IDENTIFICATION OF RIBOSOMAL PROTEINS WITH AFFINITY FOR tRNA MOLECULE BY AFFINITY CHROMATOGRAPHY ON tRNA–SEPHAROSE

Munehiko YUKIOKA and Koichiro OMORI

Department of Biochemistry, Osaka City University Medical School, Abeno-ku, Osaka 545, Japan

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1. Introduction

Interaction of tRNA molecules with ribosomes is a fundamental process in the peptide bond formation on the ribosomal machinery, which is composed of few RNA molecules and numerous different proteins.

Several approaches in vitro have been done on the topography of ribosomal binding sites for tRNA [1–4].

(i) Specific chemical or immunological blocking.

(ii) Reconstitution experiments.

(iii) Deletion or supplementation of specific proteins.

(iv) Protection by tRNA from some inactivating process.

(v) Affinity labeling technique.

However, none of these approaches, except for a few examples, gave any information about which of the ribosomal components are directly involved in the binding of tRNA to ribosomes. These difficulties might be overcome if the direct interactions of the ribosomal components with tRNA molecules could be studied.

In this report, using an affinity chromatographic technique, we studied the ribosomal proteins with affinity for tRNA molecules. Proteins S5, S9, L1 and L17 were found to be possessed of high affinity for tRNA.

2. Materials and methods

2.1. *Ribosomes and ribosomal proteins*

Ribosomes and their subunits were obtained from *Escherichia coli* Q_{13} as described previously [5]. The total protein fraction was prepared by extracting the ribosomes (70 S) or their subunits (50 S or 30 S) with 4 M urea–2 M LiCl as described by Fahnestock et al. [6]. The ribosomal protein fractions (250 A_{260} equivalent*/ml) were dialyzed against 1000 vol. 30 mM Tris–HCl, pH 7.4, 20 mM magnesium acetate, 1 M KCl, 6 mM 2-mercaptoethanol, with three changes, each for 1 h, at 4°C.

2.2. *tRNA-Sepharose*

Transfer-RNA was extracted from *E. coli* Q_{13}, and was purified by DEAE-cellulose column chromatography as described by Ehrenstein [7].

A tRNA–Sepharose, in which the tRNA molecules were covalently coupled via their 3′-terminus to a hydrazinyl–Sepharose 4B, was synthesized according to the procedure given by Remy et al. [8]. The gels used in the present experiments, were charged with 2.0 mg tRNA/ml gel. The tRNA–Sepharose was suspended in equal vol. 30 mM Tris–HCl, pH 7.4, 20 mM magnesium acetate. It could be stored at 4°C for several months without any detectable decrease in its binding capacity for the ribosomal proteins.

With RNAase treatment of the tRNA–Sepharose 0.5 ml of tRNA–Sepharose gel (containing 1.0 mg tRNA) was treated with 100 μg RNAase A (Worthington) or RNAase T_1 (Sankyo), at 37°C for 1 h.

2.3. *Affinity chromatography of ribosomal proteins on tRNA–Sepharose*

The typical reaction mixtures to study the binding of the ribosomal proteins with tRNA–Sepharose contained in 2.0 ml reaction mixture, 50 A_{260} equivalents of ribosomal proteins, 30 mM Tris–HCl, pH 7.4, 50 mM

* One A_{260} equivalent is the ribosomal proteins obtained from one A_{260} unit of 70 S ribosomes

magnesium acetate, 0.35 M KCl, 6 mM 2-mercaptoethanol and 0.5 ml of tRNA–Sepharose gel. The mixture was incubated at 37°C for 45 min with intermittent shaking to avoid settling of the Sepharose gel. After incubation, the following alternative techniques were used. All procedures were carried out at room temperature.

2.3.1. Batchwise technique

To measure quantitatively the affinity of the ribosomal proteins for tRNA–Sepharose in various conditions, namely ionic strength as demonstrated in figs 1 and 2, the reaction mixture was centrifuged at 2500 rev./min for 10 min. The pelleted gel was washed five times with 10 ml of the buffer of the same ionic composition used for the incubation. The ribosomal proteins bound to tRNA–Sepharose were eluted with 1.0 ml of 8 M urea–4 M LiCl. Protein concentrations were estimated by absorbance at 230 nm. One A_{230} unit of ribosomal protein extract contained 0.35 mg protein as determined by the Lowry reaction with bovine serum albumin as a standard.

2.3.2. Column technique

To characterize qualitatively the ribosomal proteins bound to the tRNA–Sepharose, the incubation mixture was poured onto a column with diameter of 0.9 cm. After washing the column with 100 ml of the buffer of the same ionic components used for the incubation, the ribosomal proteins retained on the tRNA–Sepharose were eluted with 3.0 ml of 8 M urea–4 M LiCl. The eluate was extensively dialyzed against 6% acetic acid at 4°C, and was lyophilized.

2.4. *Two-dimensional polyacrylamide gel electrophoresis*

Polyacrylamide gel electrophoresis of the ribosomal proteins was performed as originally described by Kaltschmidt and Wittmann [9], according to the modified procedure of Howard and Traut [10].

3. Results

3.1. *Binding of ribosomal proteins to tRNA–Sepharose*

The tRNA–Sepharose could bind effectively the ribosomal proteins of *E. coli,* as shown in figs 1 and 2. In these experiments, the effects of ionic environment on the binding of ribosomal proteins to the tRNA–Sepharose were studied using the batchwise technique as described in Materials and methods.

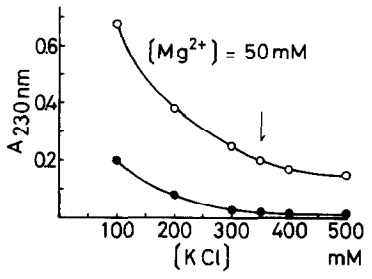

Fig.1. Effect of KCl concentrations on the binding of 70 S ribosomal protein fraction to tRNA–Sepharose (○——○) or to hydrazinyl–Sepharose (●——●). The binding was assayed as described in Materials and methods, except that the KCl concentrations were varied. The value without the ribosomal protein fraction was used as the blank, which was subtracted from each value. The arrow indicates the KCl-concentration (0.35 M) used for further experiments.

The binding of ribosomal proteins was markedly affected by the concentration of KCl (fig.1) At lower KCl concentration, higher binding was observed. But, at these low concentrations of KCl, the hydrazinyl–Sepharose devoid of the tRNA moiety also retained the ribosomal proteins. It might be that the higher binding of the ribosomal proteins at low KCl concentrations resulted from increased ionic interaction between protein and the chromatographic support. At more than 0.35 M of KCl, only slight retention of ribosomal proteins on the hydrazinyl–Sepharose was observed.

In this connection, it is interesting to note that

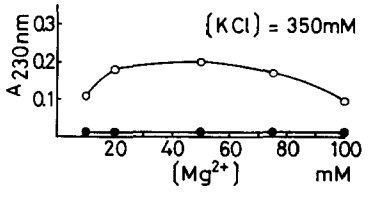

Fig.2. Effect of Mg^{2+}-concentrations on the binding of 70 S ribosomal protein fraction to tRNA–Sepharose (○——○) or to hydrazinyl–Sepharose (●——●). The procedures were the same as described in fig.1, except that Mg^{2+}-concentrations were varied.

0.25–0.35 M KCl is optimal for the specific binding of ribosomal proteins to tRNA [11] and that the KCl-dependence for the ribosomal subunit reconstruction exhibited a relatively sharp maximum around 0.30–0.35 M [3].

At 0.35 M of KCl, 0.5 ml of tRNA–Sepharose gel could bind about 70 μg of the ribosomal proteins out of 50 A_{260} equivalent of ribosomal proteins (approx. 1000 μg) corresponding to approximately one-fourteenth of the originally applied ribosomal proteins (70/1000 : 1/14). Since the number of different proteins in 70 S ribosomes is 54, then the tRNA–Sepharose binds about 4 different ribosomal proteins (54/14 : 4). This binding of ribosomal proteins to the tRNA–Sepharose seems to originate from a specific interaction between ribosomal protein and tRNA, because the RNase-treated tRNA–Sepharose did not exhibit any binding for the ribosomal protein (result not shown).

Mg^{2+}-concentration was optimal around 20–50 mM as evident in fig.2.

3.2. *Identification of ribosomal proteins specifically bind to tRNA–Sepharose*

The ribosomal proteins retained on tRNA–Sepharose at 0.35 M KCl were analyzed by two-dimensional gel electrophoresis as shown in fig.3. As expected from above mentioned results, four major spots were observed. These spots were identified as proteins S5, S9, L1 and L17, along with faint spots of proteins L18 and L22. These proteins, S5 and S9, or L1 and L17, were also identified on the tRNA–Sepharose chromatography of protein fractions from the ribosomal subunits, 30 S or 50 S, respectively.

The binding of tRNA–Sepharose with these proteins could be due solely to ionic interactions between basic groups of ribosomal proteins and acidic phosphate groups of tRNA. However, the binding is restricted to a small number of ribosomal proteins. Only S9 is a strongly basic protein ($pK > 12$), whereas other ribosomal proteins of strong basicity ($pH \geqslant 12$: S3, S7, S11, S12, S13, S15, S18, S19, S20, S21, L2, L15, L16, L19, L20, L27, L30, L33) [12] are devoid of the binding activity. These considerations advance a suggestion that the proteins S5, S9, L1 and L17 specifically interact with tRNA molecules in some way other than pure ionic-bond in nature. This notion was confirmed by the fact that the binding of these proteins was not affected in the presence of excess rRNA in the binding mixture (result not shown), except protein L1 which had been known to interact directly to 23 S RNA [11].

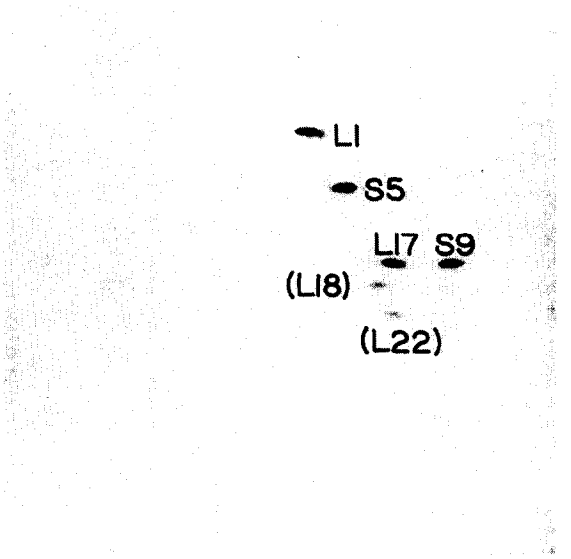

Fig.3. Two-dimensional electrophoresis of 70 S ribosomal proteins retained on tRNA–Sepharose column. The ribosomal proteins bound to tRNA–Sepharose at 0.35 M KCl were prepared using the column technique as described in Materials and methods.

4. Discussion

Present results clearly show that four ribosomal proteins, S5, S9, L1 and L17, do interact specifically with tRNA molecules, covalently coupled to a hydrazinyl–Sepharose 4B matrix via their 3′-terminus. This was demonstrated when the ribosomal proteins were not intergrated within the ribosomal structure, but free in solution. So, it is hard to correlate these ribosomal proteins with the actual ribosomal functions of tRNA binding in situ. However, it is interesting to evaluate the present findings in connection with the previously known informations about the function of these proteins in the ribosomal machinary, or about their topographical orientation in the ribosomal structure.

Concerning the 30 S ribosomal proteins, S5 and S9 are located at the 30 S – 50 S subunit interface [2, 13, 14]. Functionally, S5 is involved in the binding of fMet–$tRNA^{Met}$ to 30 S–mRNA complex; S9 participates in the EF-Tu-dependent binding of Phe–$tRNA^{Phe}$ to ribosomes [1,2]. Furthermore, these two 30 S proteins co-operatively participate in mediating the ribosome dependent GTPase-activity of EF-G [15].

Protein L1 is located at the subunit interface [2] and is involved in the association of ribosomal subunits [16]. It is also reported that protein L1 is required for the formation of the 70 S initiation complex [16].

These lines of evidences support the view that protein S5, S9 and L1 are directly involved in the binding of tRNA to ribosomes and that tRNA's bound to ribosomes are situated at or in the proximity of the 30 S – 50 S subunit interface.

As far as protein L17 is concerned, it is reported that this protein has an antibody binding-site on the convex side of an 'armchair-like structure' of the 50 S subunit and hence lies very far from the subunit interface [14]. On the other hand, this protein L17 was inferred to locate at the subunit interface, from the labeling experiments using lactoperoxidase or *N*-ethylmaleimide [17]. Considering this information together with the present findings, it might be that protein L17 constitutes a part of the 'seat' region of the 50 S subunit, positioning one of its heads to the exterior surface and the other head to the 30 S – 50 S subunit interface. The latter might be responsible for the binding of tRNA.

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