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Note

Polyacrylamide gel electrophoresis of alginic acid

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Polyacrylamide gel electrophoresis has been used extensively in the study of proteins[1] but much less in work on polysaccharides. It is an unsatisfactory method for separating classes of acid mucopolysaccharides because the sieving effect of the medium does not allow separation purely on the basis of charge density. However, in the polyuronide alginic acid the ratio of carboxyl groups to sugar residues is constant and movement on gels should be related closely to the molecular weight.

Polyacrylamide gel electrophoresis has been developed in this laboratory to provide a rapid and convenient method for assessing the "quality" of alginic acid samples.

METHODS AND MATERIALS

Various commercial alginate samples were investigated. Before study they were dialysed against 0.4% NaCl containing 0.4% EDTA, pH 8.5, then against distilled water and finally lyophilised. Samples of other polysaccharides were used without purification.

Most chemicals were obtained from BDH, Poole, Great Britain. Acrylamide and N,N'-methylene bisacrylamide were recrystallised as described by Shuster[2], Alcian Blue 8GX (now known as 8G, ref. 3) was from George T. Gurr, London S.W. 9, Great Britain.

The polyacrylamide gel for the routine study of alginate samples consisted of 6% (w/v) total monomer with N,N'-methylene bisacrylamide constituting 2% of the monomer. Gels were prepared in uniform bore glass tubes, 5 mm I.D. Monomers were dissolved in 0.25 M Tris–HCl, pH 8.3 containing 0.05% of the accelerator TEMED (N,N,N',N'-tetramethylethylenediamine) and sufficient ammonium persulphate was added to give a final concentration of 0.08%. Aliquots (1.0 ml) were transferred from the bulk to the tubes using an automatic pipette. At 20° polymerisation was complete 20 min after the addition of the ammonium persulphate.

A discontinuous buffer system, based on the strategy of Williams and Reisfeld[4] was used. The sample gel was 6% (w/v) acrylamide polymerised without cross-linker in 0.02 *M* MOPS (3-(N-morpholino)propanesulphonic acid)–NaOH, pH 7.3. Alginate samples (20–60 μg in water) were mixed with the viscous sample gel (100 μl) *in situ* in the electrophoresis tube and 5 μl of 0.01% bromophenol blue was added as a

marker. The running buffer (0.1 *M* glycine–Tris, pH 9.0) was layered on top of the sample gel.

Electrophoresis proceeded for 90 min at 20° under 100 V (approx. 4 mA per tube). Gels were removed from the glass tubes by rimming with a syringe needle lubricated with water, and stained overnight in 0.08% Alcian Blue in 7% (v/v) acetic acid. Excess Alcian Blue was removed electrophoretically.

Alcian Blue is a permanent stain and gels may be stored indefinitely in the light in 7% acetic acid without appreciable fading. Records of gels were made either photographically or by scanning in a Joyce, Loebl & Co. Chromoscan densitometer.

RESULTS AND DISCUSSION

Electrophoresis of an alginic acid sample on gels of different pore sizes (Fig. 1) produced a broad band of slow-moving material and a sharp band of anodic material that coincided with the electrophoretic front, indicated in unstained gels by a band of marker dye. The maximum spread of stained material occurred in the gels of largest pore size but, since 4% and 5% gels with 2% cross-linker are soft and difficult to handle, 6% gels with 2% cross-linker were chosen as standard gels for further studies.

Fractionation of an alginate sample on Sephadex G-200 followed by gel electrophoresis confirms that the anodic band is due to material of low molecular weight (Fig. 2). The sample used here is an extreme case, being very polydisperse; a sample of sodium alginate supplied by BDH is much less disperse.

The method has been used for enzyme studies. Fig. 3 indicates the effect of incubating alginate with an alginate lyase partially purified from a marine *Pseudomonad*. This indication of enzyme action can be achieved rapidly without the use of viscometers or chemical methods for detecting breakdown products.

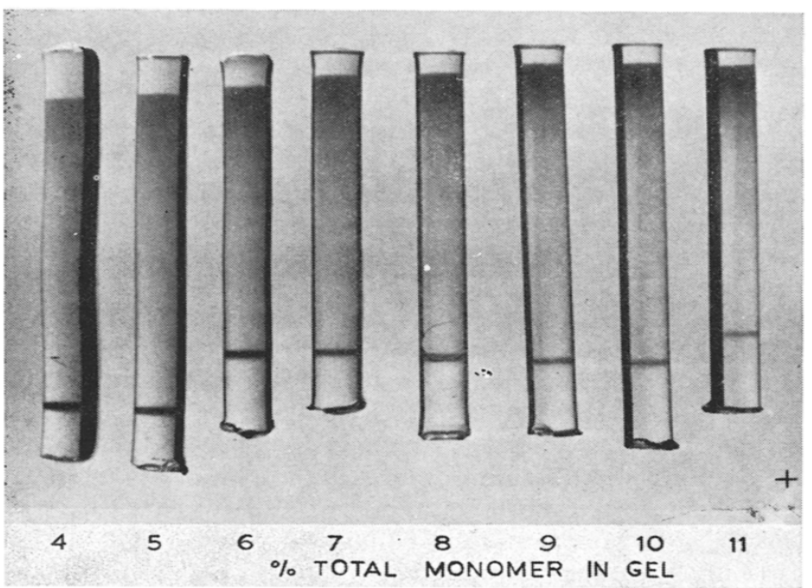

Fig. 1. Electrophoresis of sodium alginate (20 μg) with acrylamide contents from 6 to 11%. Each gel contained N,N'-methylene bisacrylamide as 2% of the total monomer.

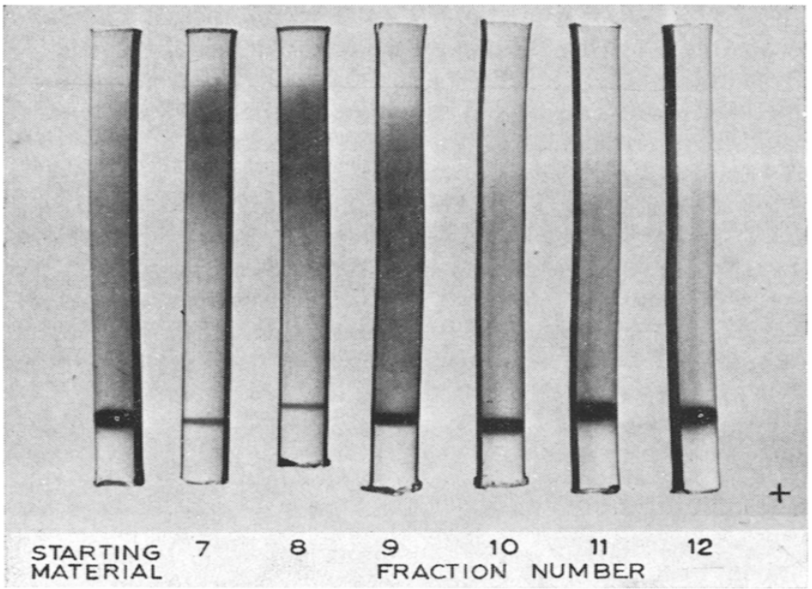

Fig. 2. Electrophoresis of sodium alginate fractionated by gel permeation on Sephadex G-200 using 6% gels.

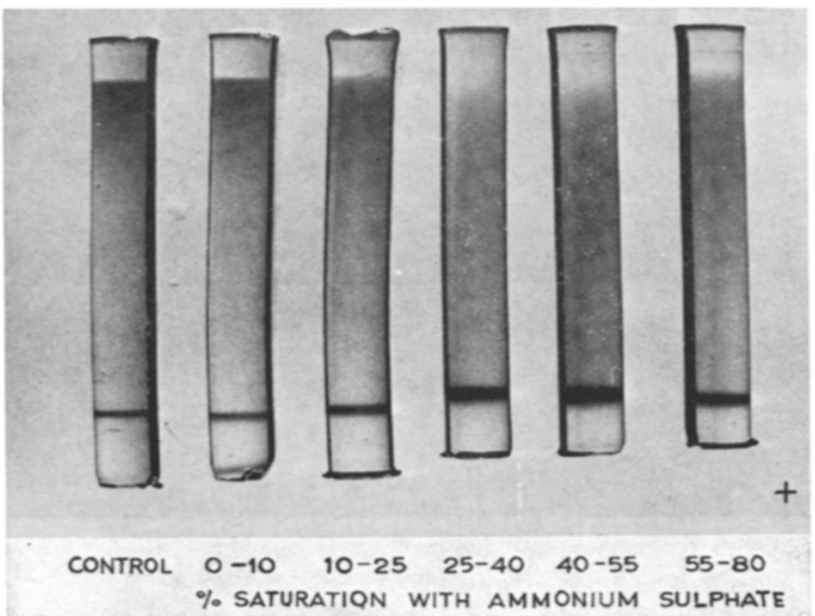

Fig. 3. Electrophoresis of alginate samples after incubation with ammonium sulphate fractions of protein from a marine bacterium.

Although the method was developed with the aim of investigating alginic acid preparations it is equally applicable to other acidic polysaccharides such as pectins and possibly carrageenans if it is known that the charge density is constant throughout the sample. The method can be used semi-quantitatively when coupled with densitometry but since the dye used, Alcian Blue 8GX, is probably a mixture of four geometrical isomers[3] the dye–alginate complex may not be stoichiometric.

The molecular weights of alginates could be determined using this method if samples of accurately-known molecular weight were available. The first fraction from Sephadex G-200 (Fig. 2) is totally excluded and will have a molecular weight in the range 400,000 to 800,000; the last fraction will have a molecular weight of around 20,000. A wider range of molecular weights can be studied using smaller pore gels in addition to those used here. Such gels would be of use in studying food-grade pectins.

REFERENCES

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