosomes where L7/L12 proteins are located are removed by the action of RNase I (4). Thus, it appears that the ordered structures responsible for the inhibitory capacity of the core particles are produced on removal of L7/L12 proteins.

Ethidium bromide is capable of binding to ribosomes (10,11) and the binding is dependent on the concentration of Mg^{++} (7). The extent of binding was measured in case of intact 50S ribosomes and L7/L12-depleted core particles at various Mg^{++} concentrations (Fig. 3). The core particles

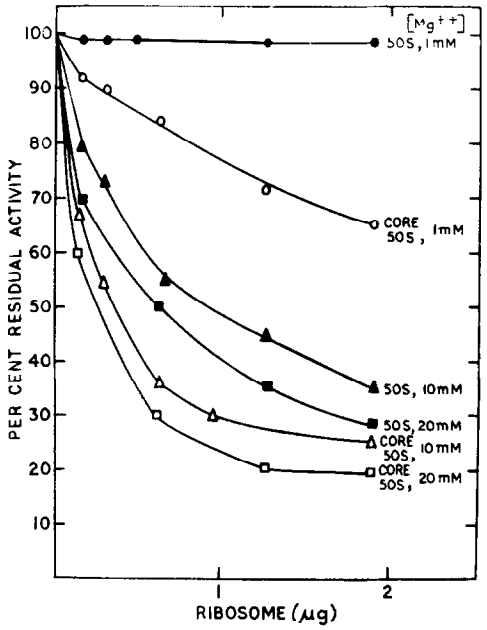

Fig. 2: Inhibition of RNase I-catalyzed hydrolysis of poly A by 50S ribosomes and core particles. The inhibition assay was carried out at 37°C for 10 min in a total volume of 0.25 ml containing 25 μmoles of Tris, pH 7, 50 μg of poly A, 0.12 unit of RNase I and indicated amount of magnesium acetate and ribosomes or core particles. The remaining procedure was the same as described by Chakraburtty and Burma (9).

bind ethidium bromide slightly more than the intact 50S ribosomes. This is particularly evident at low Mg^{++} concentrations where the binding is greater. The mechanism of binding of ethidium bromide to ribosomes is not known but it is likely that the dye intercalates between the base pairs of rRNA in the double-stranded regions. The enhanced binding of ethidium bromide to the core particles may be explained on the assumption of an increase in the double-stranded regions in the core particles as compared with the intact 50S ribosomes.

It has already been observed in this laboratory that, on treatment of 50S ribosomes with RNase I in the presence of 20 mM Mg^{++}, portions of the structure (both rRNA and protein) are removed and the RNase I becomes latent (4). Since the enzyme hydrolyzes single-stranded regions, such a

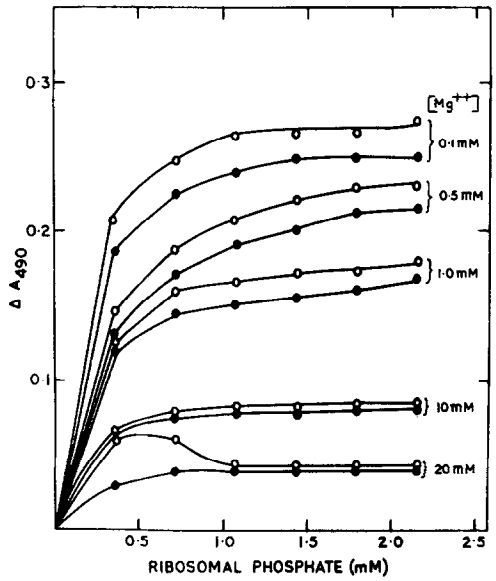

Fig. 3: Binding of ethidium bromide to 50S ribosome and core particles. The binding was studied in 1 ml containing 0.1 mM ethidium bromide, 10 mM Tris, pH 7.5, 100 mM KCl and indicated amount of magnesium acetate, by measuring the decrease in absorbancy of ethidium bromide at 490 nm. (-●-), 50S ribosome; (-O-), core particle.

region(s) of the 50S ribosome is the probable site of attack. All four copies of L7/L12 are released by the enzyme action, therefore, one of the sites is most probably the region where L7/L12 are located. There is a good deal of controversy in the literature regarding the site of location of L7/L12 proteins in the 50S ribosome (12-14). Wherever the location of these proteins, the present studies suggest that on removal of L7/L12 proteins double-stranded regions are created resulting in resistance to the hydrolytic action of RNase I as well as enhanced binding of ethidium bromide. Reconstitution experiments (Fig. 1) also support this hypothesis. The functional role of this region (15) suggests its capability of undergoing a change in the conformation under certain conditions; this is also reflected in the observations reported in this communication.

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