*Carbohydrate Research*, 128 (1984) 160–164
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

## Note

# Enzymic synthesis of oligosaccharides on a polymer support, light-sensitive, water-soluble substituted poly(vinyl alcohol)*

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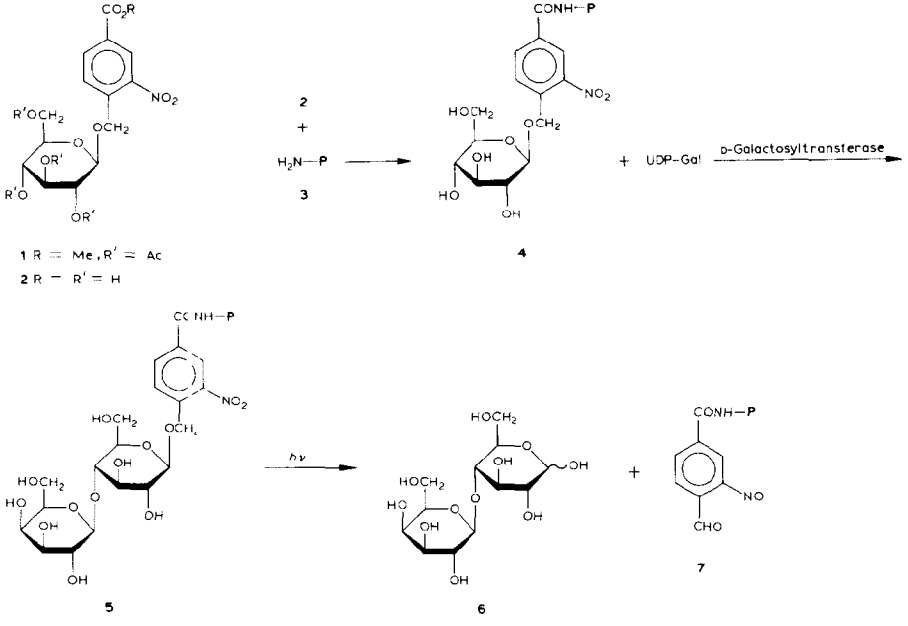
(Received April 19th, 1983; accepted for publication, September 1st, 1983)

In a preceding paper[1], we have established that saccharide derivatives can be linked to a polymer and serve as acceptors in glycosyltransferase and transglycosylation reactions. The resulting oligosaccharides were subsequently released efficiently by irradiation from the polymer in their free, reducing forms. The polymer used, which consisted of commercially available substituted poly(acrylamide)-gel beads, had, however, a low accessability that affected the




*Presented at the 38th Northwest Regional Meeting, American Chemical Society, Honolulu, HI, December 1983 (Abstr. 98).

0008-6215/84/$ 03.00 

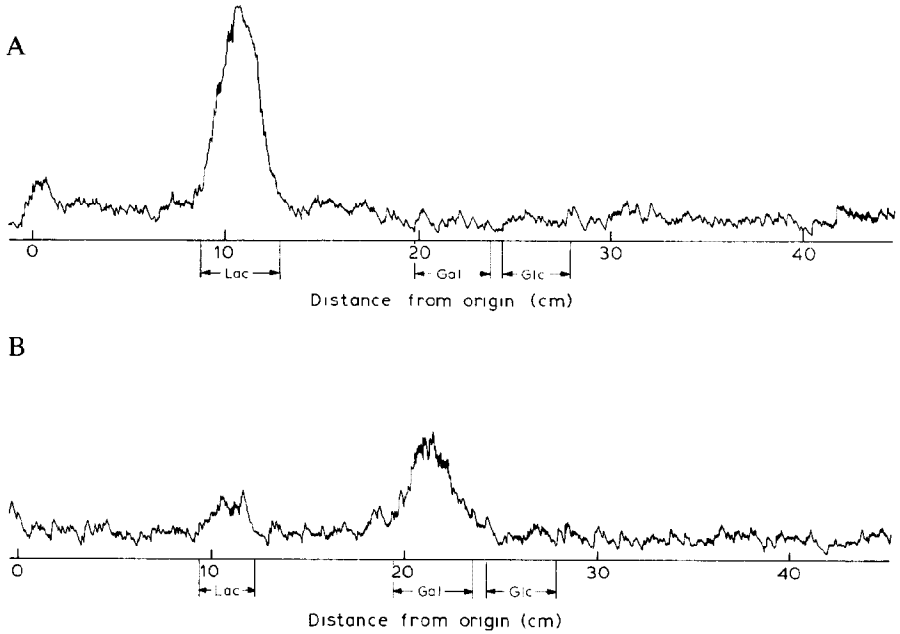



Fig. 1. Chromatographic examination of radioactive products released by irradiation of polymer **5**. Paper chromatography on Whatman 3 MM paper in descending 3:5:1:3 (v/v) pyridine–1-butanol–benzene–water (upper phase). (A) Products released (16 325 c.p.m.) by the irradiation of polymer **5**. (B) Products released (8160 c.p.m.) by the irradiation of polymer **5**, followed by β-D-galactosidase digestion.

yields of chemical and enzymic condensations. The present communication describes the use of a water-soluble polymer that provided improved accessibility. Moreover, such saccharide-bearing, water-soluble polymers may be improved acceptors in oligosaccharide-synthesis catalyzed by particulate enzyme-systems.

4-Methoxycarbonyl-2-nitrobenzyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside[1] (**1**) was deblocked to yield 4-carboxy-2-nitrobenzyl β-D-glucopyranoside (**2**). Poly(vinyl alcohol) was *p*-toluenesulfonylated, and the *p*-toluenesulfonyloxy groups were subsequently displaced by azide groups. Hydrogenation of the polymer gave amino-substituted poly(vinyl alcohol) ($H_2N$-**P**, **3**). Compound **2** and polymer **3** were condensed to yield the light-sensitive D-*gluco* polymer **4**. This polymer was an acceptor for the D-galactosyltransferase reaction[1,2] using radioactively labeled UDP-Gal which gave the *lacto* polymer **5**. Irradiation of **5** released lactose (**6**) (Fig. 1A) and polymer **7**. The two products were separated by ultrafiltration. The synthesized lactose (**6**), which was identified by chromatography and m.s. of the octaacetyl derivative, accounted for most of the radioactivity released and, as anticipated, was unaffected by α-D-galactosidase, but was readily hydrolyzed by β-D-galactosidase (Fig. 1B).

The results of the chemical and enzymic condensation steps demonstrate the

improved accessibility of the water-soluble polymers described herein as compared to the substituted poly(acrylamide)-gel beads[1], which was reflected in much higher yields. Significantly, the transferase reaction could most likely be further improved with an increased supply of UDP-Gal (~27% of which was consumed in the transfer reaction and an additional amount underwent hydrolysis). The photochemical release from the polymer proved again to be a worthwhile, efficient route for producing reducing oligosaccharides.

## EXPERIMENTAL

*General.* — Materials, methods, and equipment were as previously described[1].

*4-Carboxy-2-nitrobenzyl β-D-glucopyranoside* (**2**). — 4-Methoxycarbonyl-2-nitrobenzyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**1**) (100 mg) was deblocked[1], and the aqueous solution made neutral with Amberlite IR-120 ($H^+$), filtered, and lyophilized to yield amorphous **2** (41.2 mg, 50%) having a very low rate of migration on t.l.c. (1:1, v/v, chloroform–methanol) $R_{\alpha\text{-hydroxy-2-nitrotoluene}}$ 0.06; m.p. 60–64°, $[\alpha]_D^{25}$ +23.9 ±1.2° (*c* 0.48, water); $^1$H-n.m.r. (80 MHz, $D_2O$): δ 8.3–7.3 (aromatic), 5.33 (d, *J* 3.3 Hz, $C_6H_5CH_2$), and 4.82 ($H_2O$).

*Anal.* Calc. for $C_{14}H_{17}NO_{10} \cdot 5\ H_2O$: C, 37.41; H, 6.04; exchangeable hydrogen atoms/molecule (water determination[3]), 15.0. Found: C, 37.54; H, 6.33; 14.86 ±1.5.

*Amino-substituted poly(vinyl alcohol)* (**P-$NH_2$**, **3**). — Poly(vinyl alcohol) (88% hydrolyzed, average $M_r$ 10 000, 10 g) and *p*-toluenesulfonyl chloride (8.6 g) were stirred in pyridine (30 mL) overnight at room temperature. The solution was evaporated *in vacuo* and a solution of the residue (~3.5 g in 1.5 mL of *N,N*-dimethylformamide) was applied, in small portions, to a Sephadex LH-20 (20–100 μm; Pharmacia, Uppsala, Sweden) column (15 cm × 0.9 cm diam.), which was eluted with *N,N*-dimethylformamide. The fractions containing the polymer that stays at the origin when examined by t.l.c. in 2:1, v/v, chloroform–methanol were pooled (~90 mL). Sodium azide (4.5 g) was added to the polymer solution, and the mixture was stirred overnight in a bath (100°) and under a calcium chloride seal. The mixture was brought to room temperature, diluted with water (250 mL), dialyzed (Spectra/Por 3 bags, Spectra Medical Industries, Los Angeles, CA 90054) against water, and hydrogenated at 0.3 MPa and at room temperature in the presence of platinum(IV) oxide. The catalyst was removed by filtration, and the solution dialyzed as just described and lyophilized to yield **3** (~300 mg from a typical portion containing[4] 0.5 meq. of $NH_2$/g).

*4-(*N*-P-Amidocarbonyl)-2-nitrobenzyl β-D-glucopyranoside* (**4**). — Compound **1** (186 mg) was deblocked (without isolation) and the solution adjusted to pH 4.7 as previously described[1]. Polymer **3** (295 mg) and water (final volume 30 mL) were added, the solution stirred at room temperature, and 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride added (four portions, 100 mg in 0.6 mL of

water each). The mixture was stirred overnight, and the resulting solution was concentrated and dialyzed against a Diaflo UM2 ultrafiltration membrane (Amicon, Lexington, MA 02173). The resulting polymer **4** was used either in the aqueous solution or after lyophilization (300 mg, $\lambda_{max}^{H_2O}$ 218, 260 nm). Irradiation of polymer **4** (20 h, 7-mg samples in 4 mL of water) was carried out in an RPR-100 apparatus (Rayonet, the Southern New England Ultraviolet Company, Hamden, Connecticut 06514) with RPR 3500A lamps in Pyrex glassware. This was followed by ultrafiltration through Diaflo UM2. Determination of D-glucose[5] in the filtrate showed the release of 0.15 mmol of D-glucose/g.

*4-(*N*-**P**-4-Amidocarbonyl-2-nitro)-benzyl 4-O-β-*D*-galactopyranosyl-β-*D*-glucopyranoside* (**5**). — Polymer **4** (43.5 mg) UDP-Gal (4 mg) and UDP-D-[U-$^{14}$C]galactose (347 000 c.p.m./mg; 211 670 000 c.p.m./mmol), α-lactalbumin (5 mg), and D-galactosyltransferase (1 unit) in sodium cacodylate buffer (5 mL, pH 7.0, 25mM) containing 3mM manganese chloride and 0.1% mercaptoethanol were incubated for 18 h at 37°. The product (**5**) was purified by extensive dialysis (Diaflo UM2) until only very little radioactivity (4 × blank) emerged in the eluates, a slight turbidity was removed by filtration through a Celite filter, and polymer **5** (34.1 mg, 10 838 700 c.p.m./g; 51 μmol of lactose/g, 34% incorporation of D-galactose) was collected after lyophilization or further used as a water solution.

*Release of lactose.* — A 0.15% solution of polymer **5** in water was irradiated for 20 h as described for polymer **4**. Ultrafiltration (Diaflo UM2) and examination of the filtrate showed the release of 88% (9 522 200 c.p.m./g) of the radioactivity from the polymer into the filtrate; after dialysis (Diaflo UM2), the resulting irradiated polymer (**7**) still possessed 10% of the radioactivity. The filtrate was lyophilized and the residue examined by paper chromatography. Lyophilized filtrate samples were also incubated for 2 h at 37° with β-D-galactosidase (*E. coli*, EC 3.2.1.23; 3.6 units in 0.2 mL of 25mM Tris buffer, pH 7.3) or with α-D-galactosidase (coffee beans, EC 3.2.1.22; 0.35–3 units in 0.2 mL of 20mM sodium acetate buffer, pH 5.5) prior to their application to paper chromatography. Markers were made visible by the silver nitrate reagent[6], and radioactivity was located with a Packard Model 7201 Radiochromatogram Scanner.

Lactose (**6**, 100 μg) was dissolved in a mixture of acetic anhydride (0.15 mL) and pyridine (0.3 mL). The mixture was kept overnight at room temperature, ice was added, the mixture evaporated *in vacuo*, and the residue extracted with chloroform. The extract was applied to a t.l.c. plate of silica gel, developed in 1:1 (v/v) chloroform–ethyl acetate. The resulting acetate migrated at the same rate as an authentic sample of 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranose (lactose octaacetate); m.s. at 175° (LKB model 20910): *m/z* 618 (M − 60), 588 (M − 120), 457,414, 332; the spectrum was very similar to that of the authentic sample.

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