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Polyacrylamide gel electrophoresis and gel filtration of dyed polysaccharides

At present, polyacrylamide gel electrophoresis is a widely used method in the field of biopolymer chemistry. However, application of this useful and selective method for resolving mixtures of polysaccharides has hitherto been limited to glycoproteins[1] and mucopolysaccharides[2]. Recently, we have shown that the method can be applied to the analysis of neutral and uronic acid-containing polysaccharides[3]. The method possessed a satisfactory resolving power and reproducibility. Nevertheless, this technique had one substantial disadvantage; detection of polysaccharides

was rather difficult and insufficiently sensitive. The same disadvantage is inherent in the analytical gel filtration of polysaccharides on Biogels and Sephadex, because the most common phenol–sulphuric acid method of detection is time-consuming.

In recent years, information[4,5] concerning new types of reactive Procion dyes which form stable, covalent linkages with dyeing material has been reported. The use of these dyes in carbohydrate chemistry has also been described[6,7]. DUDMAN AND BISHOP[7], in particular, have conducted studies on the electrophoresis of dyed polysaccharides on cellulose acetate strips to eliminate the problem of detection. In the present work, the dyed polysaccharides were found to be satisfactorily resolved and characterised by the use of polyacrylamide gel electrophoresis and analytical gel filtration.

Experimental

Polysaccharides. The polysaccharides used in this study were samples which were available in our laboratory as follows: commercial samples of amylopectin, dextrans (mol. weight 15,000–20,000 and 60,000–90,000), and pectin; laminarin was isolated from *Laminaria japonica* as usual; sargassan[8], pelvetian[9], and zosterine[10] and its fragment (galacturonan) were also used.

Synthesis of the reactive dye. A solution of 3.6 g (0.02 moles) of cyanuric chloride (CC) in acetone (12 ml) was added over a period of 10 min at 0° to 6.2 g (0.01 moles) of Amido Black 10B (AB10B) dissolved in water (14 ml). The hydrochloric acid produced was neutralised with sodium carbonate. The mixture obtained was stirred for 2 h at room temperature, and the pH was then adjusted to 6.8 with phosphate buffer. The precipitate was filtered and dried *in vacuo* over P_2O_5 to furnish the dye CCAB10B; the yield was 5.6 g.

Dyeing procedure. The dyeing of the polysaccharide (50 mg) was achieved as described by DUDMAN AND BISHOP[7].

Electrophoresis. Polyacrylamide gels (4.6% acrylamide) were prepared according to the procedure of DAVIS[11]. The apparatus and technique for the electrophoresis of undyed polysaccharides has been described previously[3]. The following buffers were used: (1) boric acid (0.6 g) + EDTA sodium salt (1 g) + Tris (10 g) in 1 l of water (pH 9.2); (2) sodium tetraborate (2 g) + EDTA sodium salt (1 g) + Tris (10 g) in 1 l of water (pH 9.3).

Procedure. The solution (0.02–0.03 ml) of dyed polysaccharides (15–200 μg) in mixture of 15% Cyanogum 41 (0.7 ml) and buffer 1 or 2 (0.3 ml) was applied as layer on top of the gel, and voltage (200 V, 1 mA per tube) was immediately applied and the electropherogram run for 20–25 min. The voltage was then increased to 400 V, 7 mA per tube, and electrophoresis was continued under these conditions for 2.5–3 h; the migration of the bands was followed visibly. The coloured polysaccharide bands were sharp and easily visible on the electropherogram. The colour obtained was stable at least for 48 h.

Gel filtration. Biogel P ("Bio-Rad" Laboratories, Richmond, Calif., U.S.A.) were used for the gel filtration. The dyed polysaccharides (10–20 mg) or their mixtures dissolved in water (1–2 ml) were applied on the Biogel columns (2.0 × 25 cm) and eluted with water, and migration of the zones was followed visibly. Simultaneously, gel filtration of undyed polysaccharides was carried out under the same conditions. The fractions collected were tested using the phenol–sulphuric acid procedure[12].

Results and discussion

In the present study, the syntheses of dichlorotriazine dyes using cyanuric chloride and the chromophore groups: Amido Black 10B, Variaminic Blue and Congo Red, were carried out. The dyes CCAB10B, CCVB and CCCR, respectively, were obtained and used for dyeing polysaccharides according to the procedure of DUDMAN AND BISHOP[7]. It should be noted that the most satisfactory dye found was CCAB10B,

TABLE I

OPTICAL CHARACTERISTICS OF POLYSACCHARIDES DYED WITH CCAB10B

Compound	*Uronic acid content (%)*	λ_{max} *(mμ)*	$E^{1cm}_{1\%}$ *at* λ_{max}
CCAB10B dye	—	590	41.5
Laminarin	0	530	1.50
Amylopectin	0	530	2.15
Dextran ($15–20 \cdot 10^3$)	0	520	0.54
Dextran ($60–90 \cdot 10^3$)	0	525	0.55
Sargassan	20	520	0.74
Pelvetian	20	530	0.84
Zosterine[a]	60	600	0.23
Commercial pectin[a]	78–86	535	0.52
Galacturonan[a]	99.0	600	0.25

[a] Pretreated with CH_2N_2.

due to its good solubility in water and high extinction coefficient (Table I), while CCVB was poorly soluble in water and CCCR possessed a small extinction coefficient. The list of dyed polysaccharides and their optical characteristics are given in Table I. As can be seen from Table I, the incorporation of the dye into these polysaccharides was found to vary with the nature of polysaccharide. The dyed and undyed polysaccharides had a similar qualitative sugar composition, as was shown using acid hydrolysis and paper chromatographic examination. All of the dyed polysaccharides, which were sufficiently coloured, were subjected to electrophoretic examination. Because the samples were visible, it was a simple matter to experiment with variables to establish the most favourable conditions for electrophoresis. The most satisfactory results were obtained under the conditions described under *Experimental*. The sharpest bands of polysaccharides were observed in these circumstances. The results of the electrophoretic examination of the polysaccharide samples and some synthetic mixtures are shown in Figs. 1 and 2. Fig. 1 shows that commercial samples of amylopectin and dextrans afford several sharp and, broad bands to demonstrate hetrogeneity of these compounds on the contrary purified sargassan and pelcetian furnish sharp bands indicating a rather high homogereity of these polysaccharides. The separation of the synthetic mixtures was also excellent. Self-evidence, simplicity and reproducibility are characteristic proper- ties of the method. It is noteworthy that the dyed polysaccharides possessed higher electrophoretic mobility than the undyed ones. Nevertheless, the electrophoretic patterns were similar in both cases.

Gel filtration of dyed polysaccharides led to successful results. The separations of the mixture of dextrans and that of dextran (mol. weight 60,000–90,000) and pelvetian were run as models using Biogels P-60 and P-100 in the first and Biogel P-150 in the latter case. Satisfactory results were obtained in both cases. It is note-

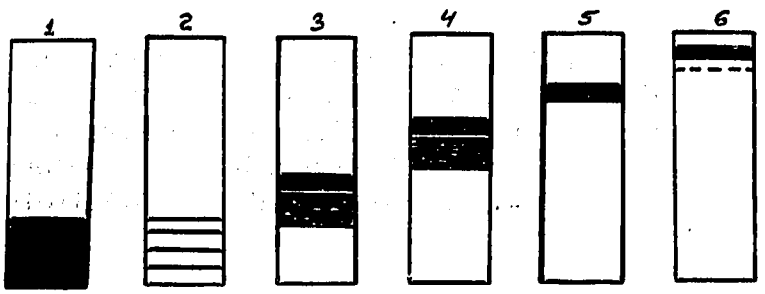

Fig. 1

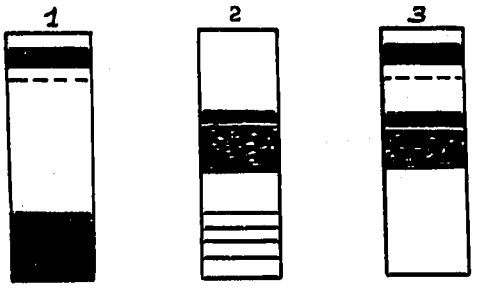

Fig. 2

Fig. 1. Polyacrylamide gel electrophoresis of dyed polysaccharides. 1 = laminarin; 2 = amylopectin; 3 = dextran (mol. weight 15,000–20,000); 4 = dextran (mol. weight 60,000–90,000); 5 = sargassan; 6 = pelvetian.

Fig. 2. Polyacrylamide gel electrophoresis of synthetic mixture of dyed polysaccharides. 1 = laminarin + pelvetian; 2 = amylopectin + dextran (60,000–90,000); 3 = dextran (60,000–90,000) + pelvetian.

worthy that the progress of gel filtration of dyed polysaccharides can be followed with a Uvicord and an automatic recorder to give the elution curve. Simultaneously, the elution curves of the gel filtration of parent polysaccharides were obtained and found to be similar.

The analytical value of gel filtration of the dyed polysaccharides is obvious. The samples were visible on the Biogel column immediately and, consequently, the process could be stopped at any time affording valid results rather than carrying out a long procedure using the phenol–sulphuric acid method of analysis of the separated fractions.

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