Separation of nucleotides, nucleosides and bases on a new gel filtration material

A polyacrylamide gel filtration material has recently been developed in this laboratory which has a smaller pore size than gels previously available. This new gel, designated Bio-Gel P-2, consists of acrylamide, methylene-bisacrylamide copolymer beads, which exclude substances above molecular weight 2000. The ability of this gel to fractionate polypeptides from 2000 to 200 molecular weight[1] and to desalt small peptides and some amino acids[2] indicates that it may be useful for separation of low molecular weight components of nucleic acids.

Other workers have fractionated larger molecules using gel filtration. Nucleic acid components have been separated on cross-linked dextrans[3,4] and polyacrylamide gels[5]. Oligonucleotides have been partially separated according to molecular weight and nucleotides have been separated from polynucleotides.

This paper presents some preliminary work on the fractionation of nucleotides, nucleosides and free bases.

Experimental

A hydrolysate of ribonucleic acid was prepared by placing 40 mg of yeast ribonucleic acid in a sealed tube containing 3 ml of 1 *N* HCl and heating to 100° for one hour. The hydrolysate was neutralized with 1 *N* NaOH and diluted to 25 ml.

Bio-Gel P-2, 100–200 mesh (lot 2834) was used as the gel filtration medium. This material has a water regain of 1.6 g water/g of dry gel and a hydrated bed volume of 3.8 ml/dry gram. A gel suspension was made by slowly pouring, with stirring, the dry Bio-Gel P-2 into an excess of 0.05 *M* phosphate buffer, pH 8. Approximately 10–15 ml of buffer per g of dry gel gave a good slurry. The gel was allowed to hydrate for 1 h. The gel suspension was poured into a column (1.3 × 40 cm) fitted with a funnel and allowed to pack with gravity flow to a bed height of 36 cm. The buffer level was drained to the top of the bed and samples were applied with a pipet. A buffer rinse of 0.5 ml followed the sample application. The column was then filled with 0.05 *M* phosphate buffer, pH 8, and attached to a Beckman Model 746 solution metering pump. Buffer was pumped through the column at a rate of 12 ml/h.

The column effluent was monitored through a Vanguard Model 1056 Automatic Ultraviolet Analyzer set at a wave length of 260 mμ. The effluent from the hydrolysate sample was tested for sodium chloride with $AgNO_3$–HNO_3 reagent. The effluent volumes corresponding to the peaks on the elution curve were collected, concentrated and spotted on microcrystalline cellulose TLC plates together with known standards. The plates were eluted in isopropanol–HCl–water solvent and the spots detected with U.V. light[6,7].

Results and discussion

Fig. 1 shows the elution curve of an acid hydrolysate of yeast ribonucleic acid obtained by gel filtration through Bio-Gel P-2 in 0.05 *M* phosphate buffer, pH 8. Sodium chloride was eluted at 41 to 49 ml. Using TLC, peak A was identified as cytidylic and uridylic acids, peak C as adenine. The middle peak, B, could not be identified, possibly because of the small amount of material which it contained.

Standard samples of nucleotide, nucleosides, and base were applied to a column

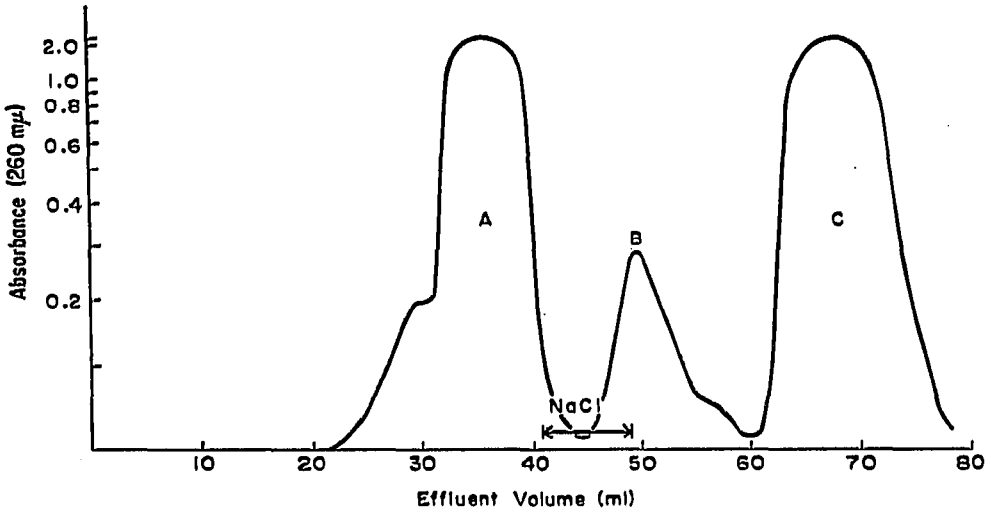

Fig. 1. Fractionation of a hydrochloric acid hydrolysate of yeast ribonucleic acid on Bio-Gel P-2, 100–200 mesh. 0.5 ml sample eluted with 0.05 *M* phosphate buffer, pH 8, at 12 ml/h through a 1.3 × 36 cm column.

of Bio-Gel P-2 and eluted under the same conditions as the hydrolysate. The R_F values of uridylic acid, uridine, adenosine, and adenine were determined and the individual elution curves were plotted on the same effluent volume axis (Fig. 2). This verified the group separation of the hydrolysate sample and suggested that peak B is probably a nucleoside.

The peak position of uridine eluted with 0.2 *M* NaCl from a column of Bio-Gel P-2 equilibrated with 0.2 *M* NaCl was the same as that obtained with 0.05 *M* phosphate buffer. Elution of hydrolysate with water from gel equilibrated with water gave an elution curve similar to Fig. 1.

During gel filtration molecules are separated according to their molecular size provided no interaction with the gel matrix occurs. Adenine (135 molecular weight) has an R_F of 0.29 on Bio-Gel P-2, while leucine (131 molecular weight) has an R_F

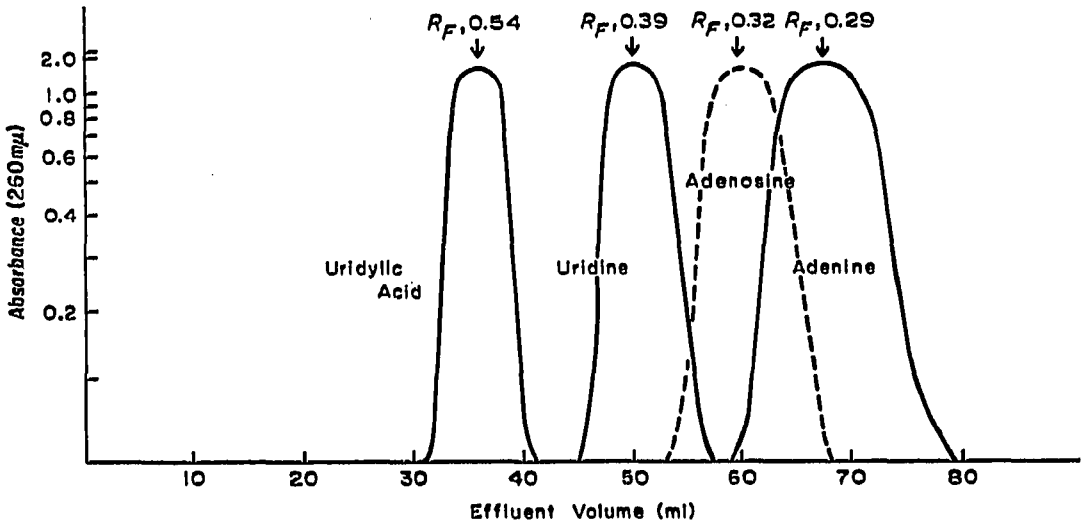

Fig. 2. Composite diagram of elution curves from a 1.3 × 36 cm column of Bio-Gel P-2, 100–200 mesh. Eluant: 0.05 *M* phosphate buffer at 12 ml/h.

of 0.5, suggesting that gel filtration of free bases is coupled with adsorption effects. In the group separation of nucleotides, nucleosides and bases, the interaction with the gel serves to enhance the separation.

Comparison of the R_F values of the two nucleosides, 0.39 for uridine (244 molecular weight) and 0.32 for adenosine (267 molecular weight) shows that the elution order expected from molecular weights is reversed, again indicating adsorption. Because of these adsorption effects, partial separation of nucleosides may be possible.

These experiments demonstrate that Bio-Gel P-2 can be used to separate nucleic acid hydrolysates into separate groups according to their molecular sizes. Although Dowex 1-X8[8], ECTEOLA cellulose and DEAE cellulose[9] are generally used for nucleic acid separations, their use is time consuming and involves large elution volumes. It is also difficult to identify the components in the eluate without a preliminary run of a set of known standards. Bio-Gel P-2 offers a rapid way of making a group separation of nucleotides, nucleosides and free bases. The entire sample can be eluted in 7 h over an elution volume of 80 ml, and the elution pattern is reproducible. Further separation of each group can be carried out with TLC techniques. This method is especially useful in the study of nucleic acid biosynthesis where one wishes to follow the path of a particular tracer.

Note added in proof

Results of a study of the desalting of nucleic acid components using Bio-Gel P-2 were reported by M. UZIEL AND W. COHN (Biology Division, Oak Ridge National Laboratory, Tennessee) at the 49th Annual Meeting of the Federation of America Societies for Experimental Biology on April 12th, 1965. Details of their work are to be published.

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