A NEW APPROACH TO THE FRACTIONATION OF RIBOSOMAL PROTEIN

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1. Introduction

Earlier work [1] in this laboratory has shown that about 95% of the ribosomal proteins can be detached from the ribosomal RNA with salt, provided that the ribosomes are unfolded before being treated with the salt. The amount of protein detached from such unfolded ribosomes is a function of the salt concentration, and at a fixed salt concentration it is a function of the ribosome concentration. We have proposed that the dependence on ribosome concentration may result from an equilibrium between the detachment and reattachment of proteins. In this case, if the detached proteins were to be continuously removed from the ribosomes, it might be possible to obtain a small group of ribosomal proteins whose detachment would depend only on the salt concentration employed, and it might then be possible to isolate different groups of proteins by employing successively different salt concentration. We have tried to do this by attaching unfolded 50 S ribosomal subunits to a fixed support and washing them with a salt gradient. The preliminary results, reported here, indicate that the ribosomal proteins can be fractionated in this way.

As the fixed support, we employed a column of DEAE-cellulose. Native folded ribosomes can be eluted from such a column with salt at neutral pH [2]. However, adsorbed ribosomal RNA cannot be eluted [3] until the ion exchanger is deionized with alkali. Since unfolded ribosomes are essentially strands of RNA partly neutralized by the attached proteins, it seemed likely that their chromatographic behaviour would resemble that of ribosomal RNA, and this proved to be the case. Therefore, unfolded 50 S ribosomal subunits were adsorbed on a DEAE-cellulose column, which was then eluted with a sodium phosphate gradient (10^{-3} M to 0.4 M) in 6 M urea at a constant pH of 6.5. The ribosomal proteins were recovered in high yield, and different fractions gave different patterns in acrylamide gel electrophoresis. No RNA was eluted until, at the end of the run, 1 M NaOH was applied.

2. Experimental

70 S Ribosomes from *E. coli* MRE 600 [4] were isolated by differential centrifugation and purified with 0.5 M NH_4Cl [1]. They were dissociated into subunits by a 40 hr dialysis against 0.2 M NH_4Cl - 10 mM tris-HCl (pH 7.4) - 1 mM magnesium acetate. The 30 S and 50 S subunits were separated in the B XV zonal rotor of an MSE (London) Super Speed 65 centrifuge in an exponential convex gradient of 9–20% sucrose in the dissociation buffer, with a 200 ml cushion of 45% sucrose and a 200 ml overlay of dissociation buffer. About 1–1.2 g of ribosomes in 20 ml of dissociation buffer was centrifuged for 19–20 hr at 25,000 rpm. Fractions of 10–12 ml were collected*.

The 50 S fractions were pooled, made 0.01 M in magnesium acetate, and concentrated from about 400 ml to about 100 ml by dialysis in an evacuated flask. The ribosomes were precipitated by the addition of solid $(NH_4)_2SO_4$ to near saturation and

* I am grateful to Drs. H.G.Wittmann, Berlin, and H.Delius, Geneva, for supplying us, before publication, with full details of the procedures used in their laboratories for preparing ribosomal subunits. The method described here is based on those procedures.

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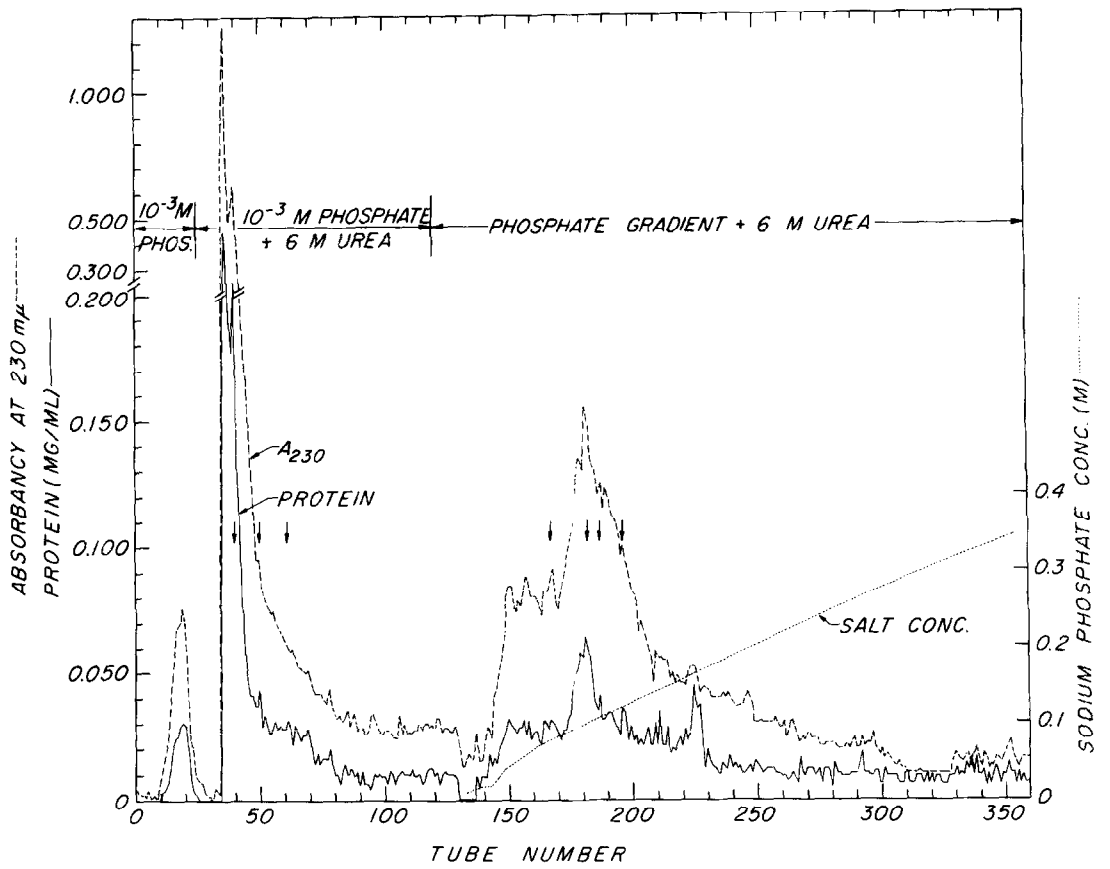

Fig. 1. Elution pattern of the proteins of the 50 S ribosomal subunit of *E. coli*. The ribosomes were unfolded and adsorbed directly on the column of DEAE-cellulose, and the proteins were eluted, as described in the text. The eluted protein was assayed by its absorbancy at 230 mμ (broken line) and also with the Folin reagent (solid line). The arrows show fractions taken for acrylamide gel electrophoresis (see fig. 2).

were dissolved in and dialyzed against an appropriate buffer. The 50 S ribosomes thus obtained were unfolded by dialysis against 1 mM tris-HCl (pH 7.4) - 1 mM EDTA for 24 hr in the cold. After that they were dialyzed against 1 mM sodium phosphate buffer (pH 6.5) for an additional 24 hr. The sedimentation constant of these unfolded ribosomes in 1 mM sodium phosphate at a concentration of 1.2 mg/ml was 9 S.

Preswollen 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 then washed on a filter with 1 mM sodium phosphate (pH 6.5) until the pH and conductivity of the wash fluid were those of the buffer, suspended in the same buffer, and packed in a column 2.2 cm in diameter and 27 cm high.

108 mg of unfolded ribosomes in 90 ml of 1 mM sodium phosphate (pH 6.5) were adsorbed and washed with an additional 80 ml of the same buffer. Elution of the ribosomal proteins was begun with 670 ml of 6 M urea containing 1 mM sodium phosphate (apparent pH 6.5) and was completed with a linear sodium phosphate gradient in 6 mM urea at the same apparent pH, formed from 990 ml 1 mM in phosphate and 950 ml 0.4 M in phosphate. The column was then washed overnight with distilled water, and the RNA was removed with 150 ml of 1 M NaOH. The flow rate was 33.5 ml/hr and fractions

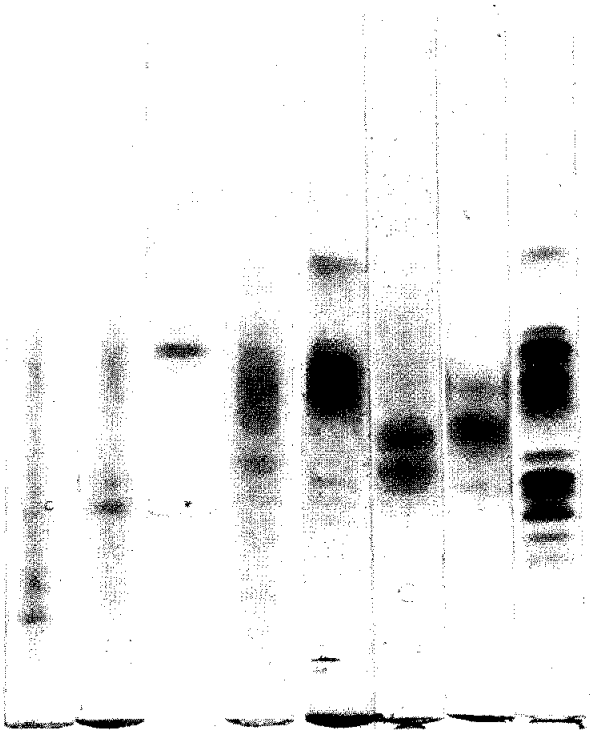

Fig. 2. Acrylamide gel electrophoresis patterns of (from left to right): total 50 S proteins, fractions 40, 50, 62, 167, 182, 187, 196. Details are given in the text.

were collected every 12 min. They were assayed for protein content (a) by measuring the absorbance at 230 mμ (A_{230}) and (b) with the Folin reagent [6]. In the latter procedure aliquots were restricted to 0.2 ml or less, since larger volumes of the elution solvents interfered with the determination. The urea solution was deionized before use and the absorbance at 230 mμ of a 6 M urea solution was never higher than 0.1. Before gel electrophoresis, the protein fractions were either concentrated by pressure filtration (Diaflo cell with a UM-2 membrane, Amicon Corp., Lexington, Mass., USA) or were dialyzed against HCl (pH 2) in the cold and lyophilized. The concentrated fractions dissolved in urea were analysed by electrophoresis in gels of 7.5% acrylamide and 0.2% methylene bisacrylamide containing 6 M urea at pH 4.5. Electrophoresis was carried out at room temperature for 2 hr at 5 mA/tube. The gels were stained with coumassie blue and destained in 10% trichloroacetic acid according to Chrambach et al. [5].

3. Results and discussion

The elution pattern is shown in fig. 1 and gel electrophoresis patterns of those fractions examined in fig. 2. These results are preliminary, and experiments are being continued aimed at finding optimal elution conditions for improved resolution. However, these preliminary results indicate that it is possible to fractionate the ribosomal proteins directly, without first having to isolate them from the ribosomes. Aside from convenience, there are several possible advantages to this. For one, the proteins are removed as they are detached from the RNA; there is therefore less chance for them to interact with each other than when all the species are present together in solution.

In this system the free phosphate groups of the RNA (those not bound to ribosomal proteins) are neutralized by the basic groups of the ion-exchanger. Under these conditions the proteins are dissociated from the RNA at low salt concentrations. It is of interest that a large portion became detached in 6 M urea at the starting sodium phosphate concentration of 1 mM. The elution of the proteins was complete by the time the phosphate concentration reached 0.3 M. The total recovery of protein was over 90% as determined with the Folin reagent, and was not increased when, in other experiments, the gradient was extended to as high as 1 M phosphate. No more than an additional 3–5% of the protein was recovered with the ribosomal RNA when it was eluted with alkali.

A more detailed study of the technique and of the protein fractions obtained is now in progress.

Acknowledgements

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