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Note

Fluorescein-labeled *O*-glycosyloxyalkenyl–aminoalkenyl–acrylamide copolymers in lectin-saccharide binding studies*,†

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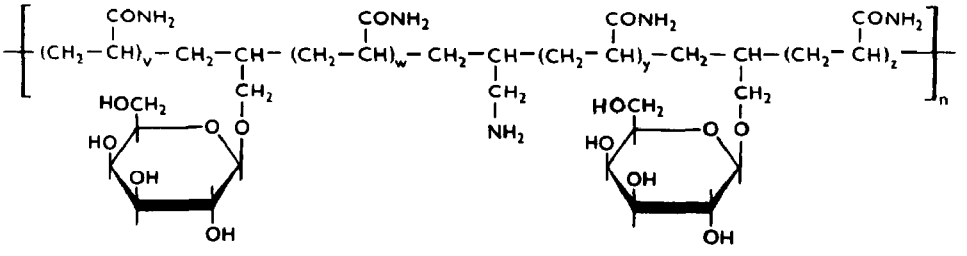
Water-soluble poly(*O*-glycosylacrylamide) [poly(*O*-glycosyloxyalkenylacrylamide)] copolymers, described by Hořejší *et al.* [1] have been widely used for the preparation of affinity gels for affinity electrophoresis of lectins[2–4] or proteins interacting with saccharides as, *e.g.*, D-galactose oxidase[5] or α-D-galactosidase[6,7]. In addition, these poly(acrylamide) copolymers are useful tools in precipitation of lectins and, in general, as synthetic model substances to study interactions of lectins with saccharides[1]. Poly(*O*-glycosylacrylamide) copolymers are substitutes for natural polysaccharides and glycoproteins, or for semisynthetic conjugates containing sugar residues attached to serum albumin in studies on sugar-binding specificities of lectins. Poly (*O*-glycosylacrylamide) copolymers are prepared by copolymerization of alkenyl glycosides and acrylamide.

By copolymerization of acrylamide, allylamine, and an allyl glycoside, *O*-glycosyl derivatives of poly(acrylamide) containing free amino groups were prepared as described in the Experimental section[8–10] (see Structure 1). The sugar content could be varied by changing the amount of allyl glycoside used in the copolymerization reaction.



Structure 1. Tentative structure of a poly(*O*-α-D-galactopyranosylacrylamide) copolymer containing free amino groups.

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TABLE I

Content of sugars and amino groups in poly(*O*-glycosylacrylamide) copolymers (COP)

Copolymer	*Sugar content*		*Amino groups (mmol/100 g COP)*
	(mmol/100 g COP)	*(g/100 g COP)*	
α-D-Glc*p*-COP-NH_2	44	8.0	6.3
α-D-Gal*p*-COP-NH_2	40	7.2	3.5
COP-NH_2			5.3

TABLE II

Degree of modification of amino groups in poly(*O*-glycosylacrylamide) copolymers (COP)

Copolymer	*FITC (mmol/100 g COP)*	*Modified amino groups (%)*
α-DGlc*p*-COP-NH-FITC	1.6	26.6
α-D-Gal*p*-COP-NH-FITC	1.0	28.0
COP-NH-FITC	2.4	45.7

Modification of the free amino groups with fluorescein isothiocyanate (FITC) yielded soluble copolymers having covalently-bound fluorescein and glycosyl groups. FITC-labeled poly(*O*-glycosylacrylamide) copolymers could be purified by affinity chromatography on immobilized lectins. The content of sugar residues, free amino groups, and FITC label in the copolymers, used in further experiments, is given in Tables I and II. The molecular masses of the FITC-labeled copolymers were estimated to be in the range of 380 000–400 000 from the measurements of the limit-viscosity number and from the behavior on a TSK-Gel SW column in h.p.l.c., in which the copolymers yielded one peak at an elution volume that was very close to the void volume of the TSK-Gel 3000 SW column.

Affinity electrophoresis showed that the presence of free amino groups and FITC-modified amino groups does not influence the ability of sugar residues to interact with lectins. The dissociation constants of the complex of pea lectin and immobilized α-D-glucopyranosyl residues (K_i) were determined by use of the following copolymers, *O*-α-D-glucopyranosyl copolymer without amino groups, *O*-α-D-glucopyranosyl copolymer with amino groups, and FITC-labeled *O*-α-D-glucopyranosyl copolymer; the values of K_i obtained were very close to each other, 28, 32, and 33mM, respectively. Affinity chromatography showed also that the FITC-labeled *O*-glycopyranosyl copolymers interact specifically with immobilized lectins according to the lectin sugar-binding specificity, *i.e.*, immobilized concanavalin A binds specifically the *O*-α-D-glucopyranosyl copolymer (Fig. 1), and the interaction is inhibited by the presence of D-glucose in the starting eluent buffer. On the other hand, the FITC-labeled *O*-α-D-galactopyranosyl copolymer of the FITC-labeled poly(acrylamide) copolymer without sugar residues is not retained by immobilized concanavalin A. Immobilized soybean

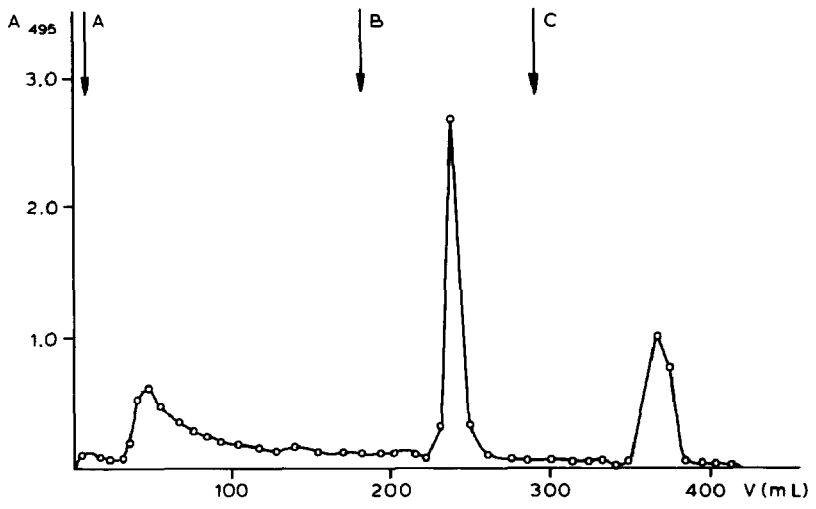

Fig. 1. Affinity chromatography on concanavalin A-Sepharose of α-D-glucopyranosyl-COP-NH-FITC eluted with: (A) 0.05M phosphate buffer, pH 7.8; (B) 0.2M D-glucose; and (C) M NaCl.

lectin specifically binds the FITC-labeled *O*-α-D-galactopyranosyl copolymer, whereas no interaction was observed for the FITC-labeled *O*-α-D-glucopyranosyl copolymer (not shown).

Both poly(*O*-glycosylacrylamide) copolymers containing free amino groups and FITC-labeled *O*-glycosyl copolymers yielded precipitates with lectins upon double diffusion in agarose gel. Lectins from the following seeds were used: *Pisum sativum, Glycine soja, Arachis hypogaea,* and *Canavalia ensiformis*. FITC-labeled poly-(*O*-glycosylacrylamide) copolymers as well as *O*-glycosyl copolymers with free amino groups yieded precipitates with lectins according to the sugar-binding specificity of the lectins *i.e.*, the lectin from seeds of *P. sativum* and *C. ensiformis* only with *O*-α-D-glucopyranosyl copolymer, and the lectins from seeds of *G. soja* and *A. hypogaea* only with *O*-α-D-galactopyranosyl copolymer. The FITC-labeled copolymer devoid of bound sugar residues yielded a precipitate only with concanavalin A.

Preliminary experiments have shown that the FITC-labeled poly(*O*-glycosyl-acrylamide) copolymers are very useful for the detection of lectins or saccharide-binding proteins after their electrophoretic separation and electroblotting on nitrocellulose membranes.

EXPERIMENTAL

Materials. – Concanavalin A and the lectin from *P. sativum* seeds were isolated by affinity chromatography on *O*-α-D-mannosyl–Spheron H-1000, and the lectins from seeds of *G. soja* (soybean) and *A. hypogaea* (peanut) on *O*-α-D-galactosyl–Spheron H-1000[11]. Poly(*O*-glycosylacrylamide) copolymers without free amino groups were

prepared as described by Hořejší *et al.*[1]. Concanavalin A and soybean lectin were bound to CNBr-activated Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) according to the procedure recommended by the producer.

Preparation of poly(O-glycosylacrylamide) copolymers containing amino groups. — Acrylamide (400 mg) and allyl glycoside (200–400 mg) were mixed with a solution containing allylamine (100–300 μL) in 0.2M phosphate buffer adjusted to pH 7.0. After dissolution, the volume was adjusted to 8 mL and $(NH_4)_2S_2O_8$ solution (200 μL of a 10% solution, freshly prepared) and *N,N,N',N'*-tetramethylethylenediamine (TEMED; 5 μL) were added. The solution was heated on a water bath so that the temperature rose from 20 to 100° within 3–5 min. After being cooled, the solution was diluted with the same volume of distilled water and exhaustively dialyzed against distilled water (at least 72 h, several changes of water), and then lyophilized. Copolymers were prepared from a mixture containing acrylamide (400 mg), allyl α-D-glucopyranoside or allyl α-D-galactopyranoside (400 mg), and allylamine (200 μL). For control experiments, a copolymer without sugar residues was prepared from a mixture of acrylamide (400 mg) and allylamine (200 μL).

Preparation of FITC-labeled poly(O-glycosylacrylamide) copolymers. — To a solution of poly(*O*-glycosylacrylamide) copolymer (500 mg) in 0.15M NaCl (50 mL), was added FITC (20 mg) and the pH of the solution was adjusted to 9.0. The solution was kept for 24 h at 4°, and after the first hour, the pH was readjusted to 9.0. The solution was dialyzed at 4° (2 × 24 h) against a 1% suspension of charcoal and then against distilled water (3 × 24 h); finally the solution was lyophilized.

Affinity chromatography on immobilized lectins. — Affinity chromatography of FITC-labeled poly(*O*-α-D-glucopyranosyl- or *O*-α-D-galactopyranosyl-acrylamide) copolymers was performed on columns of Con A-Sepharose (2.2 × 17 cm) or soybean lectin-Sepharose (2.2 × 11 cm), respectively in 0.05M phosphate buffer at pH 7.8. After elution of nonadsorbing material with the starting buffer, the adsorbed material was eluted with 0.2M D-glucose or 0.2M D-galactose, respectively, and then with M NaCl. The chromatography was monitored by measurement of A_{495}. The collected fractions were dialyzed against distilled water and lyophilized. For analytical purposes, small columns (0.6 × 4 cm) were used.

Liquid chromatography. — H.p.l.c. used a column of TSK-Gel SW type (7.5 mm d. × 30 cm) and the LKB System, equipped with an HPLC Pump 2150, 2151; the eluent was 0.05M phosphate buffer, pH 7.8, containing 0.3M NaCl at a flow rate of 60 mL/h and detection at 495 nm.

Analytical methods. — Neutral sugar content in lyophilized copolymers was estimated by the phenol–H_2SO_4 method[12] using the corresponding free sugars as standards. The content of free amino groups in lyophilized copolymers was determined by the method described by Habeb[13]. The amount of bound FITC residues in poly(acrylamide) copolymers was estimated by measurement of A_{495} (ε_{495} = 70 000 dm^3 mL^{-1} cm^{-1}). The limit-viscosity number was determined with an Ubbelohde viscosimeter for FITC-labeled copolymer solution in 0.05M phosphate buffer, pH 7.8. Affinity electrophoresis was performed and the dissociation constants of lectin–immobilized saccharide complexes were determined as described previously[3].

Double diffusion in agarose gels. — Double diffusion in agarose gels was performed as described in the LKB manual[14]. A 2% solution of copolymer and a 2% solution of lectin (5–10 μL each) were applied to wells in 3% agarose gel in Tris·barbiturate buffer, pH 8.6.

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