Are there proteins between the ribosomal subunits?

Hot tritium bombardment experiments

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The hot tritium bombardment technique [(1976) Dokl. Akad. Nauk SSSR 228, 1237–1238] was used for studying the surface localization of ribosomal proteins on *Escherichia coli* ribosomes. The degree of tritium labeling of proteins was considered as a measure of their exposure (surface localization). Proteins S1, S4, S7, S9 and/or S11, S12 and/or L20, S13, S18, S20, S21, L5, L6, L7/L12, L10, L11, L16, L17, L24, L26 and L27 were shown to be the most exposed on the ribosome surface. The sets of exposed ribosomal proteins on the surface of 70 S ribosomes, on the one hand, and the surfaces of 50 S and 30 S ribosomal subunits in the dissociated state, on the other, were compared. It was found that the dissociation of ribosomes into subunits did not result in exposure of additional ribosomal proteins. The conclusion was drawn that proteins are absent from the contacting surfaces of the ribosomal subunits.

Ribosomal protein *Ribosome surface* *Tritium bombardment* *Ribosome interface*

1. INTRODUCTION

Ribosomal RNA is known to be involved in the contact between two ribosomal subunits [1–6]. At the same time, a number of ribosomal proteins have been located on or near the ribosome interface [7–11]. The question remains open, however, as to which ribosomal proteins are positioned directly on the contacting surface of the ribosomal subunits and which are just on the interface periphery.

To elucidate this question the technique of hot tritium bombardment [12,13] has been used. The energy of hot tritium atoms is adjusted to a level which ensures the substitution of ^3H for ^1H in the CH groups of amino acid residues of proteins, without polypeptide backbone rupture [14]. The reaction results from the first collision of tritium atoms with the target. The straightforwardness of tritium atom flow and small depth of penetration of the reactive atoms (3–5 Å) allow labeling of only an exposed surface of proteins [15]. Evidently, the degree of tritium labeling of different ribosomal proteins within the ribosome (in situ) must be proportional to the accessibility of the proteins to the tritium atom flow, i.e. to the degree of exposure of the proteins on the ribosome surface.

The ribosomal proteins situated on the contacting surfaces of the ribosomal subunits are expected to be shielded in the 70 S ribosome and exposed after ribosome dissociation into 30 S and 50 S subunits. Correspondingly, these proteins should be labeled by hot tritium bombardment only in the dissociated ribosomes. It is found here that the dissociation of 70 S ribosomes into 30 S and 50 S subunits does not result in the exposure of any additional proteins as compared with the exposed proteins in the original 70 S ribosome. The conclusion is suggested that there are no proteins between ribosomal subunits.

We dedicate this paper to Professor S. Prakash Datta, the first Managing Editor of FEBS Letters, in recognition of his efforts and outstanding contribution to the international cooperation of scientists

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2. MATERIALS AND METHODS

Ribosomes were isolated from *Escherichia coli* MRE-600 using the procedure of Staehelin et al. [16] with minor modifications. The ribosomes were suspended in a standard buffer containing 20 mM Tris-HCl, pH 7.4, 100 mM NH_4Cl, 1 mM dithiothreitol, 0.1 mM Na_2EDTA and varying concentrations of $MgCl_2$. In all experiments the concentration of ribosomes was adjusted to 1 mg/ml.

The Mg^{2+} dependence of ribosome dissociation was determined by light scattering at 400 nm in an Aminco spectrofluorimeter using the standard buffer with Mg^{2+} from 0.1 to 10 mM at 2°C. The physical state of the ribosomes was also checked by analytical sedimentation in a Spinco E ultracentrifuge. It was found that in the range 4–10 mM Mg^{2+} the ribosomes were represented by 70 S particles, while at 1 mM Mg^{2+} and below only dissociated particles existed (fig.1). The point of half-dissociation corresponded to about 2 mM Mg^{2+} (at the given ionic strength, temperature and ribosome concentration).

Tritium bombardment experiments with ribosomes and ribosomal subunits were performed using the standard buffer containing 10 and 1 mM $MgCl_2$, respectively. The principal scheme of the tritium bombardment device is shown in fig.2. Preliminarily, a suspension of ribosomal particles was frozen and ground in liquid nitrogen. The

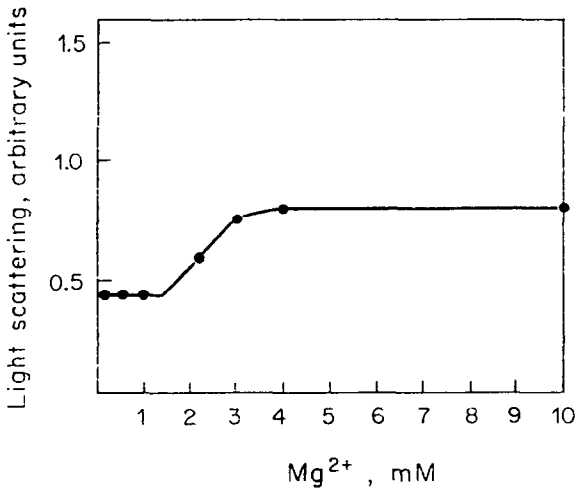

Fig.1. Mg^{2+} dependence of ribosome dissociation as measured by light scattering at 400 nm. Ribosome concentration, 1 mg/ml.

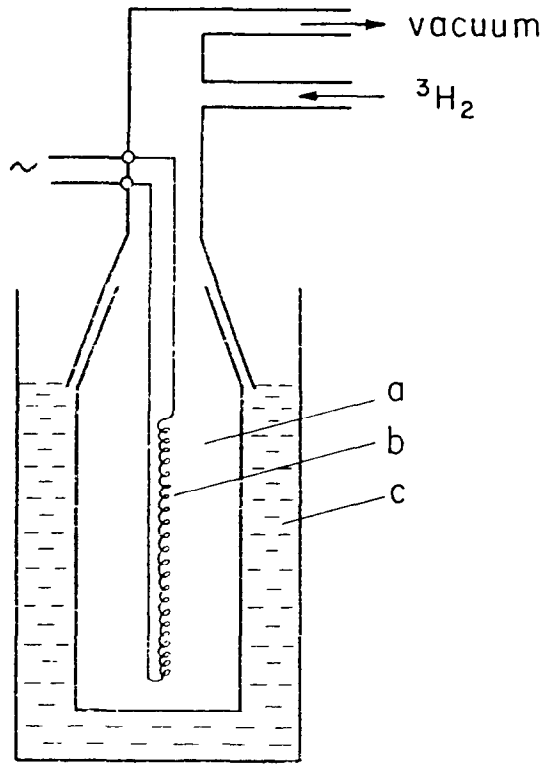

Fig.2. Scheme of the device for bombardment of ribosomes by hot tritium atoms. (a) Reactor flask, (b) tungsten wire, (c) thermostatting jacket with liquid nitrogen. Dissociation of tritium gas into tritium atoms occurs on the tungsten wire heated to 1000 K.

frozen powder was deposited on the inner wall of the glass reactor (fig.2). The reactor was thermostatted at liquid nitrogen temperature. A vacuum, with a residual pressure of 10^{-4} Torr, was produced in the reactor, and then tritium gas ($^3H_2$) was injected to adjust the pressure to 10^{-2} Torr (this tritium gas pressure ensured the straightforwardness of the tritium atom flow from the heated wire to the target [12]). Tritium atoms (^3H) were generated in the reactor by heating the tungsten wire to 1000 K. The distance from the wire to the target (frozen ribosome suspension) was 35 mm and the bombardment time 3 min. As a result, the ribosomal particles were labeled with unexchangeable tritium, so that the specific activity of ribosomal protein was (6–30) × 10^7 dpm/mg protein. The labeled ribosomal particles retained their physical integrity and intactness of the covalent structures of their RNA and proteins [13].

As controls, the unfolded ribosomal subunits

[18] in the standard buffer without Mg^{2+} ($s^{o}_{20,w}$ = 17 and 25 S), as well as the extracted total ribosomal protein [17] under denaturing conditions (5% acetic acid), were also subjected to similar hot tritium bombardment.

For analysis, the ^{3}H-labeled ribosomal protein was extracted from ribosomal particles with 67% acetic acid and precipitated with acetone [17]. The acetone pellet was dissolved in the electrophoresis buffer. The proteins were separated by two-dimensional gel electrophoresis according to Traut et al. [19] with small modifications. The degree of tritium labeling of individual ribosomal proteins was measured by fluorographic analysis of the electrophoretic slabs [20].

3. RESULTS

Fig.3a represents a control fluorogram of the electrophoretic slab of the total ribosomal protein labeled by tritium bombardment under denaturing conditions. It is seen that all the ribosomal proteins are labeled. Most of the ribosomal proteins are labeled more or less proportionally to their molecular masses.

In fig.3b a control fluorogram of the electrophoretic slab is given where the ribosomal proteins in the unfolded ribosomal particles have been labeled by tritium bombardment. Again, all proteins are labeled. In this case, however, the label distribution is not always proportional to the molecular masses of the proteins. It is possible that different proteins can be shielded by other proteins and by RNA to varying degrees in the unfolded ribosomal ribonucleoproteins.

Fig.4 demonstrates fluorograms of the two-dimensional electrophoretic slabs of ribosomal proteins which have been labeled by tritium bombardment of ribosomes in the standard buffer containing either 10 mM Mg^{2+} (a, 70 S ribosomes) or 1 mM Mg^{2+} (b, 30 S and 50 S ribosomal subunits). First of all, only a certain set of the ribosomal proteins is found to be labeled to a significant extent in the ribosomes and their subunits, namely S1, S4, S7, S9 and/or S11, S12 and/or L20, S13, S18, S20, S21, L5, L6, L7/L12, L10, L11, L16, L17, L24, L26 and L27; they comprise no more than half of the 30 S proteins and only about one-third of the 50 S proteins. It is likely that these proteins are the most exposed on the ribosome surface. The rest of the proteins are shown to be labeled either to an essentially lesser degree or virtually unlabeled.

It should be mentioned that the extent of labeling of spots S7, S13, S12/L20 and L5 varied in different experiments (with different ribosome samples) though it was always high enough to distinguish them from the weakly labeled proteins.

Fig.3. Fluorograms of two-dimensional gel electrophoretic slabs with ribosomal proteins labeled by hot tritium bombardment: control experiments. (a) Totally extracted ribosomal protein under denaturing conditions (5% acetic acid); (b) unfolded ribosomal subunits (17 S and 25 S) containing intact RNA and a complete set of proteins.

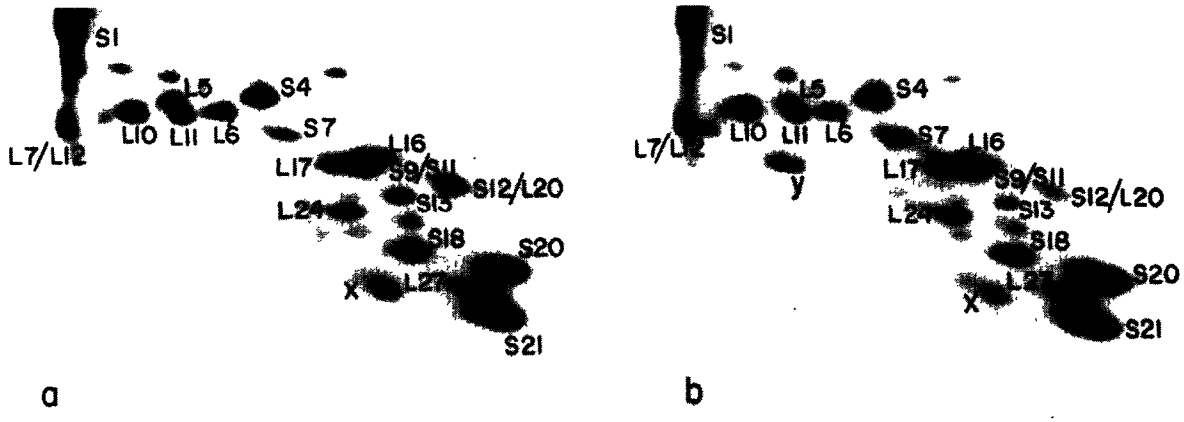

Fig.4. Fluorograms of two-dimensional gel electrophoretic slabs with ribosomal proteins labeled by hot tritium bombardment: assay experiments. (a) 70 S ribosomes at 10 mM $MgCl_2$; (b) mixture of 30 S and 50 S ribosomal subunits in 1 mM $MgCl_2$.

The other exposed proteins were labeled very reproducibly.

The second result following from tritium bombardment of the 70 S ribosomes (fig.4a) and their 30 S and 50 S subunits (fig.4b) is that the dissociation of ribosomes into subunits does not lead to exposure of additional ribosomal proteins. This strongly suggests that no ribosomal proteins are buried between the ribosomal subunits (between their RNAs). It should be mentioned, however, that some samples of dissociated ribosomes display an unknown labeled spot Y (see fig.4b) which is not identified as a ribosomal protein.

4. DISCUSSION

Immunoelectron microscopy studies demonstrated that all (or almost all) ribosomal proteins have antigenic determinants on the surface of ribosomal particles [21–24]. Consistently, the electron density distribution in ribosomal particles [25,26] and the neutron scattering inhomogeneity of ribosomes [26–28] indicated a predominantly peripheral localization of ribosomal proteins and a central position of the RNA in ribosomal subunits. Those results, however, did not yield quantitative information on the degree of exposure of different individual ribosomal proteins on the particle surface. The present results show that a number of ribosomal proteins (half of the 30 S proteins and one-third of the 50 S proteins) are exposed on the ribosome surface to a significantly greater degree than others.

Results principally consistent with ours were obtained by using the technique of enzymic iodination of ribosomes [29]. It was found that only a part of the ribosomal proteins in the ribosomes was accessible for enzymic iodination, namely S3, S7, S9, S10, S18, L2, L5, L6, L10 and L11. The difference between the set of iodinated proteins and that of the tritiated proteins on the ribosome surface is not surprising because the former is determined by the number of exposed tyrosine residues in the proteins whereas the latter reflects exposure of any amino acid residues.

The main result of the hot tritium bombardment experiments seems to be that the dissociation of 70 S ribosomes into the constituent 30 S and 50 S subunits does not lead to the appearance of additional exposed proteins (fig.4). This suggests that the contacting surfaces of the ribosomal subunits are organized entirely by ribosomal RNA. The involvement of ribosomal RNA in the ribosomal interface has been demonstrated in experiments on chemical and enzymic modifications of 16 S and 23 S ribosomal RNAs in 70 S ribosomes and in 30 S and 50 S ribosomal subunits [1–6]. Besides, the ability of isolated ribosomal 16 S and 23 S RNA to interact directly with each other has been reported [30–32]. Finally, electron microscopy studies of ribosomes under contrast variation conditions have revealed a united RNA core in the whole ribosome, with no visible protein material between the RNAs of the two ribosomal subunits [33].

On the other hand, many ribosomal proteins

have been localized by chemical cross-linking techniques on or near the ribosomal interface [7–11]. Taking into account the direct results obtained here, as well as the observations cited above, these proteins should be attributed rather to the periphery of the interface. The same conclusion can be made from analysis of immunoelectron microscopy results [21–24] taking into consideration the most recent morphological models of the ribosome [34–36]: most of the ribosomal proteins seem to be positioned in the vicinity of the interface periphery and no proteins can be definitely localized between the ribosomal subunit cores.

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