USE OF POLYACRYLAMIDE GEL COLUMNS FOR THE SEPARATION OF NUCLEOTIDES

Michael JOHN, Hans SKRABEI and Hanswerner DELLWEG
Institut für Gärungsgewerbe und Biotechnologie, Berlin 65, Seestr. 13, Germany

Received 6 October 1969

1. Introduction

The methods most commonly used to achieve separation of nucleotides are ionexchange chromatography, paper chromatography, and paper electrophoresis. The separation of nucleotides by gel chromatography has also been used with success [1–10].

In the course of our experiments using polyacrylamide gel as chromatographic support, we found elevated temperatures to increase the resolution of gel columns [11]. A preceding paper [12] reported the separation of polyglucoses up to a D.P. of 13 on Bio-Gel P-2 (minus 400 mesh). This finding suggested that chromatography on polyacrylamide gel would provide a useful method for the separation of nucleotides as well as sugars.

The present communication describes the separation of mono- and oligonucleotides on a column of Bio-Gel P-2 (minus 400 mesh) and their detection by ultraviolet absorption.

2. Experimental

Bio-Gel P-2 (minus 400 mesh), control number 3397, date 8-29-65, was obtained from Calbiochem, Luzern, Switzerland. In order to get a more uniform particle size the gel was fractionated by settling as described previously [13]. Polyuridylic acid and mononucleotides were purchased from Boehringer, Mannheim, Germany. Uracil, adenine and cytosine were supplied by Merck, Darmstadt, Germany. Ribonuclease from pancreas was obtained from Serva, Heidelberg, Germany. Partial digestion of poly U with pancreatic ribonuclease was carried out for 4 min in 0.01 M EDTA, pH 5.2 at 37°C. 10 μg of enzyme was used to hydrolyse 3 mg of poly U in 4 ml EDTA. During incubation the pH was kept constant by means of an autotitrator. The reaction was stopped by adding uranyl acetate.

For the analytical procedure two glass-jacketed columns (1.5 X 150 cm and 1.5 X 127 cm) both fitted with flow adapters are connected in series. The two columns are connected at the top by a Teflon capillary tubing using Swagelok fittings. The column temperature of 65°C is controlled by a Haake constant temperature circulator. The total gel bed is 270 cm. The columns were packed as described recently [12]. In order to get a more closely packing, Brij-35 was added to the eluent and each column was eluted overnight at a flow rate of about 50 ml per hour. Afterwards Brij-35 was omitted. For the automatic application of the sample a Swagelok Union Tee (1/8″), covered with a rubber septum, is connected by a Teflon tubing to the column and is used as an injection port. In a typical run the sample was dissolved in the eluent buffer and was applied to the column by means of a 250 μl Hamilton syringe. The column is eluted with 0.25 M citrate buffer, pH 4.4, at a flow rate of 25 ml per hour controlled by a Milton-Roy Minipump. The effluent is monitored by measuring its transmittancy at 254 nm in an Uvicord II (LKB) equipped with a 5 mm flow cell and a strip-chart recorder (Moseley/ Hewlett-Packard, 7101 BM).

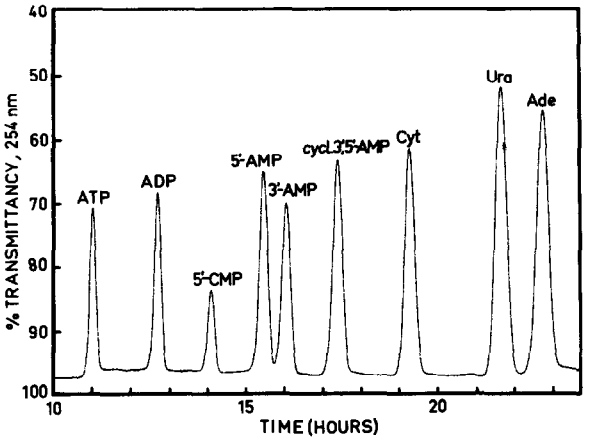

Fig. 1. Separation of a mixture of nucleotides and bases, chromatographed on Bio-Gel P-2 (minus 400 mesh). Gel bed, 1.5 X 270 cm. Flow rate, 25 ml per hour. Column temperature, 65°C. Elution was carried out with 0.25 M citrate buffer, pH 4.4.

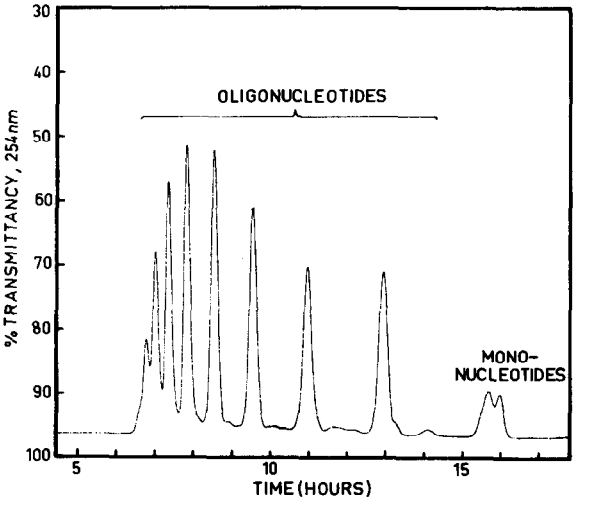

Fig. 2. Fractionation of poly U after partial hydrolysis with ribonuclease from pancreas, chromatographed on Bio-Gel P-2, minus 400 mesh. Chromatographic conditions identical to those described for fig. 1.

3. Results

Figure 1 shows the separation of a mixture of nucleotides and bases on polyacrylamide gel. The order in which the substances are eluted from the column was established by adding one compound at a time to a previously established mixture. Complete resolution of the mixture was obtained and the symmetrically peaks would promise to use this method also for quantitative purpose.

It is well established that gel chromatography separates the molecules mainly according to their size and shape. Thus, mononucleotides are eluted before the smaller bases cytosine, uracil and adenine as can be seen in fig. 1. Furthermore it was observed that the separation of nucleotides on polyacrylamide gel is pH dependent and partition effects as a secondary separation principle may be assumed.

To study the separation of the oligonucleotides on Bio-Gel P-2, poly U was subjected to partial hydrolysis with pancreatic ribonuclease and the hydrolyzate was fractionated on a polyacrylamide gel column.

Pancreatic ribonuclease catalyzes cleavage of the phosphodiester bond of poly U with the formation of oligonucleotides terminating in 2′,3′-cyclic phosphate derivatives which may be hydrolyzed to the corresponding 3′-phosphates. The chromatogram in fig. 2 shows the separation of a mixture of mono- and oligonucleotides obtained after partial hydrolysis of poly U by pancreatic ribonuclease.

References

[1] B.Gelotte, Naturwissenschaften 48 (1961) 554.
[2] S.Zadrazil, Z.Sormová and F.Sorm, Coll. Czech. Chem. Commun. 26 (1961) 2643.
[3] V.M.Ingram and J.G.Pierce, Biochemistry 1 (1962) 580.
[4] M.Litt and V.M.Ingram, Biochemistry 3 (1964) 560.
[5] O.W.Jones, E.E.Townsend, H.A.Sober and L.A.Heppel, Biochemistry 3 (1964) 238.
[6] A.N.Schwartz, A.W.G.Yee and B.A.Zabin, J. Chromatog. 20 (1965) 154.
[7] H.G.Zachau, Biochim. Biophys. Acta 108 (1965) 355.
[8] H.Birnboim, Biochim. Biophys. Acta 119 (1966) 198.
[9] R.Braun, Biochim. Biophys. Acta 142 (1967) 267.
[10] J.Lerner and A.I.Schepartz, J. Chromatog. 39 (1969) 132.
[11] G.Trénel, M.John and H.Dellweg, FEBS Letters 2 (1968) 74.
[12] M.John, G.Trénel and H.Dellweg, J. Chromatog. 42 (1969) 476.
[13] H.Dellweg, G.Trénel, M.John and C.C.Emeis, Mschr. Brauerei 22 (1969) 177.