THE INTERACTION OF TRANSFER RIBONUCLEIC ACID WITH 30 S RIBOSOMAL SUBUNIT PROTEINS

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1. Introduction

That tRNA interaction with the 30 S ribosomal subunit in the presence of mRNA can be characterized by equilibrium constants of the order of 10^{-8} M and higher [1] is inexplicable in terms of codon–anticodon interaction alone. There must be some additional mechanism through which codon–anticodon binding can be supplemented by interaction with the ribosome. This may be achieved by RNA–RNA interactions or by tRNA–ribosomal proteins (s) interaction or both. Because universal sequences in the molecule of tRNA, TΨC in loop IV and CCA at its 3′-terminus, seem to be involved in the binding of tRNA to 50 S subunit [2,3], some RNA–RNA interactions are almost certainly involved.

Data obtained so far by affinity-labeled tRNAs (review [4]) can, in principle, give information only about physical proximity of certain proteins to tRNA. Here we report the direct binding of *Escherichia coli* 30 S ribosomal subunit proteins S4, S6, S7, S9, S12, S13, S18 and S20 to tRNA. These results demonstrate the possibility that ribosomal proteins can interact with tRNA.

2. Materials and methods

2.1. *Preparation of 30 S ribosomal subunits, ribosomal proteins and RNA*

Subunits, 30 S, of ribosomes from *E. coli* MRE 600 were prepared according to Hardy et al. [5] or purchased from IREA (Olaine, USSR). Proteins from 30 S subunits (TP 30) were prepared according to Mora et al. [6] and dialysed against an appropriate buffer with various concentrations of potassium chloride (0.1–0.45 M) in 10 mM Tris–HCl, pH 7.5, 20 mM $MgCl_2$ and 6 mM 2-mercaptoethanol. Proteins were identified by urea–urea [8] and urea–SDS [9] two-dimensional gel electrophoresis. Protein concentrations were measured by the method of Hartree [10] using lysozyme as a standard.

Transfer ribonucleic acid from *E. coli* MRE 600 (Boehringer Mannheim) was purified by Sephadex G-75 gel filtration. 5 S RNA and tRNA from rat liver were isolated as described earlier [7].

2.2. *Preparation of Sepharose–RNA gels and affinity chromatography*

Adipic acid dihydrazide was prepared according to the procedure of Lamed et al. [11]. Epoxy-activated Sepharose 6B (Pharmacia, Uppsala), washed with distilled water, was suspended in 1 vol. saturated adipic acid dihydrazide solution in 0.1 M sodium carbonate buffer, pH 9.5 and the reaction was allowed to proceed 16 h at room temperature on an end-over-end mixer. Epoxy-activated hydrazide Sepharose 6B was washed throughly with water and 0.2 M NaCl to remove non-bound dihydrazide and finally with 0.1 M sodium acetate, pH 5.8.

Ribonucleic acid, oxidized with sodium periodate [12] was added to the gel in 0.1 M sodium acetate, pH 5.8. The coupling reaction was allowed to proceed for 24 h at 6°C, whereafter the gel was washed with buffer and 2 M sodium chloride. Routinely,

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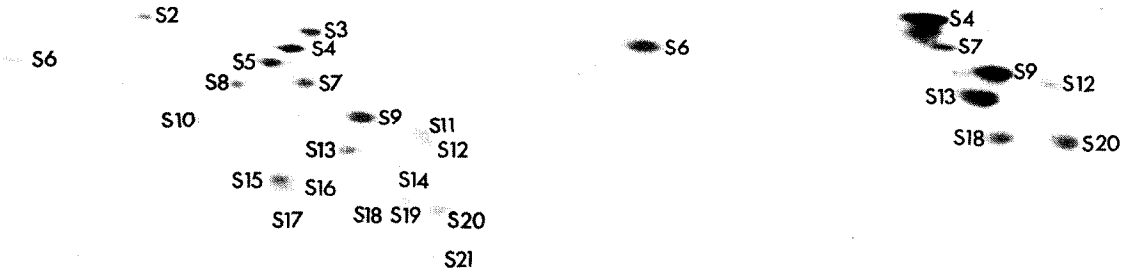

Fig.1. Identification of the 30 S ribosomal subunit proteins bound to the immobilized tRNA. Buffer: 150 mM KCl, 20 mM $MgCl_2$, 10 mM Tris–HCl, pH 7.5 and 6 mM 2-mercaptoethanol. 1.48 mg 30 S subunit proteins in vol. 6 ml were passed through a 0.35 ml Sepharose column containing 1.3 mg rat liver tRNA. 0.48 mg protein was retarded in the column and was washed out with 1 M KCl in 10 mM Tris–HCl buffer, pH 7.5, containing 5 mM EDTA·Na_2, 6 mM 2-mercaptoethanol. (A) 0.28 mg 30 S subunit proteins and (B) 0.16 mg tRNA-bound proteins were analysed by a two-dimensional polyacrylamide gel electrophoresis system as described by Howard and Traut [8].

10–15 mg oxidized tRNA was linked per one gram dry gel.

tRNA–Sepharose columns of various sizes were prepared and affinity chromatography of 30 S subunit proteins was performed as described [13].

3. Results and discussion

Proteins from the smaller ribosomal subunits of *E. coli* ribosomes able to bind to tRNA immobilized via its 3′-end to adipic acid dihydrazide epoxy-activated Sepharose 6B are S4, S6, S7, S9, S12, S13, S18 and S20 (fig.1). tRNAs both from *E. coli* and rat liver gave qualitatively identical results. Two types of control experiments were performed. Firstly, Sepharose columns were prepared without tRNA, and these did not bind proteins. Secondly, to rule out that RNA, as such, works as an unspecific ion-exchanger, binding of *E. coli* ribosomal proteins to 5 S RNA from rat liver was studied. Again, essentially no binding of *E. coli* ribosomal proteins to rat liver 5 S RNA immobilized to Sepharose was observed (data not shown).

Dependence of the binding of proteins to tRNA upon KCl concentration was investigated at concentrations from 0.1–0.45 M in the presence of a fixed $MgCl_2$ concentration (20 mM) because there is a possibility that at low ionic strength RNA starts to bind proteins in an unspecific manner (e.g. [14]). However, in our case we did not find any new protein at the lowest ionic strength studied, while the amount

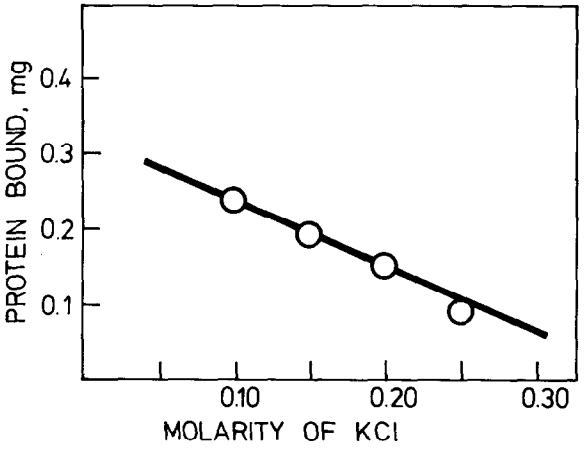

Fig.2. KCl concentration dependence of the binding of the 30 S subunit proteins to tRNA–Sepharose. 1.25 mg TP30 chromatographed through 0.2 ml affinity column, containing 0.4 mg tRNA.

of proteins bound depended significantly upon KCl concentration (fig.2).

In a similar study, recently reported ribosomal proteins S5 and S9 were found to bind to tRNA in addition to some 50 S subunit proteins [15].

Binding of the ribosomal protein S4 to tRNA was mentioned already earlier (Zimmermann, unpublished, cf. [14]) however, at very low ionic strength and therefore considered to be unspecific. Here we found S4 bound to immobilized tRNA even in 0.35 M KCl. One difference should be noted: in our experiments S4 binds together with a number of other proteins and therefore direct comparison of our results with Zimmermann's (where it was tested as a single species) is not justified.

We note that crosslinking data available on those proteins support the results presented in this paper. Thus, proteins S4–S6, S4–S9, S4–S12 [16], S4–S13 [17], S6–S18 [9,18], S7–S9 [19], S9–S13 [20], S7–S13, S12–S13, S12–S20 [21] have been crosslinked with various bifunctional reagents. Such data suggest that this group of proteins we find associated in tRNA–protein complexes could make up a tRNA-binding site. Moreover, proteins S7 and S13 [22] as well as S18 [23] have been found to crosslink to tRNA in situ while proteins S4, S12 and S18 to codon analogues [24,25].

There is additional evidence indicating that proteins S4, S5, S12 and S17 effect translational fidelity [26]. Two of these, S4 and S12, are members of the tRNA–protein complex described here, while S5 and S17 are known to give crosslinks with S4, S9, S13 and S4, S13, respectively [9,21,27]. Such correlations lend credence to the possibility, that the present results reflect a function of the eight 30 S proteins in tRNA binding by the 30 S subunit. We would be remiss, however, were we not careful to stress that experiments of the sort described here cannot establish the functions of the above-named proteins in tRNA binding. What they do establish is the possibility of such a function for the 30 S proteins: S4, S6, S7, S9, S12, S13, S18 and S20.

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