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BINDING OF ALDITOLS TO THE HYDROXYL FORM OF DOWEX-1 X8, A STRONGLY BASIC ION-EXCHANGE RESIN: AN IMPROVED METHOD FOR ESTIMATION OF THE DEGREE OF POLYMERIZATION OF NEUTRAL OLIGOSACCHARIDES AND POLYSACCHARIDES

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ABSTRACT

The hydroxyl form of a strongly basic ion-exchange resin, Dowex-1 X8, had been used by Yamaguchi *et al.* to separate alditols from monosaccharides for estimation of the degree of polymerization (d.p.) of neutral oligosaccharides and polysaccharides, because alditols passed through a column of the resin, but monosaccharides were bound to it. However, the resin has now been found to adsorb not only monosaccharides but also alditols, and it is difficult to separate them readily. Therefore, this method for estimation of the d.p. of oligosaccharides has been improved. Elution of alditols from a column of Dowex-1 X8 (OH^-) resin is influenced by methanol, ammonium acetate, and ammonium formate.

INTRODUCTION

Estimation of the degree of polymerization (d.p.) of oligosaccharides and polysaccharides is important for studies on the structure of glycans. Among the various methods used, that devised by Yamaguchi *et al.*[1–3] is the simplest and most practical. It is conducted as follows. (A) The reducing-end, carbohydrate residue of a glycan is reduced with a reducing agent to change it into an alditol residue. Then, the alditol is released from the carbohydrate chain of the reduced glycan by acid hydrolysis, and separated from large amounts of monosaccharides (derived from the intrachain carbohydrate residues) by use of the hydroxyl form of Dowex-1 X8, the fractions of eluate collected being alditol. Subsequently, a volatile derivative of the alditol is used for determination by gas–liquid chromatography (g.l.c.). (B) The total amounts of carbohydrate residues of the glycan are determined as alditols after hydrolysis of the reduced glycan, prior to additional reduction, and the d.p. is calculated from the ratio of the value determined in (B) to that from (A).

In this procedure, the strict separation of a trace of alditol from large amounts of monosaccharides, and the quantitative recovery of it prior to g.l.c., are essential. The method of Yamaguchi *et al.*[1–3] was based on the assumption that the alditols

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pass completely through the hydroxyl form of Dowex-1 X8, but that all of the monosaccharides is tightly adsorbed to the resin[1].

However, when this method was applied to estimation of the d.p. of arabinose oligomers, the recovery of arabinitol was always lower than the value expected, and showed no reproducibility. When lactose and maltotriose were used as references, it was found difficult completely to recover alditols derived from the reducing end, and this was traced to the fact that alditols are also adsorbed on Dowex-1 X8 (OH^-).

The interaction of alditols with Dowex-1 X8 resin under various conditions, and an improved method for the estimation of d.p. are now described.

RESULTS

Interaction of alditols with Dowex-1 X8

(1) *Separation of alditols on a column of Dowex-1 X8 (OH^-).* — A mixture of arabinitol, galactitol, glucitol, inositol, and glucose was applied to a column of Dowex-1 X8 (OH^-), followed by elution with distilled water. Unexpectedly, every alditol was temporarily adsorbed on Dowex-1 X8 (OH^-), as shown in Fig. 1. The binding strength decreased in the order galactitol $\approx$ glucitol > arabinitol > inositol. Galactitol or glucitol was completely recovered from the column with $\sim$12 column-volumes of water. Glucose was not eluted, even with 20 column-volumes of water.

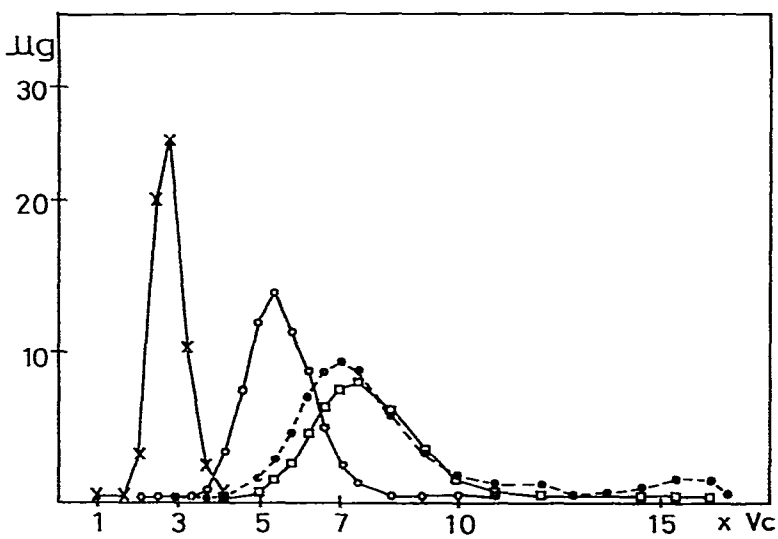

Fig. 1. Elution pattern of a sugar and alditols from a column of Dowex-1 X8 (OH^-). [A mixture of arabinitol, glucitol, galactitol, inositol, and glucose (200 μg each) was applied to a column of Dowex-1 X8 (OH^-). After elution with distilled water, xylitol was added to each 270-μL fraction as the standard. Each sample was dried, and acetylated, and the acetates were applied to a g.l.c. column as described in the Experimental section. The positions, and amounts, of eluted inositol (—×—×—), arabinitol (—○—○—), glucitol (—□—□—), and galactitol (—●—●—) were plotted. The horizontal axis is expressed as a multiple of the column volume (Vc).]

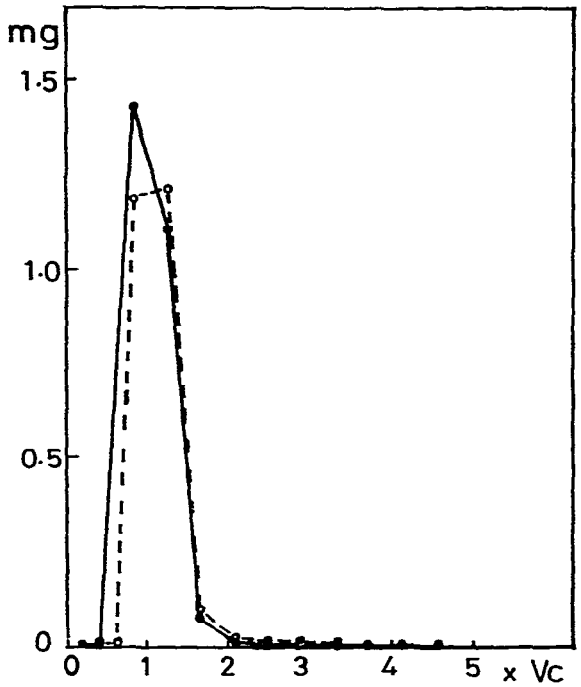

Fig. 2. Elution pattern of galactitol and glucose from a column of Dowex-1 X8 (Cl^-). [A mixture of galactitol (5 mg) and glucose (5 mg) was applied to a column of Dowex-1 X8 (Cl^-). After elution with distilled water, the galactitol (—●—●—) and glucose (--○--○--) in each fraction were determined as described in the Experimental section.]

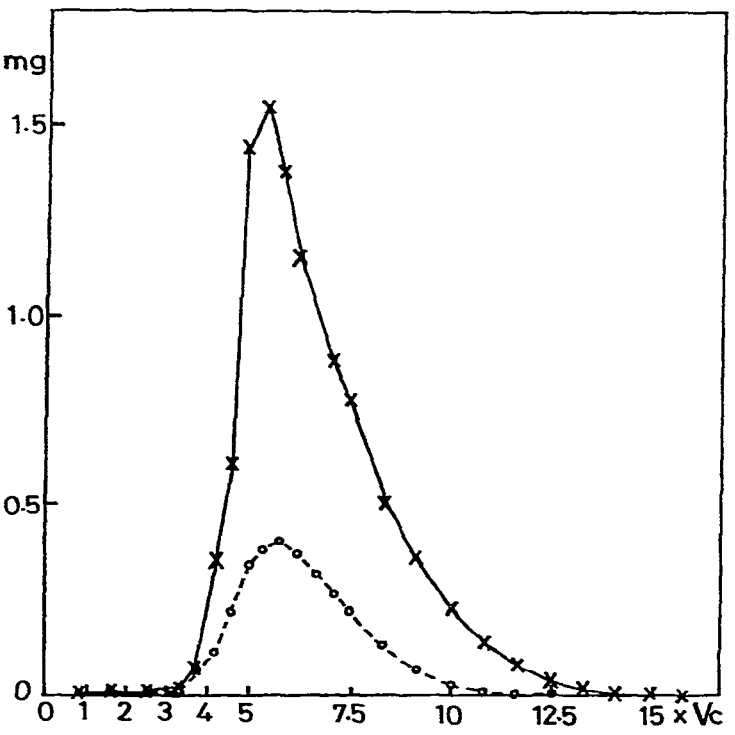

Fig. 3. Capacity of adsorption of galactitol on Dowex-1 X8 (OH^-). [Galactitol, 10 mg (--○--○--), or 40 mg (—×—×—) in 250 or 500 μL of water, respectively, were applied to a column of Dowex-1 X8 (OH^-), followed by elution with distilled water.]

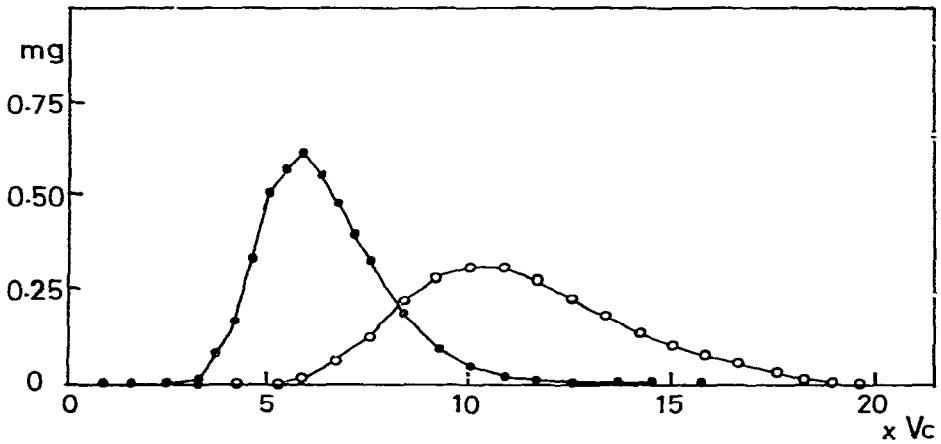

Fig. 4. Effect of methanol on the elution of galactitol from a column of Dowex-1 X8 (OH^-). [Galactitol (10 mg in 250 μL) was applied to a column of Dowex-1 X8 (OH^-) pre-equilibrated with 20% MeOH, followed by elution with the same solvent. The open circle (○) shows the effect of methanol, and the closed circle (●), the control elution with distilled water.]

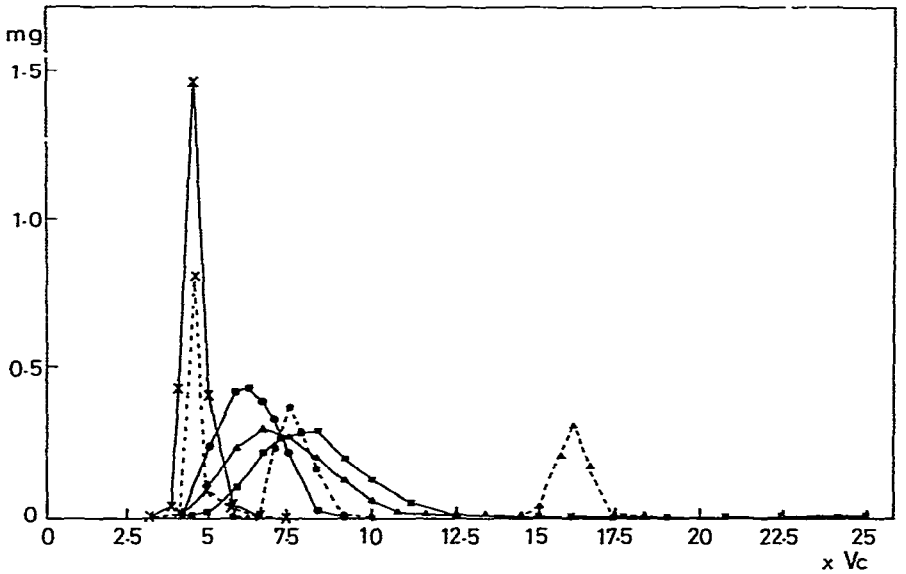

Fig. 5. Effect of ammonium acetate on the elution of galactitol and glucose from a column of Dowex-1 X8 (OH^-). [A mixture of galactitol (4 mg) and glucose (2 mg) was applied to a column of Dowex-1 X8 (OH^-), followed by elution with 50 mM (■), 100 mM (▲), 200 mM (●), or 500 mM (×) ammonium acetate. The solid lines show galactitol, and the dotted lines, glucose.]

When the acetate, hydrogencarbonate, or chloride form of Dowex-1 X8 was used instead of the hydroxyl form, all of the alditols (and, also, the monosaccharides) passed through the column. Fig. 2 shows, typically, that both galactitol and glucose passed through a column of Dowex-1 X8 (Cl^-), and the interaction of alditols and monosaccharides with the resin occurred only in the case of the hydroxyl form.

An oligosaccharide was reduced with $NaBH_4$, followed by hydrolysis under appropriate conditions dependent on the stability of the sample. The hydrolyzate, containing both an alditol and monosaccharides, was applied to a column of Dowex-1 X8 (OH^-) for separation. The alditol was recovered with 15 column-volumes of water, and then the monosaccharides were eluted with a high concentration of ammonium acetate, and also reduced to alditols. The alditol derived from the reducing-end residue and those from the intrachain monosaccharides were separately determined by g.l.c. as alditol acetates. The d.p. was calculated from the ratio of the total amount of carbohydrates to the amount of alditol derived from the reducing-end residue (see Table I).

DISCUSSION

As alditols were found to be adsorbed on Dowex-1 X8 (OH^-) resin, it was concluded that accurate estimation of d.p. by the method of Yamaguchi *et al.*[1-3] was impossible without modification thereof.

The improved method has the following advantages in comparison with the original one. In the original method, two samples were used separately for determination of the reducing-end residue and of the total carbohydrates, and so more than one column of resin was required. In contrast, the alditol and the monosaccharides were separated on a single column of Dowex-1 X8 (OH^-) in the improved method, which was so simple that samples, resin, and time were saved. In the original method, the alditol was collected as the eluate with 3.5 column-volumes of water. It was, however, likely that part of the alditol remained on the resin, whereas complete recovery of the alditol was possible by elution with 15 column-volumes of water in the improved method. For arabinose oligomers, appropriate choice of conditions of reduction and acid hydrolysis was made, to prevent degradation of arabinose and arabinitol.

Other methods were also considered for the simple separation of an alditol from a large proportion of monosaccharides. Both alditols and monosaccharides could be adsorbed on the sulfate form of strong, anion-exchange resins or the Li^+, Na^+, and K^+ form of strong, cation-exchange resins, and all of them were mutually separable under appropriate conditions[4-8]. However, these forms of resins were not suitable for the simple separation of the former from the latter group. Also, the acetate, hydrogencarbonate, or chloride form of Dowex-1 X8 was not suitable for the purpose, because they do not adsorb alditols and monosaccharides at all. Despite the aforementioned disadvantage, the use of Dowex-1 X8 (OH^-) appears to be the best yet found for the simple separation of alditols from monosaccharides as a pretreatment to g.l.c.

The binding of alditols to Dowex-1 X8 (OH^-) resin may be an example of partition chromatography. The effect of ammonium acetate may result from replacement of the hydroxyl group by the acetoxyl group, making the alditols lose their affinity for the resin, independent of the ionic strength.

(2) *The capacity of adsorption of alditols on Dowex-1 X8 (OH^-).* — Solutions of galactitol (10 or 40 mg) in water (250 or 500 μL, respectively) were applied to a mini-column containing 1.3 mL (wet volume) of Dowex-1 X8 (OH^-). Even in the case of 40 mg, the elution of the whole of the galactitol was delayed by the column, as shown in Fig. 3. The capacity of adsorption was larger than the aforementioned value, although larger amounts of galactitol could not be examined, in consideration of the solubility of galactitol and the column volume.

(3) *Effect of methanol as an eluant.* — Methanol (5 to 20%) as the eluant generally delayed the elution of alditols from a column of Dowex-1 X8 (OH^-) pre-equilibrated with the same concentration of methanol, as shown in Fig. 4.

(4) *Effect of salts on the elution of alditols.* — The elution of galactitol from the column was influenced by the concentration of ammonium acetate, as shown in Fig. 5. In addition, elution of glucose from the column was also hastened with increasing concentration of the salt. Simultaneous elution of galactitol and glucose with 500mM ammonium acetate was observed. However, they did not pass through the column, even at this high concentration of the salt. Ammonium formate showed a similar effect.

An improved method for estimation of the d.p. of oligosaccharides

Attempts were made to improve the method for estimation of the d.p. of oligosaccharides, based on the finding that the hydroxyl form of Dowex-1 X8 adsorbs both alditols and monosaccharides.

TABLE I

OBSERVED D.P., AND RECOVERY OF CARBOHYDRATES IN ESTIMATION OF THE D.P.

Carbohydrate	*D.p.*		*Recovery of carbohydrates*	
	Presumed	*Observed*	*from reducing end* (%)	*from other portion* (%)
Arabino-disaccharides				
Ara*f*-(1→3)-Ara	2	1.92	102.8	94.2
Ara*f*-(1→5)-Ara	2	2.06	95.2	101.0
Ara*p*-(1→5)-Ara	2	2.15	89.2	102.8
Arabinose oligomers				
(A)	5	4.84	98.3	94.3
(B)	12	12.1	96.8	97.2
Lactose	2	1.97	102.5	99.6
[a]	2	2.23	89.9	97.6
Maltotriose	3	2.90	91.1	86.6
[a]	3	3.19	93.9	102.9

[a]Reduced oligosaccharide was hydrolyzed in M H_2SO_4 for 5 h at 100°. The others were hydrolyzed in 0.75M HCl for 30 min at 100°.

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