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Ribosomal protein S1 induces a conformational change of the 30S ribosomal subunit

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Abstract A comparative study of the 30S ribosomal subunit in the complex with protein S1 and the subunit depleted of this protein has been carried out by the hot tritium bombardment method. Differences in exposure of some ribosomal proteins within the 30S subunit depleted of S1 and within the 30S–S1 complex were found. It was concluded that protein S1 binds in the region of the neck of the 30S ribosomal subunit inducing a conformational change of its structure.

Keywords: Ribosome; Ribosomal protein S1; Protein topography; Tritium labeling

1. Introduction

Protein S1 is the largest ribosomal protein of eubacterial ribosomes. The protein is required for the efficient *in vivo* translation of natural mRNAs in *Escherichia coli* cells and extracts. It is reputed that protein S1 participates in retention of mRNA on the ribosome during initiation and, maybe, elongation of translation [1,2]. The protein binds to the 30S ribosomal subunit relatively weakly. The details of interaction of the 30S subunit with protein S1 are still poorly understood. Even the crystallographic structure of the 30S ribosomal subunit does not yield information about protein S1 because of the absence of the protein in the preparations crystallized [3,4]. Therefore, other experimental approach would be useful in studying the protein S1–30S subunit interaction.

Here we applied the hot tritium bombardment technique which allows to determine exposed proteins and, in some cases, to detect conformational rearrangements on the surface of macromolecular complexes [5–7]. We have carried out a comparative investigation of the complex of the 30S ribosomal subunit with protein S1 and the 30S ribosomal subunit completely depleted of this protein.

As a source of protein S1 we used the extreme thermophilic bacterium *Thermus thermophilus* [8]. Earlier it was shown that protein S1 from this organism is more compact and stable in solution as compared with its homologue from *E. coli* and, therefore, more attractive for structural investigations [9].

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The data presented here allow us to conclude that protein S1 is located near ribosomal proteins S7 and S11, probably, between the head and the platform (side bulge) of the 30S ribosomal subunit which is in good agreement with the earlier results of immunoelectron and cryoelectron microscopy methods [10,11]. The most interesting conclusion following from our experiments is that the binding of protein S1 induces a conformational change of the 30S ribosomal subunit structure.

2. Materials and methods

2.1. Preparation of 30S ribosomal subunits and total ribosomal protein

E. coli MRE-600 ribosomes were prepared as described in [6] and separated into subunits according to [12]. The total preparation of ribosomal proteins of the 30S ribosomal subunit was obtained using the standard procedure of acetic acid extraction [13] in the presence of 100 mM $MgCl_2$. Precipitation with 7.5 volumes of acetone was applied, and the protein precipitate was washed with 80% acetone for desalting the samples.

2.2. Preparation of 30S subunits depleted of protein S1

Removal of protein S1 from 30S ribosomal subunits was done according to [1]. S1–free 30S subunits were prepared by passing the ribosome solution through a poly(U)-Sepharose column equilibrated by a buffer containing 20 mM Tris–HCl, pH 7.6, 10 mM $MgCl_2$, 1 M NH_4Cl, and 2 mM 2-mercaptoethanol.

2.3. Preparation of Themus thermophilus protein S1

Recombinant ribosomal protein S1 of *T. thermophilus* was isolated from the *E. coli* overproducing strain BL21(DE3)pET21d-tthS1 and purified as described earlier [8].

2.4. Preparation of the complex 30S subunits with protein S1

To prevent non-specific binding of protein S1, a relatively high salt concentration was used in the incubation mixture. The complex formation was performed in buffer A (20 mM Tris–HCl, pH 7.6, 10 mM $MgCl_2$, 200 mM NH_4Cl, and 1 mM DTT). Protein S1 and 30S subunits were mixed at a molar ratio S1:30S = 2:1 and incubated at 37 °C for 10 min. To remove the unbound protein, the mixture was loaded onto 10% sucrose cushion and pelleted by centrifugation in a TLA 100.3 rotor of TLA-100 centrifuge (Beckman) at 100 000 rpm for 90 min. The amount of protein S1 in the complex was estimated by SDS–PAGE electrophoresis. It was shown that these conditions allow obtaining the S1–30S complex with stoichiometry close to 1:1 (data not shown).

2.5. Two-dimensional gel electrophoresis of the 30S ribosomal proteins

To separate individual ribosomal proteins, the two-dimensional electrophoresis method described in [7] with minor modifications was used. In the second dimension, a non-gradient separating gel was used (16.5 T, 6%C). Gels were fixed with 15% formaldehyde in 60% methanol and stained with Coomassie blue G-250 in 15% formaldehyde.

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2.6. Hot tritium bombardment procedure

Samples of the 30S subunit and its complex with S1 subjected to tritium bombardment contained 0.5 mg/ml of the subunit in 2 ml of buffer A. The samples were first frozen in liquid nitrogen drop by drop and the frozen drops were ground with liquid nitrogen in a cooled mortar. Then, the suspension of the sample powder in liquid nitrogen was poured into a siliconized reactor flask. The reactor flask was rotating on its axis in horizontal position until nitrogen has evaporated and particles of the sample powder stuck to the inner wall of the flask. Labeling conditions were the same as in [6]. The incorporation of tritium into ribosomal proteins was analyzed by two-dimensional gel electrophoresis. Gel pieces containing individual proteins were solubilized with hydrogen peroxide, and the radioactivity was measured in a liquid scintillation spectrometer [6].

3. Results and discussion

Separation of individual ribosomal proteins by two-dimensional gel electrophoresis after tritium bombardment is shown in Fig. 1. Protein spots stained were cut from the gel, and the radioactivity of each protein was determined in a scintillation counter. On the whole, five independent experiments were done for both (30S + S1) and (30S − S1) samples. The data for all of the experiments were averaged. The averaged values of tritium radioactivity are presented in Fig. 2. As it was shown earlier [5,6], some 30S ribosomal proteins did incorporate tritium to a considerable degree, some proteins did not. The data presented in this work also confirm the observation indicating that individual ribosomal proteins are differently exposed on the ribosomal surface. Here, in order to ensure sufficient reliability in the interpretation of the results, we will consider only well labelled proteins (with 5% or more of total protein radioactivity).

The association of protein S1 with the 30S ribosomal subunit results in a change of the exposure of some ribosomal proteins. Proteins S7, S11 and S21 become less labelled in the presence of protein S1. It means that protein S1 interacts with these proteins or, at least, is at an immediate proximity to them. Protein S7 is located on the head of the 30S ribosomal subunit while protein S11 on its platform (side bulge) [3]. The exact location of protein S21 is not known since there is no counterpart to S21 in *T. thermophilus* 30S subunit which crystal structure is available [3], while position of S21 in crystal structure of *E. coli* 70S ribosome is not indicated [4]. However, in the assembly map of the 30S ribosomal subunit the binding of S21 is mediated by S11 [14]. At the same time, hydroxyl radical footprinting experiments indicate that protein

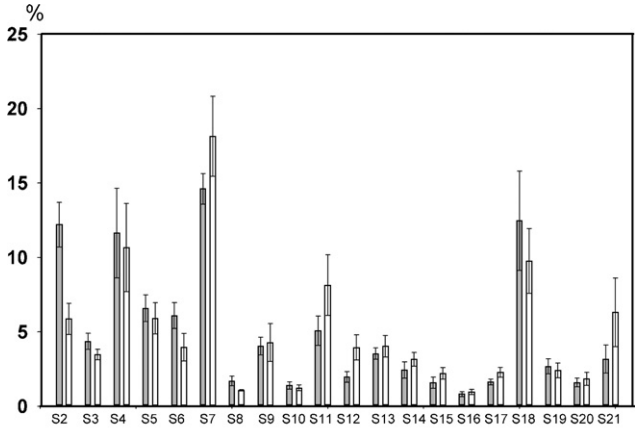

Fig. 2. Labeling of individual ribosomal proteins of the 30S subunit. Radioactivity of each protein is shown by bars in percents of the total radioactivity found in all the protein spots on the gel. Radioactivity of protein S1 was eliminated. Each bar represents results of averaging of five independent experiments. Gray bars, proteins labeled within complex 30S subunit with S1; white bars, within 30S subunit depleted of S1.

S21 contacts with central (platform) domain of 16S RNA [15]. Thus, our results suggest with high probability that protein S1 is located between the head and the platform, somewhere in the region of the neck of the 30S ribosomal subunit (Fig. 3).

On the contrary, proteins S2 and S6 become more exposed in the presence of protein S1. This observation cannot be explained by just an immediate proximity of proteins S1, S2 and S6. The only reasonable explanation of this experimental fact is that the binding of protein S1 causes a conformational change of the 30S ribosomal subunit resulting in increasing of the exposure of the two ribosomal proteins. It is possible that the exposure of protein S2 induced by protein S1 binding facilitates the interaction of the mRNA SD duplex with protein S2 upon initiation of translation [16].

We also observed small (in absolute terms) effect of S1 on protein S12. Protein S12 is located close to the "neck" between the body and the head of the 30S ribosomal subunit, at the subunits interface. In this case we also cannot explain a decrease of the exposure of S12 in the presence of S1 by an immediate proximity of these proteins. It is not excluded that this difference may also reflect the same conformational change

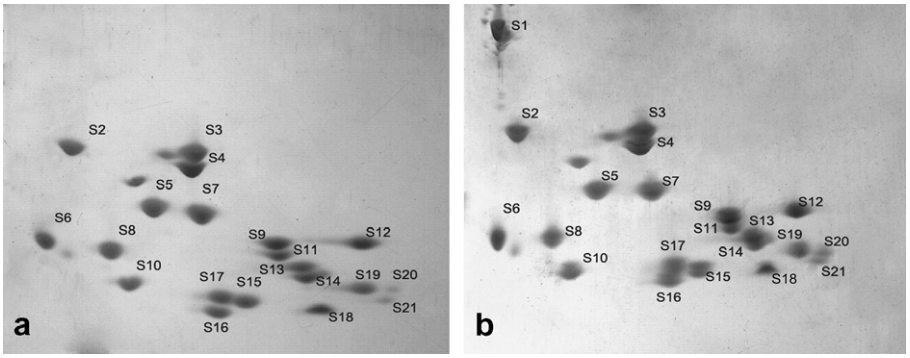

Fig. 1. Separation of individual ribosomal proteins by two-dimensional gel electrophoresis: photographs of Coomassie stained gels of proteins from 30S subunits depleted of protein S1 (a) and complex 30S with S1 (b).

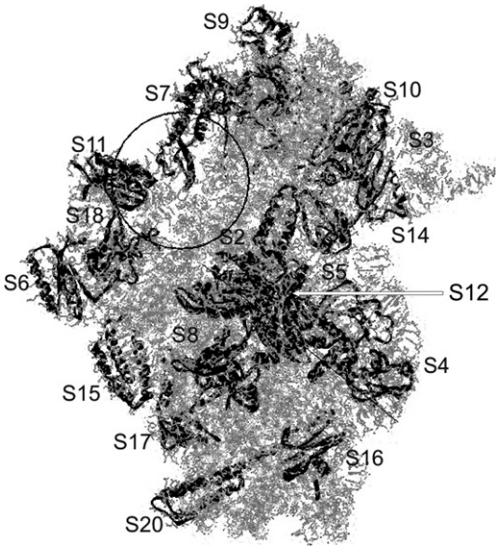

Fig. 3. Putative localization of protein S1 on the 30S ribosomal subunit. The subunit model is adapted from [18], PDB1N34. Position of protein S1 is shown by a cycle. Note that protein S12 is not visible from this view; the arrow shows its position on the opposite (interface) side of the subunit.

of the subunit, namely by a shift the head relative to the body towards the subunits interface.

Our conclusion on the localization of protein S1 is in good agreement with the results obtained previously by both immunoelectron microscopy and cryoelectron microscopy methods [10,11]. It should be noted, however, that the use of the hot tritium bombardment method provides revealing both protein localization and some conformational rearrangement in the 30S ribosomal subunit induced by protein S1. This may be a slight turning or tilting of the head of the subunit about its body. In any case, it should be admitted that the principal conformation of the 30S subunit in the complex with protein S1 does differ from that of the 30S subunit depleted of the protein, and the change in the exposure of some ribosomal proteins may imply that the structure of the subunit acquires a more open or closed state [17,18].

The data presented here confirm that the ribosome is a dynamic mobile macromolecular complex changing its conformation during the ribosomal working cycle. We believe that the conformational changes of the 30S ribosomal subunit induced by protein S1 reflect one of the steps of this combined process.

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