THE DETACHMENT OF RIBOSOMAL PROTEINS BY UREA: EVIDENCE FOR NON-ELECTROSTATIC RNA-PROTEIN INTERACTION IN THE RIBOSOME

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Received 19 July 1971

1. Introduction

Native ribosomes may be adsorbed on a DEAE-cellulose column and eluted with salt without distroying their structure or biological activity [1]. We have shown, however, that if the ribosomes are first unfolded by magnesium depletion at low ionic strength [2] and are then adsorbed on DEAE-cellulose, the ribosomal proteins can be detached without dissociating the RNA from the column [3]. Under these conditions, where the negative potential of the RNA is largely neutralized by the positively charged groups of the ion exchanger, proteins were detached at ionic strengths much lower than those required when the unfolded ribosomes are free in solution [4]. We also observed that 6 M urea detached a large part of the ribosomal protein at a salt concentration (1 mM sodium phosphate) which removed no protein in the absence of urea. These observations were made with 50 S ribosomal subunits [3].

The experiments reported in the present communication were performed to obtain information on two points: (a) the behavior of the 30 S ribosomal subunit under similar conditions and (b) the identity of the proteins in the two groups, those detached by urea and those not detached by urea alone. For this purpose unfolded 30 S subunits in 6 M urea – 1 mM sodium phosphate were applied to a DEAE-cellulose column equilibrated with the same solvent. Under these conditions the unfolded ribosomes were completely adsorbed, but 34% of the proteins came out with the adsorption solvent. The rest of the proteins were detached with a salt gradient in urea, leaving the RNA still adsorbed. The proteins in the two fractions were identified by two-dimensional polyacrylamide gel electrophoresis [5].

2. Experimental

30 S ribosomes were prepared from *E. coli* MRE 600 as described elsewhere [6]. They were unfolded at a concentration of 10 mg/ml by dialysis against 1 mM Tris-HCl (pH 7.4 – 1 mM EDTA (pH 7.4) for 48 hr in the cold, and were then dialyzed for 24 hr against 1 mM sodium phosphate (pH 6.5). Their sedimentation constant was 7.5 S (3.3 mg/ml in 1 mM sodium phosphate). Before application to the column they were brought to a concentration of about 1 mg/ml in 1 mM sodium phosphate – 6 M urea (apparent pH 6.5) and were kept in the cold for 13 hr.

Diethylaminoethyl-cellulose (DE 52, Whatman) was suspended in 0.5 M sodium phosphate (pH 6.5) and the fine particles were removed by decantation. The DEAE-cellulose was left overnight in 0.5 M sodium phosphate (pH 6.5), washed on a filter with water, equilibrated with 1 mM sodium phosphate (pH 6.5) until the pH and conductivity reached those of the medium, packed in a column 1.57 cm in diameter and 18 cm high, and equilibrated with 1 mM sodium phosphate – 6 M urea (apparent pH 6.5) by passing this solvent through the column overnight.

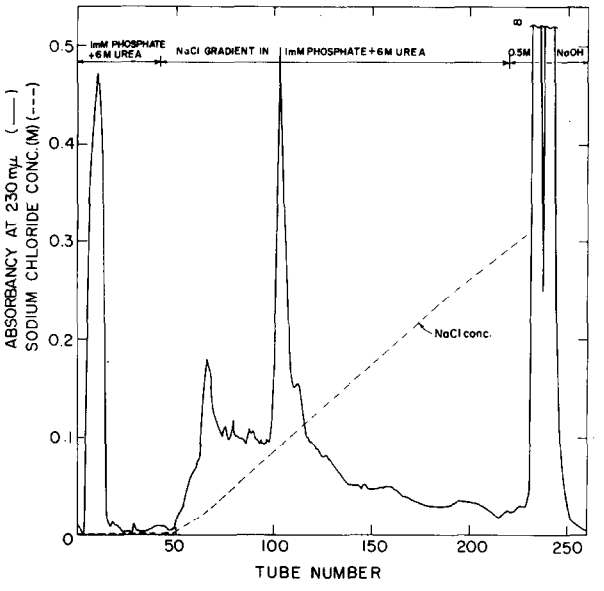

Fig. 1. Elution obtained with unfolded 30 S ribosomal subunits bound to DEAE-cellulose. See text for experimental details.

All urea solutions were decolorized with activated charcoal and deionized by passage through a mixed bed ion exchange resin (Amberlite MB-3, analytical grade). 6 M solutions had an absorbance of 0.05 at 230 mμ.

48 mg of unfolded ribosomes in 45 ml of 1 mM sodium phosphate – 6 M urea (apparent pH 6.5) were applied to a column containing 34 ml of DEAE-cellulose equilibrated with the same medium. The column was washed with 200 ml of the same medium followed by a linear salt gradient formed from 488 ml of the medium and 495 ml of 0.3 M NaCl in the medium. Finally, the column was washed with 0.5 M NaOH to detach the ribosomal RNA. 12 min fractions were collected at a flow rate of 26 ml/hr. Protein content was estimated in individual fractions from the absorbance at 230 mμ and in pooled fractions by the Folin reaction [7]. The latter assay was used to calculate recovery; it was performed with aliquots of 0.2 ml or less, since larger volumes interfered with the assay.

For gel electrophoresis, the protein fractions were dialyzed in the cold against HCl (pH 2), lyophilized, and dissolved in 6 M urea at a protein concentration

Table 1
30 S ribosomal proteins: identification and presence in elution fractions.

Wittmann code[a]	Nomura code[b]	Fraction	
		Urea	Urea-salt
S_1	P_1	–	+
S_2	P_2	–	+
S_3	P_3	+	–
S_4	P_{4a}	+	+
S_5	P_4	+	–
S_6	$P_{3b}+P_{3c}$	–	+
S_7	P_5	+	trace
S_8	P_{4b}	+	–
S_9	P_8	trace	+
S_{10}	P_6	+	–
S_{11}[c]	P_7[c]	–	+
S_{12}	P_{10}	+	–
S_{13}	P_{10a}	+	–
S_{14}	P_{11}	–	+
S_{15}	P_{10b}	+	+
S_{16}	P_9	+	–
S_{17}	P_9	+	–
S_{18}	P_{12}	–	trace
S_{19}	P_{13}	trace	+
S_{20}	P_{14}	–	+
S_{21}	P_{15}	–	trace

[a] Kaltschmidt and Wittmann [5].
[b] Essentially according to Mizushima and Nomura [9]. The key connecting the two codes was kindly supplied by Prof. H.G. Wittmann (personal communication).
[c] S_{11} is identified with P_7 on the basis of the fact that both have been identified with the same protein (No. 11) in the separation scheme of Kurland (Voynow and Kurland, [11]; Wittmann, personal communication).

of 300 μg/20 μl. The proteins were analyzed by two-dimensional gel electrophoresis according to Kaltschmidt and Wittmann [5] in an apparatus developed in this laboratory [8]. Electrophoresis was carried out in a cold room. The first dimension was run at pH 8.6 for 23 hr at 100 V in a gel 2 mm thick, 6 mm wide and 12 cm long containing 8% acrylamide and 0.26% bisacrylamide. The gel was then equilibrated

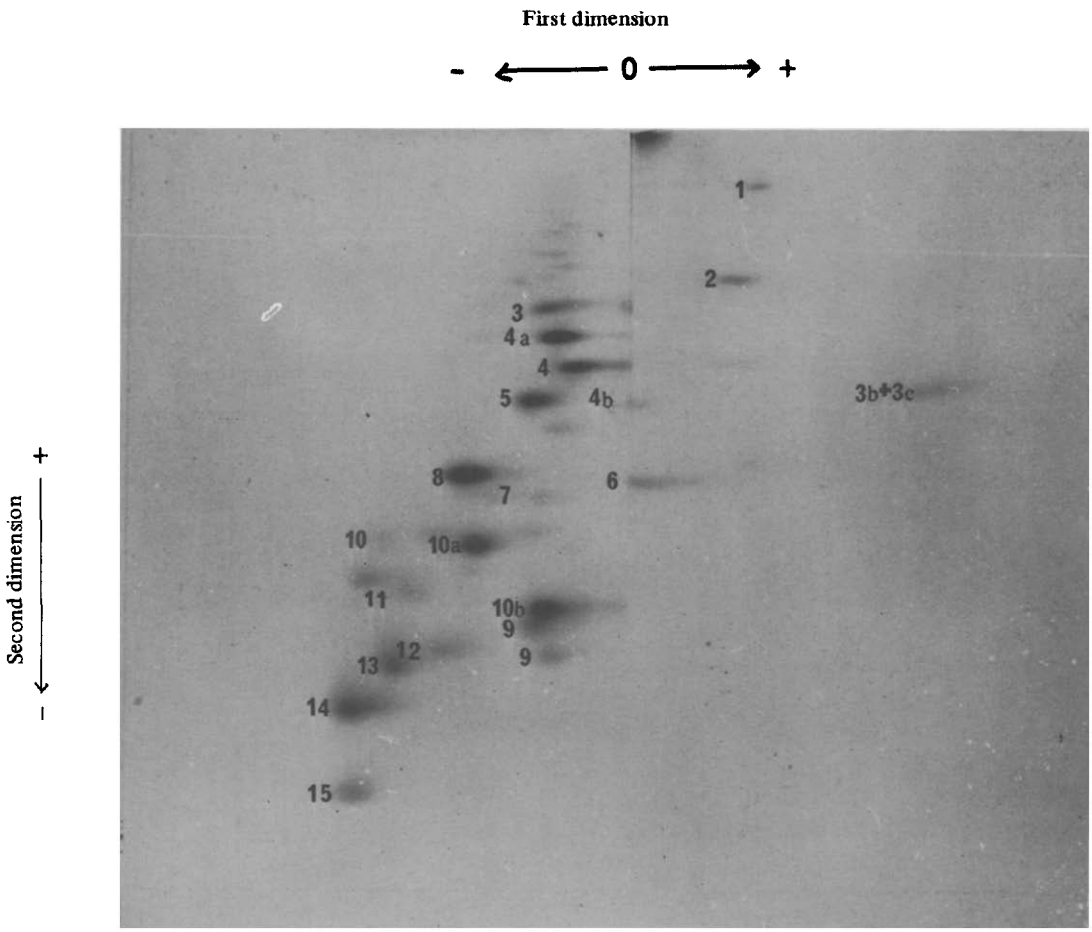

Fig. 2. Total 30 S ribosomal proteins: two-dimensional acrylamide gel electrophoresis pattern. See text for experimental details. The proteins were prepared according to Spitnik-Elson [12]. The proteins are numbered according to Nomura (see table 1).

for 2 hr with the buffer of the second dimension (pH 4.5) and incorporated into the second gel, which contained 18.6% acrylamide and 0.52% bisacrylamide, and was 2 mm thick and 14 cm square. The run was for 42 hr at 140 V. The buffers were those described by Kaltschmidt and Wittmann [5] except that urea was omitted from the buffers in the electrode vessels. Gels were stained with 0.2% amido black in 7.5% acetic acid for 1 hr and were destained by prolonged washing in 7.5% acetic acid until the background was fully decolorized.

Individual proteins were identified according to Kaltschmidt and Wittmann [5] whose analytical gel system was used. However, in the text and figures they are numbered by the code of Mizushima and Nomura [9] in order to facilitate comparison with their results. The correspondence between the two numbering systems is shown below in table 1.

3. Results and discussion

Fig. 1 shows the elution of proteins from unfolded 30 S ribosomal subunits adsorbed on DEAE-cellulose.

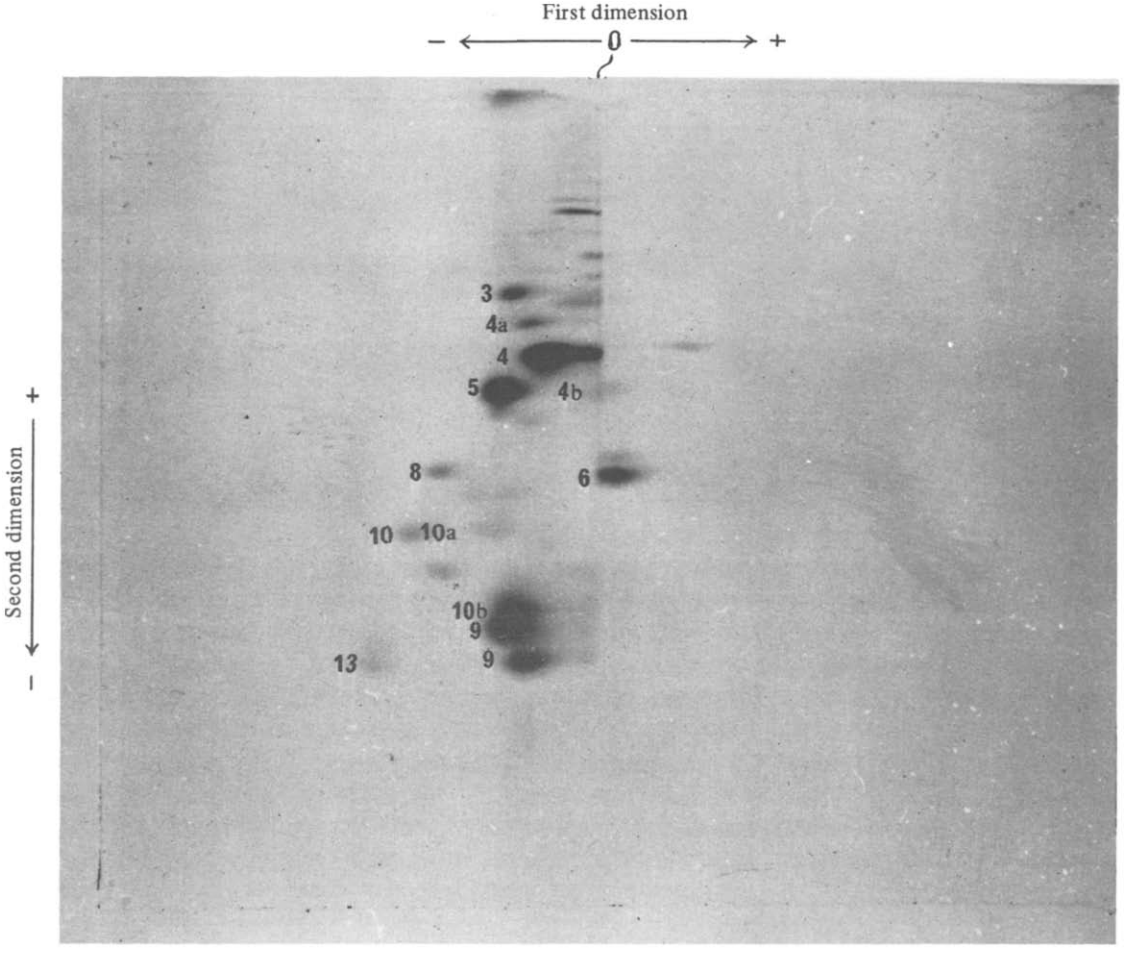

Fig. 3. Proteins of the urea fraction (tubes 8–13, fig. 1); two-dimensional gel pattern.

Recovery was quantitative. 34% of the protein was removed by 6 M urea in 1 mM sodium phosphate (urea fraction). 63.5% was then detached by a NaCl gradient in the same medium (urea-salt fraction); and, finally, about 2% was recovered together with the RNA in the NaOH wash.

Fig. 2, 3 and 4 show two-dimensional gel patterns of the total 30 S ribosomal proteins and the proteins of the urea and urea-salt fractions. The results are summarized in table 1. They show that all of the 30 S ribosomal proteins are present in the two fractions and, in nearly all cases, each can clearly be assigned to one of the two fractions.

It is of interest to examine our results in the light of the studies of Mizushima and Nomura [9] on the binding of individual proteins during reassembly of the 30 S subunit. Our urea-salt fraction consists of those proteins whose detachment from the column-bound RNA requires a higher salt concentration than those of the preceding urea fraction. In general the majority of the more basic proteins are present in the urea-salt fraction; however, three acidic proteins are also present. This fraction contains the independently binding protein P_{14} ; and also the proteins $P_{3b} + P_{3c}$, P_7, P_8, P_{12} and P_{15} whose binding is interrelated in the sequential assembly map of Mizushima and Nomura [9].

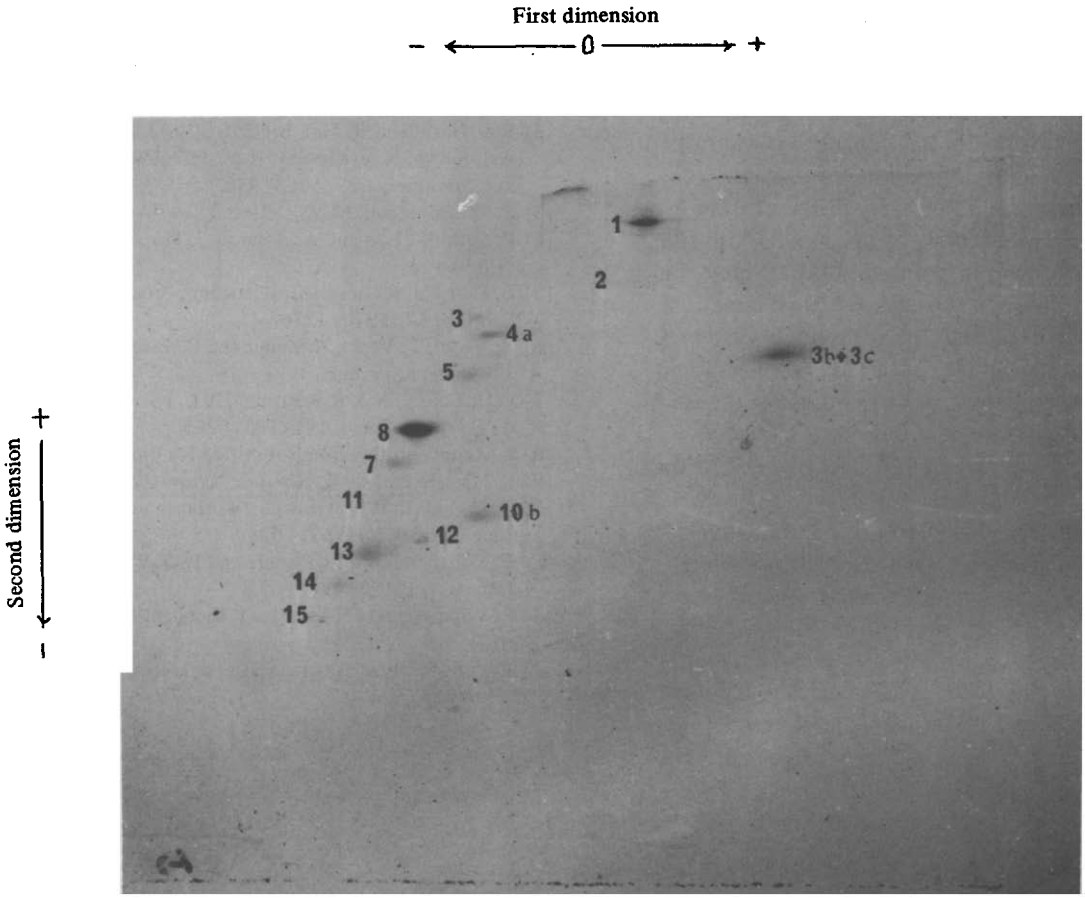

Fig. 4. Proteins of the urea-salt fraction (tubes 57–170, fig. 1); two-dimensional gel pattern.

The urea fraction consists of those proteins that are detached from the DEAE-cellulose-bound unfolded ribosomes by 6 M urea in the near absence of salt. It contains five of the six non-cooperatively binding proteins [9]: P_{4a} and P_{4b}, the two that bind quantitatively to the RNA in the absence of other proteins, and three other proteins that show some measure of direct and independent binding to the RNA, P_5, P_9 and P_{10a}. The urea fraction also contains groups of proteins which show a mutual binding relationship during ribosome assembly [9]. For example, P_4, whose binding is abolished completely in the absence of P_9, is present in the urea fraction together with P_9. The same is true of P_3, whose binding depends on P_4 and P_6; all three are eluted in the urea fraction.

The proteins of the urea fraction are those which are dissociated by 6 M urea alone when the negative potential of the RNA is neutralized, as in our case by the basic groups of DEAE-cellulose. Although most of these proteins are basic, they are all characterized by a high content of hydrophobic residues [10]. Our results show that under our experimental conditions the interaction of these proteins with the RNA or with each other is mainly through bonds which are dissociated by high concentration of urea.

The composition of these proteins suggests that hydrophobic bonds may be predominant. As pointed out above, there is a certain resemblance in the relationship among various proteins during elution under our conditions and during ribosome assembly under the conditions of Mizushima and Nomura [9]. Although our adsorption-elution experiments are performed under conditions very different from those used for the assembly of ribosomes, both systems have one factor in common. Both employ conditions where non-specific electrostatic interactions are substantially reduced. In the assembly system this is achieved by employing a relatively high ionic strength, and in our system by neutralizing the acidic groups of the unfolded ribosomal RNA with a basic ion exchanger. When the "background" of the electrostatic interactions is so reduced, it becomes possible to discern non-electrostatic interactions. Our results provide direct evidence that non-electrostatic interactions do in fact exist between the ribosomal RNA and at least some of the ribosomal proteins and, taken together with the known composition of the proteins [10], suggest that these interactions may be largely hydrophobic in nature.

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