THE FRACTIONATION OF RIBONUCLEIC ACID ON A PREPARATIVE SCALE BY POLYACRYLAMIDE GEL ELECTROPHORESIS

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1. Introduction

Polyacrylamide gel electrophoresis provides an analytical method of very high resolution; it permits the identification of minor components not readily resolved by other techniques [1-3]. Our interest in minor components of reticulocyte RNA led us to attempt to fractionate it on a preparative scale with a commercially available apparatus for polyacrylamide gel electrophoresis.

2. Materials

Acrylamide and *N,N'*-methylenebisacrylamide (Eastman Organic Chemicals, New York) were recrystallised as described by Loening [3]. *N,N,N',N'*-tetramethylethylenediamine (Kodak, Ltd., London) and ammonium persulphate (May and Baker, Dagenham, England) were used without further purification.

RNA was isolated from mouse reticulocytes or reticulocyte polyribosomes using the phenol-*m*-cresol-4-aminosalicylic acid method described by Kirby [4]. It was dissolved in the standard electrophoresis buffer after sedimentation from ethanol. It is very important to ensure that the RNA is completely free from protein, otherwise streaking or binding to the gel surface occurs.

3. Methods

A Büchler Preparative Polyacrylamide Gel Electrophoresis Apparatus ('Polyprep') (Buchler Instruments, Inc., Fort Lee, New Jersey, 07024, USA) was used. In this apparatus the polyacrylamide gel is formed in an annulus bounded by a glass water cooling jacket inside and outside. The gel is polymerized with a solid insert beneath it; the insert is then replaced by a porous glass disc, under which is the lower buffer compartment. The upper buffer compartment is directly in contact with the upper surface of the gel. A space of 1—2 mm is left between the porous disc and the lower surface of the gel; as mobile components pass through the gel they are flushed from this buffer-filled space at a constant, slow rate regulated by a peristaltic pump (Buchler). Gels were prepared by mixing acrylamide, methylenebisacrylamide, *N,N,N',N'*-tetramethylenediamine and ammonium persulphate in buffer immediately before pouring into the column. The most useful gels for total RNA separation proved to be those containing 2.4% acrylamide. A bisacrylamide concentration of 0.24% was found necessary for short gel lengths (less than 5 cm) to give a reasonably stiff, though somewhat opalescent gel. The resolution and mobility of RNA components were not affected by varying the bisacrylamide concentration from 0.12% to 0.24%.

Initially, difficulties were encountered because as the elution buffer level in the reservoir fell, the reduction of hydrostatic pressure in the elution chamber caused the rather soft gel to collapse into it. It therefore proved necessary to use a simple constant level device for the buffer reservoir (fig. 1).

When a standard low ionic strength buffer (tris, 0.04 M; sodium acetate, 0.02 M; EDTA, 0.002 M; pH 7.8 [3] was used throughout the system the electrical resistance increased progressively and the cur-

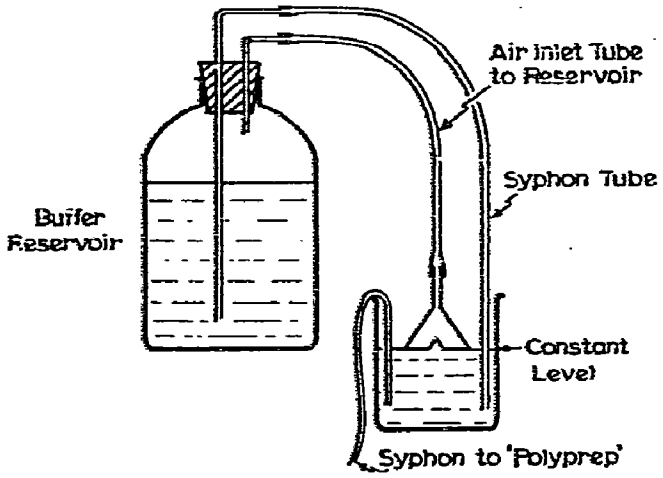

Fig. 1. Constant level device for buffer reservoir.

rent dropped sharply during a run. This was found to be due to the relatively high electrical resistance across the glass membrane in the floor of the elution chamber; this caused local heating resulting in bubble formation in the elution chamber and particularly in the membrane itself, which in turn increased the resistance still further. This difficulty was overcome by raising the buffer concentration in the lower reservoir and membrane holder to ten times the standard concentration. The gel was made up in the standard buffer; this was also used for the top reservoir and for elution.

Gels were normally polymerized in the apparatus at room temperature (21°C). After polymerization, cooling water was pumped through the jacket until the entire experiment had been completed. The RNA sample was dissolved in standard electrophoresis buffer containing 5% sucrose (w/v) usually in a volume of approximately 5 ml and was carefully layered on top of the gel after the apparatus had been set up and the buffer compartments filled. With the Buchler power pack set for constant current, a current of 200 ma was used, giving an applied voltage of 130 V between the upper, cathode, compartment and the lower, anode, compartment. An elution buffer flow rate of approximately 1 ml/m was found to be satisfactory. Water at 3°C was pumped through the water-jacket of the apparatus from a water bath kept cool by a Grant CC 15 Cooler Unit. The eluate was monitored with an LKB Uvicord II and was recorded on a Yokogawa chart recorder (Yokogawa Electric Works, Ltd., Japan). Fractions were collected at 5 m intervals.

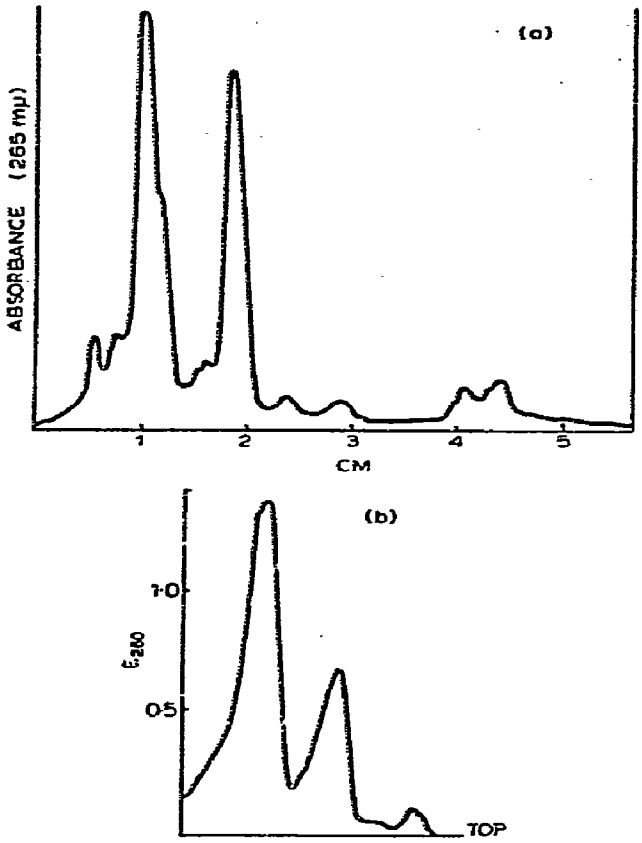

Fig. 2a. Joyce Loebl Chromoscan record of analytical polyacrylamide gel electrophoretogram of mouse reticulocyte RNA. 50 μg RNA on a 2.4% gel, electrophoresis for 1½ hr at 4°C, potential difference 10 V/cm, current 3.5 ma. Gel scanned at 265 mμ. 2b. Sucrose density gradient of mouse reticulocyte RNA. 175 μg RNA layered on 5–20% sucrose gradient, centrifuged 2 hr, 30 min in 3 × 5 ml swing out rotor, MSE superspeed 50 ultracentrifuge at 39 000 rpm. Gradient analysed using a flow cell with a Unicam SP 800 spectrophotometer.

4. Results

The RNA samples used in the Polyprep Apparatus gave the expected patterns on sucrose gradients and analytical polyacrylamide gels (fig. 2a, b). An elution pattern for mouse reticulocyte RNA from the 'Poly-

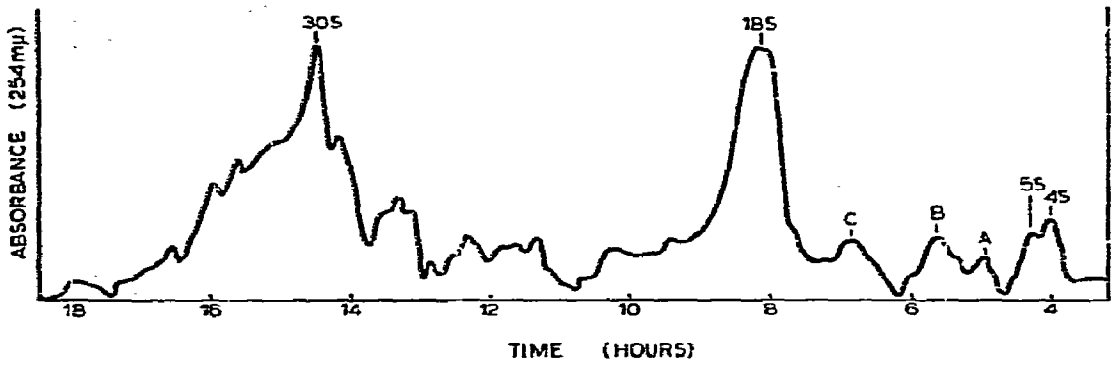

Fig. 3. Elution trace of mouse reticulocyte polysomal RNA effluent from the Buchler Polyprep. For details see text.

prep' is shown in fig. 3. In this experiment, 5 mg of total RNA were applied to a gel 5 cm long. (In other experiments up to 14 mg of polysomal RNA have been successfully separated; it is estimated that this is still well below the capacity of the gel.)

The isolated RNA components were pooled after collection, precipitated with ethanol, and run in analytical gels [3]. Quantitative isolation of the 18 S and 30 S components was not attempted; a cut was taken from the centre of each peak. Recovery of the total component C, component B and 5 S + 4 S RNA after alcohol precipitation was 60.5 μg, 50 μg and 264 μg respectively. Even the minor components, such as components B and C showed no cross-contamination, a result to be expected from the sharpness of the separation in the elution profile (fig. 4a–e).

The isolated 18 S component when run in an analytical gel shows one minor peak moving ahead of the main peak and at least one running more slowly (fig. 4b). This result has been consistently obtained with 18 S RNA fractions from gel electrophoresis, and also with 18 S RNA isolated on a sucrose gradient (B-15 zonal rotor). The cuts taken in all cases preclude contamination of 18 S with material of these sizes. The large 'slow' component does not correspond in mobi-

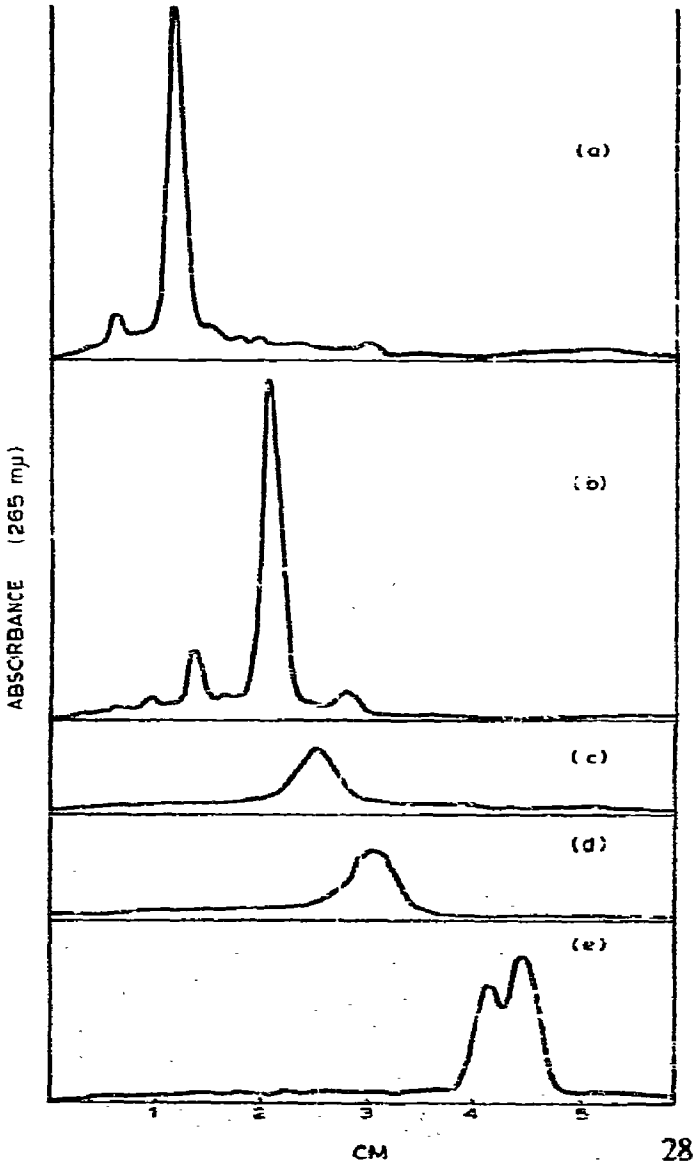

Fig. 4a–e. Joyce Loebl Chromoscan records of analytical polyacrylamide gel electrophoretogram of RNA fractions from the Buchler Polyprep Apparatus. Electrophoretic conditions as in legend to fig. 2a. (a) 12.5 μg of 30 S RNA. (b) 7.5 μg of 18 S RNA. (c) 40 μg of Compound C. (d) 40 μg of Compound B. (e) 6.0 μg of a mixture of 4 S and 5 S RNA.

lity to 28S RNA, but to a shoulder often seen running just on the 18S side of the 28S peak (fig. 2a) and may represent an aggregate. The 'fast' component has a mobility in between components B and C, and corresponds to neither of them. The nature of these minor components is being investigated.

The method thus provides a rapid technique for separating minor RNA components in preparative amounts without degradation.

5. Summary

Total RNA from mammalian reticulocytes has been fractionated on a preparative scale using polyacrylamide gel electrophoresis. Minor RNA components are separated at high resolution and undegraded material is recovered in good yield.

Acknowledgements

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