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Ribosome fractionation by gel filtration

Gel filtration has been successfully used for the fractionation of a wide variety of macromolecules[1] including such nucleoprotein particles as viruses[2]. Little, however, has been done in applying this technique to studies involving ribosomes; the latter have generally been fractionated by sucrose density gradient centrifugation[3,4] and more recently, by gel electrophoresis[5,6]. Some workers have employed gel filtration for the purification of ribosomal preparations, for example, by passing ribosomes through a column of Sephadex G-25[7,8]. To our knowledge only one report of ribosome fractionation by gel filtration has appeared in the literature[9]. That study reported that, when 1 % Agarose was used, the 50 S *Escherichia coli* ribosomes could be separated from large aggregates but that the 40 S and 60 S yeast ribosomes could not be resolved. In view of this paucity of information it seemed desirable to reinvestigate the applicability of gel filtration to the fractionation of ribosomes. This note describes such studies.

Experimental

Ribosomes. The organism used in this study was *Bacillus licheniformis* (NRS 236). Growth of the cells and isolation of the ribosomes were as described previously[10]. The ribosomes were suspended in buffer I which consisted of 0.01 *M* tris(hydroxymethyl)aminomethane (Tris), pH 7.4, 0.01 *M* magnesium acetate, and 0.06 *M* ammonium chloride. Prior to use the buffer was made 0.006 *M* in spermidine and 0.006 *M* in 2-mercaptoethanol. For some experiments, the ribosomes, after having been suspended in buffer I, were dialyzed *versus* buffer II, which consisted of 0.005 *M* Tris (pH 7.4) and 0.002 *M* magnesium acetate.

Chromatography. A 2.5 × 100 cm column (Pharmacia Fine Chemicals) was used for these experiments. The column was generally equipped with a flow adaptor. The column was packed with Bio-Gel A-15m, 100–200 mesh (Bio-Rad), and approximately 0.4–0.8 ml of ribosome solution (absorbance of about 300 at 260 mμ) was layered onto the column. The ribosomes were eluted from the column with the appropriate buffer and fractions were collected at 2 min intervals using a flow rate of about 30 ml/h. These operations were performed at 4°. The absorbance of the fractions was measured at 260 mμ in a Zeiss PMQ-II spectrophotometer.

Ultracentrifugation. All ultracentrifugal analyses were conducted in a Spinco model E analytical ultracentrifuge equipped with rotor temperature indicator control and with schlieren and UV optics. Measurements of the schlieren patterns were performed with a Nikon model 6C microcomparator. The films obtained with the UV optics were analyzed by means of a Joyce and Loebl Chromoscan densitometer. All the sedimentation coefficients were corrected to 20° for the viscosity of water.

Results and discussion

In order to assess the usefulness of gel filtration, the ribosomes were studied under two conditions, depending on the buffer in which they were suspended. In buffer I, the ribosomes consisted primarily of 70 S and 100 S ribosomes. In buffer II, 30 S, 50 S, 70 S, and 100 S ribosomes were present. This latter condition represented

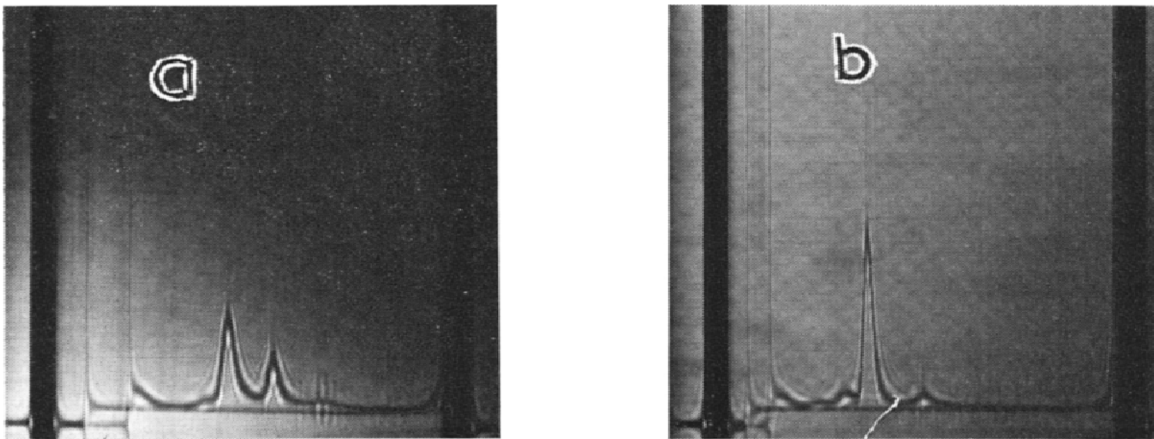
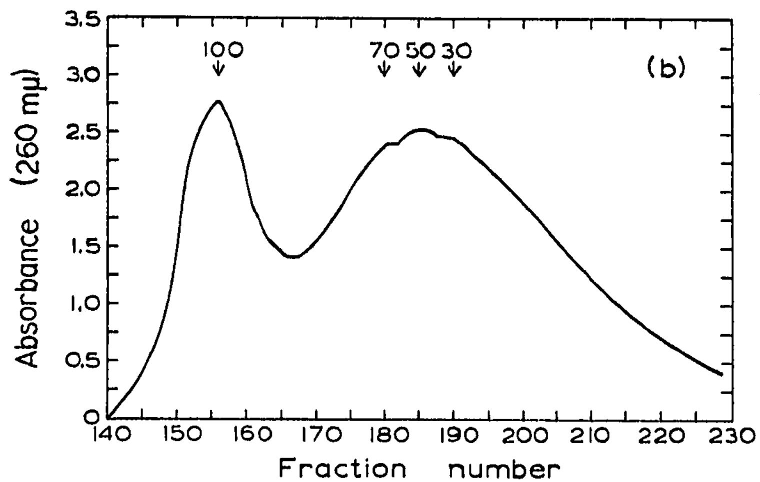


Fig. 1. Ultracentrifuge patterns of unfractionated ribosomes. Sedimentation is from left to right. Pictures were taken 20 min after reaching speed (29500 r.p.m.). Sedimentation coefficients of the major components are given in parentheses. (a) Buffer I (66, 93); (b) Buffer II (30, 49, 63, 102).



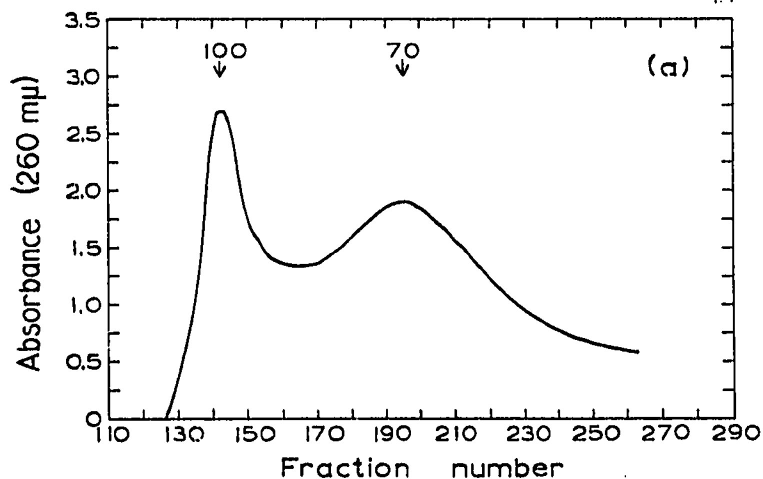


Fig. 2. Gel filtration fractionation of ribosomes. (a) Buffer I, flow rate 39 ml/h; (b) Buffer II, flow rate 30 ml/h.

a complex mixture of ribosomes, low magnesium ion concentrations, and the absence of stabilization by spermidine[11]. The ultracentrifuge patterns of the ribosomes obtained under the above two conditions are shown in Fig. 1. The fractionation of these two ribosomal preparations by gel filtration is shown in Fig. 2.

The results show that, using Bio-Gel A-15m and buffer I, good fractionation of 70 S and 100 S ribosomes could be achieved. The gel filtration pattern consisted of two well-separated peaks. Aliquots from these peaks were examined by means of analytical ultracentrifugation (using UV optics). Each peak was shown to consist of only one component; the first peak consisted of 70 S ribosomes, and the second peak consisted of 100 S ribosomes. The relative amounts of 70 S and 100 S ribosomes corresponded to those in the unfractionated sample. The recovery of applied ribosomes was approximately 100 % in terms of absorbance units at 260 mμ. The ribosomes were eluted after the void volume which amounted to about 150 ml as determined with Blue Dextran 2000 (Pharmacia Fine Chemicals).

Using the same gel but shifting to buffer II demonstrated that gel filtration can be used for the fractionation of ribosomes even at low magnesium ion concentrations and in the absence of stabilization by spermidine[11]. The gel filtration pattern, when examined by ultracentrifugal analysis (UV optics), was shown to consist of a well-separated 100 S peak and a second peak containing 30 S, 50 S, and 70 S ribosomes. The proportion of 100 S ribosomes corresponded to that in the unfractionated sample and the recovery of applied ribosomes was again approximately 100 % in terms of absorbance units. The ribosomes in the second peak were only partially resolved which is not surprising in view of the large excess of 70 S monomers (Fig. 1). When the buffer composition, the type of gel, and the flow rate are varied, it should be possible to obtain purified fractions of 30 S and 50 S ribosomes from *B. licheniformis* as well as of ribosomes from other sources.

Gel filtration has an advantage over density gradient centrifugation in that it eliminates the dialysis step which may be required for recovery of fractions from such experiments. In addition, in gel filtration the entire ribosomal sample is investigated while in density gradient centrifugation there is generally some loss of material which pellets out at the bottom of the tube. Thus gel filtration should prove to be a useful alternative procedure for the fractionation of ribosomes.

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