STRIPPING OF RIBOSOMAL PROTEINS FROM *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

A number of investigators, employing different techniques, have shown that high concentrations of salt cause the detachment of roughly half of the protein of *E. coli* ribosomes [1–4]. The detached proteins represent different molecular species than those remaining bound to the ribosomal RNA [5–9]. These findings raise the question of whether the two groups of proteins are bound differently in the intact ribosome or whether the selective detachment may be due to the structure of the ribosome.

We have therefore been interested in the question of whether the configuration of the ribosome is a significant factor in determining how much ribosomal protein can be removed by salt. It is known that magnesium plays an important role in determining the shape of the ribosome [10]. Magnesium ions can be removed with a sequestering agent such as EDTA [11], or displaced by monovalent cations when the latter are in large excess [12,13]. Such magnesium-depleted ribosomes maintain a compact form if they are kept in 0.5 M NH_4Cl or if magnesium is restored to them. If exposed to low ionic strength in the absence of magnesium, however, they undergo a drastic change in configuration, marked by a drop in sedimentation constant from 30S and 50S to about 10S or less. Protein does not become detached during this unfolding process [11,14].

Accordingly, we have begun a study of the effect of magnesium depletion and the concomitant shape changes on the detachment of ribosomal proteins by salt. The results presented below show that the degree of protein stripping is a function of both ionic strength and magnesium concentration, and that once magnesium has been removed, large amounts of protein are detached by relatively low concentrations of salt. The results also suggest quite strongly that the configuration of the ribosome influences the amount of protein detached under given conditions.

2. Methods

Protein was determined with the Folin reagent according to Lowry et al. [15] with crystalline bovine serum albumin (Armour) as standard. RNA was determined by measuring the optical density of total alkaline hydrolysates. Samples were incubated in 0.4 M NaOH at 37° for 24–48 hours and neutralized with more than two volumes of 1M sodium phosphate buffer of pH 7.0. RNA concentration was computed from the difference in optical density at 260 mμ and 290 mμ with a factor calculated for *E. coli* ribosomal RNA.

3. Experimental

3.1. *Preparation of ribosomes*

Ribosomes were prepared from *E. coli* MRE-600, a ribonuclease I-deficient strain [16], essentially according to Tissieres et al. [17], in 10 mM magnesium acetate – 1 mM Tris-HCl, pH 7.4. For further purification they were sedimented, dissolved in cold 0.5 M NH_4Cl – 1 mM Tris-HCl, pH 7.4, left for 15 hours at 0–4°, and resedimented. This treatment was repeated twice, after which the ribosomes were dissolved in 10 mM magnesium acetate – 1 mM Tris-HCl, pH 7.4, and dialyzed 48 hours against the same medium. Their composition was 60% RNA and 40% protein.

3.2. *Stripping of proteins*

The experiments were carried out at 0–4°. When ribosomes were to be depleted of magnesium, this was accomplished by one of two pretreatments: (a) *1 mM EDTA*, dialysis for 24 hours against 1 mM EDTA – 1 mM Tris-HCl, pH 7.4; ribosome concentration 4 mg/ml. (b) *0.5 M NH_4Cl*, dialysis for 24 hours against 0.5 M NH_4Cl – 1 mM Tris-HCl, pH 7.4, and for 48 hours against deionized water; ribosome concentration 10 mg/ml. Dialysis fluids were changed several times. Samples of (a) and (b) taken at this stage showed a single component sedimenting sharply at 5–12S, indicating an opening up of the ribosomal structure.

In order to remove protein, both pretreated and untreated ribosomes were brought to the desired final salt and magnesium concentrations by dilution with a concentrated solution of salt. 10 mM Tris-HCl, pH 7.4, was present in all cases. After at least 5 hours, the solutions were centrifuged at 50,000 RPM in the no. 50 rotor of a Spinco Model L preparative ultracentrifuge for 5 hours when 10 ml centrifuge tubes were used or 2.5 hours with 2 ml tubes. The pellets were dissolved in water and analyzed for RNA and protein.

4. Results

The results of representative experiments are shown in tables 1 and 2. Table 1 shows that the amount of protein detached from ribosomes by salt is a function of the concentration of both salt and magnesium. At a fixed NaCl concentration, the stripping of protein rose as magnesium concentration decreased. In the absence of magnesium, stripping increased with salt concentration, reaching 90% or more in 2 M LiCl. (This latter reagent is particularly convenient, since the RNA precipitates after 1 hour in ice and can be collected by low speed centrifugation.) Table 2 compares the effectiveness of NaCl and NH_4Cl in detaching protein from ribosomes depleted of magnesium by different pretreatments. Under the conditions employed, NH_4Cl was somewhat less effective than NaCl at 0.5 M, but was equally effective at 1M. The amount of protein detached by 0.5 M salt is as high as, or higher than, that detached by 2 M LiCl or 5M CsCl in the presence of magnesium.

Table 1
Detachment of protein from *E. coli* ribosomes as a function of salt and magnesium concentration.

Expt.	Mg^{++} conc. (mM)	Pretreatment	Final salt conc. *	Composition of particles			% of original protein detached
				RNA (%)	Prot. (%)	Prot./RNA	
	Original ribosomes			60	40	0.67	0
1.	10.0	None	0.5 M NaCl	64	36	0.56	16
2.	2.0	None	0.5 M NaCl	69	31	0.45	33
3.	1.0	None	0.5 M NaCl	69	31	0.45	33
4.	0.1	None	0.5 M NaCl	73.5	26.5	0.36	46
5.	0	1 mM EDTA	0.5 M NaCl	80	20	0.25	63
6.	0	1 mM EDTA	1 M NaCl	89	11	0.12	82
7.	0	1 mM EDTA	2 M LiCl	94.5	5.5	0.058	91

* 10 mM Tris-HCl, pH 7.4, was present.

See *methods* for experimental details.

Table 2
Detachment of protein from *E. coli* ribosomes by salt: comparison of NaCl and NH_4Cl.

Expt.	Pretreatment *	Final salt conc. **	Composition of particles			% of original protein detached
			RNA (%)	Prot. (%)	Prot./RNA	
	Original ribosomes		60	40	0.67	0
1.	1 mM EDTA	0.5 M NaCl	80	20	0.25	63
2.	1 mM EDTA	0.5 M NH_4Cl	74	26	0.35	48
3.	0.5 M NH_4Cl	0.5 M NaCl	81	19	0.24	64
4.	0.5 M NH_4Cl	0.5 M NH_4Cl	76	24	0.32	52
5.	0.5 M NH_4Cl	1 M NaCl	88	12	0.14	79
6.	0.5 M NH_4Cl	1 M NH_4Cl	88	12	0.14	79

* Magnesium was absent.
** 10 mM Tris-HCl, pH 7.4, was present.

See *methods* for experimental details.

5. Discussion

The results presented here show that the amount of protein detached from ribosomes by salt is related directly to salt concentration and inversely to magnesium concentration. At magnesium depletion, where the ribosome structure opens up, considerable quantities of protein were detached at salt concentrations not higher than those employed to remove histones from DNA [18]. Under these conditions, 0.5 M salt stripped off 50–65% of the protein; 1 M salt, 80%; and 2 M LiCl, 90%. These results indicate that almost all of the ribosomal proteins are bound to the ribosomal RNA in a similar way, and suggest that electrostatic forces play an important role in the binding.

The structure of the ribosome appears to be a major factor in determining the amount of protein detached at a given salt concentration, with considerably more protein stripped off if the ribosomes have previously been unfolded. Such an open structure would readily equilibrate with the salt of the medium. In contrast, when the ribosome is in a compactly closed configuration, the equilibration between the outside salt solution and the salt inside the ribosome would be a much slower process. Since the stripping of proteins is a function of salt concentration, one must make sure that the internal salt concentration (i.e., inside the compact structure of the ribosome) is the same as that in the external medium. The normal compact structure of the ribosome is known to be stabilized by magnesium. It is also stabilized in the absence of magnesium by 0.5 M NH_4Cl [19], and ribosomes can be subjected to extended treatments with this reagent without the loss of protein (see section 2). Nevertheless once the magnesium-depleted ribosome has been allowed to unfold, NH_4Cl is about as effective as NaCl in detaching protein (table 2). Its slightly lower effectiveness may be related to the specific cation effects observed by Latt and Sober [20], who showed that equal concentrations of these cations have unequal effects on oligolysine – polynucleotide interactions.

It seems likely that the reason that protein stripping is considerably less than maximal under the usual conditions of particle formation (exposure of compact ribosomes to, e.g., 5 M CsCl or 2 M LiCl in the presence of magnesium) is that the prevailing magnesium and high ionic strength prevent the full unfolding of the ribosome. If this is so, the fact that certain species of ribosomal protein are preferentially detached under these conditions may say more about the location of these proteins in the ribosome than about the mode or strength of their attachment to the RNA. This is now under investigation.

In an earlier communication from this laboratory [21], it was shown that 3 M LiCl detaches essentially all of the protein from *E. coli* ribosomes, but only if 4 M urea is also present. Our present results indicate that the role of urea is to disrupt the compact ribosomal structure, and that this reagent may be replaced by any treatment that accomplishes the same end. It may be noted that 2 M LiCl alone suffices to remove all of the protein of reticulocyte [22] and of yeast ribosomes [23]. This suggests that these ribosomes are structurally different from *E. coli* ribosomes.

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