Carbohydrate Research, 66 (1978) 299–302

Note

Preparation of affinity-chromatography media from soluble polysaccharides by cross-linkage with divinyl sulfone*†

N. MARTIN YOUNG and MYRON A. LEON

Division of Biological Sciences, National Research Council of Canada, Ottawa K1A OR6 (Canada), and Dept. of Immunology and Microbiology, Wayne State University School of Medicine, Mich. 48201 (U.S.A.)

(Received November 7th, 1977; accepted for publication, December 18th, 1977)

The availability of simple preparative methods based on affinity chromatography has been a stimulus to research on lectins[1], beginning with the use of Sephadex G75 by Agrawal and Goldstein[2] to prepare concanavalin A. For the purification of such D-galactose-specific lectins as those from *Bandeiraea simplicifolia*[3] and *Arachis hypogaea*[4] (the peanut), two types of affinity-chromatography media have been described. In the first, derivatives of 2-acetamido-2-deoxy-D-galactose[5] or D-galactose[6] are prepared and either coupled to agarose beads *via* activation with cyanogen bromide[5], copolymerized with acrylamide[6], or coupled to aminoethyl-poly(acrylamide) by reductive amination[7]. The second approach avoids the multi-stage syntheses of the first by employing guaran (guar gum), a soluble galactomannan. The guaran may be physically entrapped in beads of poly(acrylamide) gel[6,8] or cross-linked by epichlorhydrin[9]. Beads of agarose gel may also be used directly as affinity media, but their capacity is low unless they are first subjected to mild acid hydrolysis[10].

Divinyl sulfone has been used to couple materials to beads of agarose gel[11,12] and to improve the mechanical properties of agarose beads[13,14]. We report a simple procedure for making cross-linked gels from such soluble polysaccharides as guaran by using this reagent. These gels have good flow properties and chemical stability, and are therefore suitable for affinity chromatography.

The appropriate level of reagent for cross-linking was explored in preliminary experiments with the dextran B1355S1,3. The capacity of gels prepared from this dextran with various amounts of divinyl sulfone was measured for concanavalin A. The capacity fell rapidly as the amount of cross-linker was increased, until a plateau was reached where capacity seemed relatively insensitive to the concentration of cross-linker (Fig. 1). The amount of divinyl sulfone may therefore be chosen in this region on the basis of mechanical properties rather than capacity. Guaran cross-linked with 5% of divinyl sulfone proved a suitable medium for preparing the lectins from *B. simplicifolia* (Fig. 2) and *A. hypogaea*. The yields were similar to those by other methods in the literature and the products were of high purity, as judged by disc-gel electrophoresis.

*Dedicated to Dr. Allene R. Jeanes on the occasion of her retirement.

†N.R.C.C. Publication No. 16843.

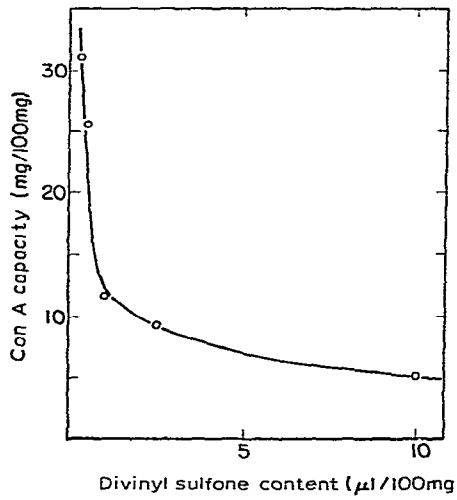

Fig. 1. Capacity of divinyl sulfone cross-linked gels (prepared from dextran B1355S1,3) for concanavalin A. The ordinate represents the amount of protein released by methyl α-D-glucoside from 100 mg of cross-linked dextran, and the abscissa the amount of divinyl sulfone (μl) added to the dextran to cross-link it.

We have also successfully prepared divinyl sulfone–cross-linked gels from other soluble polysaccharides, including the yeast phosphomannan Y1842 and a number of dextrans. These products were used in immunochemical work on the bovine-serum protein conglutinin, which reacts with Y1842 (N.M. Young and M.A. Leon, unpublished results) and the dextran-specific murine myeloma protein[15] MOPC 104E. During these experiments, agglutination of the gel particles by the proteins was observed, and it is possible that cross-linked polysaccharides could provide a stable material for agglutination assays of lectins and antibodies.

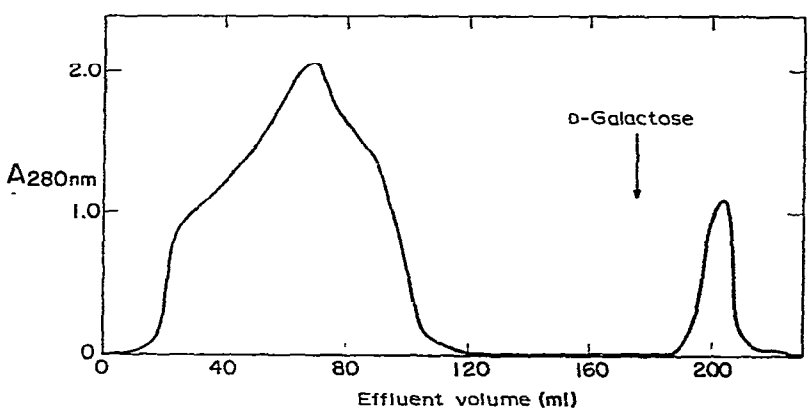

Fig. 2. Purification of *B. simplicifolia* lectin by affinity chromatography on guaran, cross-linked by divinyl sulfone.

EXPERIMENTAL

Materials. — Guaran was obtained from Sigma Chemical Co., divinyl sulfone from Aldrich Chemical Co., Inc., and *B. simplicifolia* seeds from Calbiochem. Concanavalin A was prepared by the method of Agrawal and Goldstein[2]. Dextran B1355S1,3 was kindly provided by Dr. Allene Jeanes.

Divinyl sulfone–dextran. — Aliquots of a solution of dextran B1355S1,3 (200 mg/ml) in 0.2M sodium hydroxide were treated with various amounts of divinyl sulfone. The required amount of reagent was measured onto the tip of a spatula and thoroughly mixed into the solution. After 1 h at room temperature, the solid products were broken up, mixed with water, and ground in a Ten Broeck tissue grinder until finely divided. The products were washed with water, and dehydrated by successive washes with 50% aqueous 2-propanol, 100% 2-propanol, and finally ether.

The capacities of the divinyl sulfone–dextran gels for concanavalin A were measured by incubating 1 ml of a 2% suspension of the gel with 1 ml of 10 mg/ml concanavalin A in triethanolamine-buffered saline[16], pH 7.4, for 15 min at 37°. The gels were washed with the buffer by centrifugation, and the adsorbed concanavalin A was eluted with 0.5M methyl α-D-glucoside. The amounts of concanavalin A were determined from the absorption at 280 nm of the extracts by using the extinction coefficient $E^{1\%}_{280\,nm} = 12.6$.

Divinyl sulfone–guaran. — Guaran (2 g) was made into a smooth paste with 20 ml of 0.2M sodium hydroxide and 100 μl of divinyl sulfone was thoroughly mixed into it. After 90 min, the gel was broken up and washed as already described. The gel particles were roughly fractionated by suspending them in 500 ml of water in a 500-ml measuring cylinder and collecting the material settling after 1 h. The yield was approximately 50 ml of settled gel. Upon drying, 30 mg of crosslinked polysaccharide was recovered per ml of settled gel (an overall yield of ~ 75%). A column (1.6 × 19 cm) was prepared from the gel and equilibrated with 0.9% sodium chloride. The flowrate was 20 ml/h.

Purification of A. hypogaea *lectin.* — An extract was prepared from 95 g of milled, defatted peanuts as described by Lotan *et al.*[4]. The material precipitated by 60% saturation with ammonium sulfate was redissolved and dialyzed against 0.9% sodium chloride. After centrifugation, the extract (175 ml) was applied to the divinyl sulfone–guaran column, which was then washed with 0.9% sodium chloride. The lectin was eluted with 0.1M D-galactose in 0.9% sodium chloride; yield 152 mg. Lotan *et al.*[4] obtained 147 mg from 100 g of defatted meal. The product showed a single band in disc-gel electrophoresis.

Purification of B. simplicifolia *lectin.* — An extract was prepared from 100 g of defatted *B. simplicifolia* meal by the method of Hayes and Goldstein[3] and the material precipitating between 55 and 75% saturation by ammonium sulfate was collected, redissolved and dialyzed against phosphate-buffered saline containing calcium[3]. The dialyzed extract was fractionated on the guaran column in the same buffer. The

lectin was eluted with 0.1M D-galactose in buffer; yield 88 mg. From the same amount of meal, Hayes and Goldstein[3] obtained 110 mg and Horisberger[8], 96 mg. In disc-gel electrophoresis, the product gave a single band at acid pH, and multiple bands at alkaline pH, as previously reported[3].

ACKNOWLEDGMENTS

We thank Karen Tippett, an NRCC summer student, for technical assistance. This work was supported in part by Grant CA-20812 from the National Cancer Institute.

REFERENCES

1 H. Lis, R. Lotan, and N. Sharon, *Ann. N.Y. Acad. Sci.*, 234 (1974) 232–238.
2 B. B. L. Agrawal and I. J. Goldstein, *Biochim. Biophys. Acta*, 147 (1967) 262–271.
3 C. E. Hayes and I. J. Goldstein, *J. Biol. Chem.*, 249 (1974) 1904–1914.
4 R. Lotan, E. Skutelsky, D. Danon, and N. Sharon, *J. Biol. Chem.*, 250 (1975) 8518–8523.
5 J. A. Gordon, S. Blumberg, H. Lis, and N. Sharon, *FEBS Lett.*, 24 (1972) 193–196.
6 K. Sutoh, L. Rosenfeld, and Y. C. Lee, *Anal. Biochem.*, 79 (1977) 329–337.
7 R. J. Baues and G. R. Gray, *J. Biol. Chem.*, 252 (1977) 57–60.
8 M. Horisberger, *Carbohydr. Res.* 53 (1977) 231–237.
9 J. Lönngren, I. J. Goldstein, and R. Bywater, *FEBS Lett.*, 68 (1976) 31–34.
10 H. J. Allen and E. A. Z. Johnson, *Carbohydr. Res.*, 50 (1976) 121–131.
11 J. Porath and L. Sundberg, *Nature New Biol.*, 238 (1972) 261–262.
12 L. Sundberg and J. Porath, in H. Peeters (Ed.), *Protides of the Biological Fluids*, 23 (1975) 517–523.
13 J. Porath, T. Laas, and J.-C. Janson, *J. Chromatogr.*, 103 (1975) 49–62.
14 T. Laas, in ref. 12, pp. 495–503.
15 M.A. Leon, N. M. Young, and K.R. McIntire, *Biochemistry*, 9 (1970) 1023–1030.
16 J. J. Kent, A. G. Otero, and R. E. Harrigan, *Am. J. Clin. Pathol.*, 27 (1957) 539–545.