EXTRACTION OF RIBOSOMAL PROTEINS BY DISPLACEMENT WITH PROTAMINE

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1. Introduction

The methods commonly used for solubilizing ribosomal proteins are fairly vigorous, involving acid extraction at low pH [1] or prolonged treatment with concentrated solutions of LiCl and urea [2]. These conditions may possibly be unfavourable for the preservation of the enzymic and structural activities which according to current concepts are associated with the ribosomal proteins *in situ* [3,4]. As will be described below, effective solubilization of proteins in rat liver ribosomes can be achieved in ordinary buffered media by displacement with protamine. By varying the experimental conditions the effect of protamine can be made selective, as indicated by disc-electrophoresis on polyacrylamide gel. The method may therefore be useful not only for preparative purposes, but also for studies of the ribosomal structure.

2. Experimental

Rat liver ribosomes, prepared by the method of Rendi and Hultin [5], were suspended in 0.015 M ice-cold Tris HCl buffer, pH 7.7 (measured at 25°), or in 0.015 M phosphate buffer, pH 6.5, at a concentration of 1 mg protein [6] per ml. Under standard conditions these buffers also contained 0.025 M EDTA, previously adjusted to pH 7.7 or 6.5, respectively. The suspensions were mixed thoroughly for 30 min (0°) in a homogenizer with 0.8 volumes of 0.12 – 1.0% salmine sulfate (Sigma Chemical, St. Louis, Mo.) in plain buffer. (The pH of the protamine solutions was adjusted with dilute NaOH.) After centrifuging for 90 min at 60,000 *g* the clear extracts were dialyzed for at least 3 hours (4°) against 200 volumes of 0.01 M HCl, and were analyzed by disc-electrophoresis at pH 4.3 as described by Östner and Hultin [7]. In control experiments the ribosomal residues were re-extracted with 0.2 M HCl [1,7]. After dialysis these extracts were analyzed by disc-electrophoresis in parallel tubes.

3. Results and discussion

In the presence of EDTA, protamine in more than twice the stoichiometric amounts effectively solubilized the proteins of rat liver ribosomes (figs. 1A, 2A). The disc-electrophoretic pattern of the solubilized proteins was essentially similar to that obtained after extraction with 0.2 M HCl (fig. 3A) or with 3 M LiCl in 4 M urea [7]. Of the main protein fractions only the fraction referred to as No. 3 was obtained in significantly lower yield. No appreciable difference was observed between the two buffer systems used, despite the difference in pH.

When the particles were treated with protamine in amounts only slightly above the stoichiometric proportions, the extraction became less effective, and at the same time increasingly selective (figs. 1B, C, 2B, C). With both buffer systems protein bands 8-10 were the first to decrease in staining intensity. These proteins are probably located in the larger ribosomal subunit [8]. Available data suggest that protein No. 10 is to a great extent hidden inside the particles, except for a minor loop reaching the ribosomal surface [7,8]. The higher resistance of this protein to solubilization by protamine is probably a manifestation of this structural shielding.

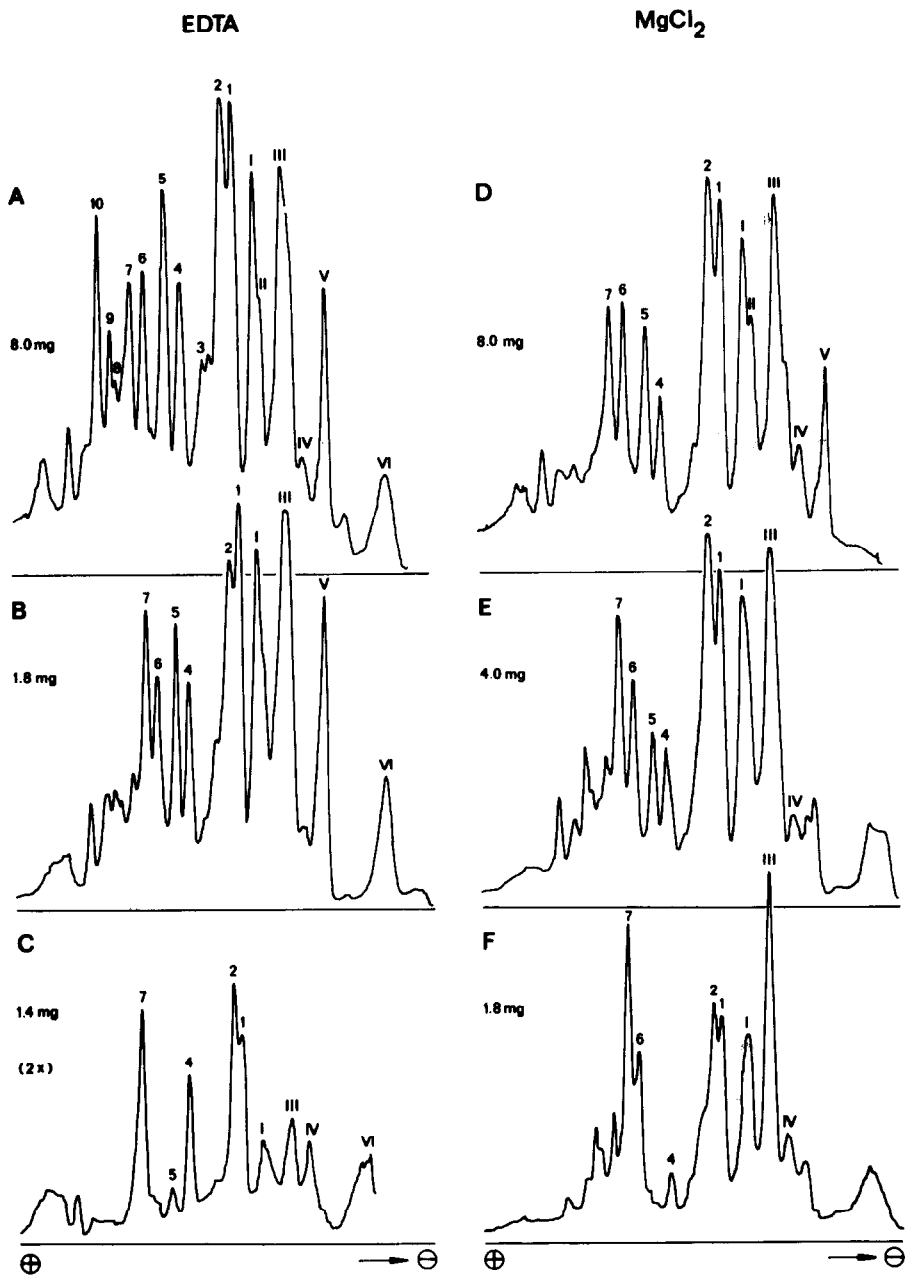

Fig. 1. Solubilization of rat liver ribosomal proteins by displacement with protamine in alkaline buffer. Equal samples of ribosomes containing 1.0 mg protein were suspended in 0.015 M Tris HCl buffer, pH 7.7, containing 0.025 M EDTA (curves A, B, C) or 5 mM $MgCl_2$ (curves D, E, F), and were treated for 30 min (0°) with protamine at the concentrations indicated (mg protamine per mg ribosomal protein). After centrifuging and dialysis 0.15 ml samples of the extracts were analyzed by disc-electrophoresis on 5 × 100 mm columns of 12.5% polyacrylamide gel, pH 4.3. After staining with amido black the protein bands were recorded by use of a Joyce-Loebl microdensitometer, operated with optical wedge 0-2 (except in experiment C, where the peak height was increased by a factor of 2 by use of wedge 0-1). The electrophoretic patterns are directly comparable, but because of the protamine the exact amount of ribosomal protein added to each column could not be assessed.

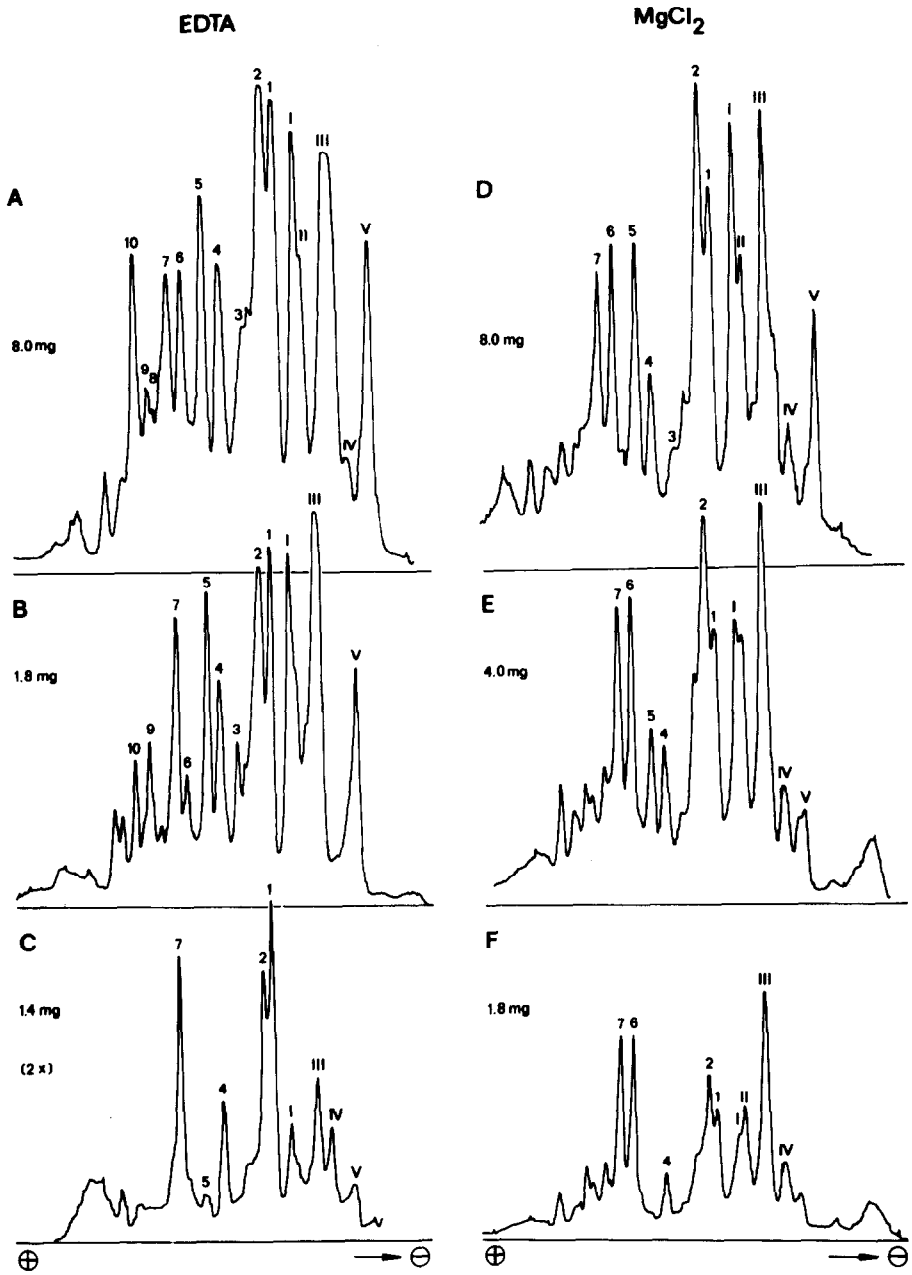

Fig.2. Solubilization of rat liver ribosomal proteins by displacement with protamine in acid buffer. The experiments were as described in fig. 1, with the exception that 0.015 M phosphate buffer, pH 6.5, was used.

The proteins most readily extracted were those referred to as Nos. III, I, 1, 2, 4 and 7. Of these, proteins III, I, 1 and 2 occur in the minor ribosomal subunit appearing in the presence of EDTA [8]. The fragility of the minor subunit [9] may account for the rapid solubilization of these proteins. Of the ribosomal proteins discussed, protein 4 seems to have the most superficial location [7]. This may account for the

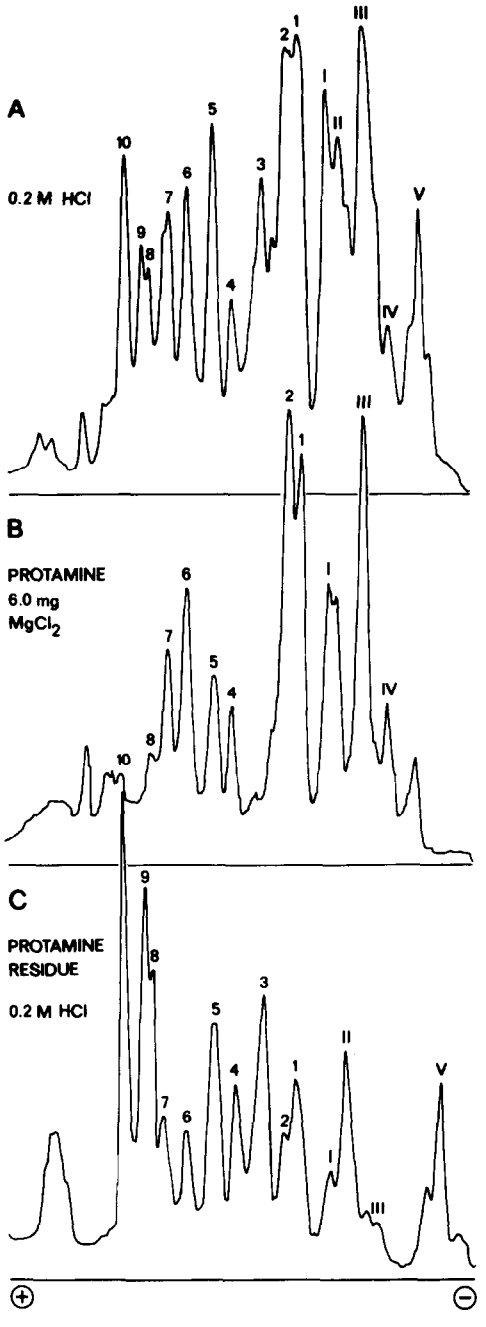

Fig.3. Efficiency of protamine extraction. A. Electrophoretic pattern of ribosomal proteins extracted with 0.2 M HCl [7]. The amount of protein added to the column was 85 μg. B. Electrophoretic pattern of ribosomal proteins extracted with protamine (6 mg per mg ribosomal protein) in the presence of 5 mM $MgCl_2$. C. Ribosomal residues from experiment B, re-extracted with the same volume of 0.2 M HCl. From similar residues, extracted with protamine in the presence of EDTA (fig. 1A), protein fraction 3 was the only fraction obtained in significant amount after re-extraction with 0.2 M HCl.

ease with which it is solubilized under these conditions.

In buffers containing 5 mM $MgCl_2$ the particles were more resistant to protamine than in the presence of EDTA. Proteins 8-10 were little affected even at high concentrations of protamine, but were readily solubilized from the ribosomal residues by subsequent HCl-extraction (fig. 3C). Of special interest was the effect of Mg^{++} on the solubilization of proteins V and 5 (figs. 1B, F, 2B, F). Previous experimental data indicate that these proteins are relatively resistant to proteolytic attack as long as the Mg^{++} concentration of the medium is sufficiently high to prevent ribosomal dissociation [8]. They may be located inside Mg^{++}-stabilized ribosomal grooves (possibly in the cleft between the ribosomal subunits). The striking effect of Mg^{++} on their solubilization by protamine would be compatible with this interpretation.

Tests of the released proteins for proteolytic activity using benzoyl L-arginine amide, N-acetyl L-tyrosine or hemoglobin were negative.

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