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Molecular imprinting of acetylated carbohydrate derivatives into methacrylic polymers☆

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Abstract

The non-covalent imprinting procedure was used for the preparation of polymers selective for various carbohydrate derivatives, i.e. peracetylated phenyl α- and β-D-glycosides of galactose. The selectivities of the resulting polymers were tested in a HPLC procedure. The selectivity was influenced by the anomeric configuration of the glycoside (α- or β-configuration). Thus, a polymer prepared with *p*-aminophenyl β-galactoside as print molecule, gave a relatively high selectivity ($\alpha = 1.27$) for the β-galactoside over the α-galactoside, whereas a polymer prepared with the corresponding α-galactoside showed no or low selectivity towards the α-glycoside ($\alpha = 1.02$). Moreover, the structure of the aglycon was important. Thus, the use of aminophenyl glycosides as print molecules resulted in polymers with induced selectivity, whereas the use of the corresponding nitrophenyl- and acetaminophenyl-galactosides gave no induced selectivity.

1. Introduction

The method of preparing polymers with different selectivities, named molecular imprinting, has recently been demonstrated to be a powerful technique to prepare chromatographic adsorbents suitable for the chiral separation of biologically active compounds [1]. Thus, results from our laboratory have shown that polymers with high selectivity (α-values of 3 or higher when tested in the HPLC mode) for either L- or D-amino acid derivatives can be obtained by polymerising methacrylic acid as functional monomer and ethylene glycol dimethacrylate (EDMA) as crosslinker together with either the L- or D-amino acid derivative (the "print molecule"), respectively [2,3]. In an alternative and also successful approach, covalent interactions between monomer and print molecule have been used to prepare selective polymers [4].

In the present study, the non-covalent imprinting procedure for the preparation of polymers selective for various carbohydrate derivatives, i.e. peracetylated phenyl α- and β-D-glycosides of D-galactose, was investigated. Previously, a covalent imprinting procedure for unprotected sugar glycosides, which require unprotected vicinal hydroxyl groups for the formation of boronate esters between sugar hydroxyl groups and

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phenyl boronic acid groups on the polymer, has been described [5]. However, in general, protected mono- or oligosaccharides are of importance as they are widely used as intermediates in the chemical synthesis of biologically active carbohydrates found in glycoproteins and glycolipids [6].

The phenyl and amino-groups of print molecules, governing non-covalent and ionic interactions with functional monomers (methacrylic acid), have been shown previously to promote a high enantiomeric selectivity or specificity of recognition of imprinted polymers against derivatized amino acids [3,7]. Thus, in this investigation we were interested to compare the selectivity of polymers prepared by imprinting of aminophenyl, nitrophenyl and acetamidophenyl groups as aglycons of acetylated glycosides.

2. Experimental

2.1. Materials

The *p*- and *o*-nitrophenyl α-D- and β-D-galactopyranosides were obtained from Sigma (St. Louis, MO, USA), ethylene glycol dimethacrylate (EDMA) was obtained from Polysciences (Warrington, FL, USA), methacrylic acid (MAA) and 2,2″-azobis (2-methyl-propionitrile) (AIBN) were from Janssen Chimica (Beerse, Belgium). Solvents and other reagents were of either HPLC grade or analytical reagent grade.

2.2. Preparation of print molecules

Peracetylation of D-galactoside anomers was performed by treatment with pyridine and acetic anhydride and the nitrophenyl group was converted to the aminophenyl group with H_2/Pd, active carbon [8]. The structures and purity of the corresponding print molecules (Table 1) were confirmed by means of TLC (ethylacetate–isooctane; Kieselgel 60 F 254; Merck), UV–Vis (Hitachi, Model 3200) and NMR spectra (^{1}H, ^{13}C, XL-300 MHz, Varian).

Table 1
Polymers prepared employing different carbohydrate derivatives as template molecules

Polymer[a]	Structure of template molecule
I	*p*-aminophenyl tetraacetyl α-D-galactoside
II	*p*-aminophenyl tetraacetyl β-D-galactoside
III	*p*-nitrophenyl tetraacetyl α-D-galactoside
IV	*p*-nitrophenyl tetraacetyl β-D-galactoside
V	*p*-acetamidophenyl tetraacetyl α-D-galactoside
VI	*p*-acetamidophenyl tetraacetyl β-D-galactoside
VII	*o*-aminophenyl tetraacetyl α-D-galactoside
VIII	*o*-aminophenyl tetraacetyl β-D-galactoside

[a] All polymers were prepared using EDMA (52.4 mmol) as crosslinking monomer, chloroform (16 ml) as solvent and AIBN (0.76 mmol) as the initiator at 0°C as described in Section 2.

2.3. Polymer preparation

Methacrylic polymers were prepared according to a slightly modified standard method [7] using methacrylic acid (MAA) as functional monomer and ethylene glycol dimethacrylate (EDMA) as crosslinker. The molar ratio of crosslinker to functional monomer to print molecule was within the range of 44:8.8:1–38.4:7.7:1 employing peracetylated galactosides as print molecules. The bulk polymers were ground to particles, size-separated (25–50 μm) and packed into 250 × 5.0 mm I.D. stainless-steel columns with acetonitrile as solvent at 150 bar using an air-driven fluid pump (Haskel Engineering Supply, Burbank, CA, USA).

2.4. HPLC analyses

The column was washed on-line with methanol–acetic acid (9:1, v/v) until a stable baseline was obtained. The print molecule was quantitatively removed from the polymer by this treatment as judged from the elution profiles. HPLC analyses (LKB 2150, Bromma, Sweden) were performed with the solvent compositions and flow-rates indicated in Table 2 and Fig. 2. Detection was at either 290 or 300 nm. An amount of 20–40 μg of each of the anomers/isomers of a given compound, prepared in the

Table 2
Capacity factors for the different *ortho*- and *para*-aminophenyl galactoside derivatives on different polymers[a]

Polymer	Capacity factor k'		α_{12}
	α-anomer	β-anomer	
I	1.34	1.32	1.02
II	1.09	1.38	1.27
VII	1.61	1.61	1.00
VIII	1.88	2.00	1.06

[a] Each of the anomers of the galactoside derivatives was injected (20–40 μg) onto the polymer-containing columns in a total volume of 20 μl of aqueous acetonitrile and eluted at room temperature using the mixture of acetonitrile–water (50:50); the void volume was measured with acetone.

mobile phases, was injected for analysis in a total volume of 20 μl. The void volumes of the columns were determined by injection of acetone. Capacity factors (k') and separation factors (α), were calculated according to standard chromatographic theory [3].

3. Results and discussion

In this study, the acetylated carbohydrate moiety of the print molecules was kept constant, i.e. the galactose structure was used throughout the experiments, whereas the anomeric configuration and aglycon structure were varied (cf. Fig. 1 and Table 1).

As mentioned above, previous data obtained with amino acid derivatives show the importance of the amino group as well as of the phenyl group for the induction of specificity in this type of polymers. Therefore, in this study, we investigated the *o*- and *p*-aminophenyl glycoside structures shown in Fig. 1 as template molecules. Control polymers were also prepared, employing template molecules which did not contain the amino function, i.e. *p*-acetamidophenyl glycosides and nitrophenyl glycosides (Table 1).

Moreover, all polymerizations were performed under equivalent conditions to ensure that the physical properties of the polymers were as equivalent as possible, exhibiting comparable pore volumes (0.04–0.05 cm^3/g) to the previously reported for other polymers (0.08 cm^3/g) [5]. The molar ratio of crosslinker (EDMA) to functional monomer (MAA) was 5:1. The ratio of functional monomer to print molecule, was in the range of 7.7–8.8:1 as determined by the solubility of the print molecule.

3.1. Chromatography and specificity of recognition

Polymers were evaluated in the HPLC mode using isocratic elution. The composition of the eluent (acetonitrile–water) was chosen to give a capacity factor, k', for the β-form of the print molecule of ca. 1 (data shown in Table 2).

Table 2 summarizes the results obtained with polymers I, II, VII and VIII, which were prepared using the various aminophenyl tetraacetyl galactosides shown in Fig. 1 as print molecules. The use of *p*- or *o*-aminophenyl β-galactosides as print molecules resulted in polymers II and VIII which exhibited higher selectivity than polymers I and VII prepared from the corresponding α-glycosides. Thus, polymer II, prepared with *p*-aminophenyl β-galactoside (struc-

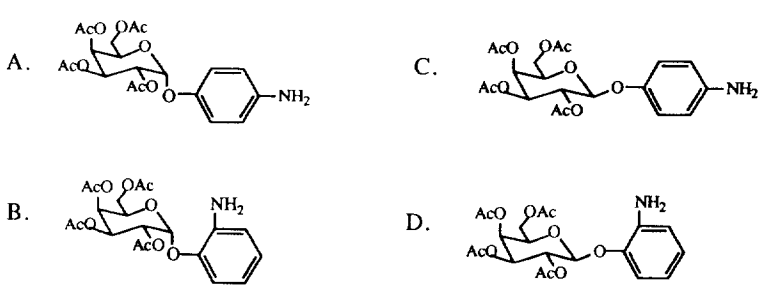

Fig. 1. Structure of the carbohydrate derivates employed as print molecules in this study.

ture C in Fig. 1) as print molecule, gave a relatively high selectivity ($\alpha = 1.27$) for the β-glycoside over the α-glycoside, whereas polymer I prepared with *p*-aminophenyl α-galactoside (structure A in Fig. 1), showed no or low selectivity towards the α-glycoside ($\alpha = 1.02$).

Similar results were obtained using the *ortho*-substituted aminophenyl α- and β-galactosides as print molecules (polymer VII and VIII, respectively). Thus, polymer VIII prepared with the β-galactoside (structure D in Fig. 1) exhibited an α-value of 1.06, whereas polymer VII prepared with the α-galactoside did not show discrimination. Interestingly, polymer VIII also showed selectivity towards the *para*-amino substituted phenyl β-galactoside (i.e. as compared with the corresponding *para*-amino substituted α-galactoside).

Importantly, none of the above polymers, including polymer II, exhibited selectivity against the galactosides which did not contain an amino group, i.e. the *p*-acetamido or nitrophenyl tetraacetyl galactosides. Moreover, none of the four different control polymers (polymers III, IV, V, and VI in Table 1) prepared with the latter galactosides as print molecules, showed any induced selectivity and the capacity factors (k'-values) for these columns were practically zero for the compounds tested in this study.

The chromatographic performance of the imprinted polymers is illustrated in Fig. 2, which shows a chromatogram obtained upon injection of the positional isomers *o*- and *p*-aminophenyl tetracetyl β-galactoside on a column containing polymer VIII (prepared with the *o*-aminophenyl tetraacetyl β-galactoside as the print molecule). As can be seen, a complete separation was obtained ($\alpha = 1.51$). No separation of these two compounds was obtained with the polymers prepared with the corresponding amino-phenyl α-galactosides (polymers I and VII; Table 1), nor with the polymers prepared with the nitrophenyl or acetamidophenyl glycosides as print molecules (polymers III–VI).

From the data it is obvious that the amino function is required both for the preparation of polymers with induced selectivity as well as for the recognition of the injected carbohydrate

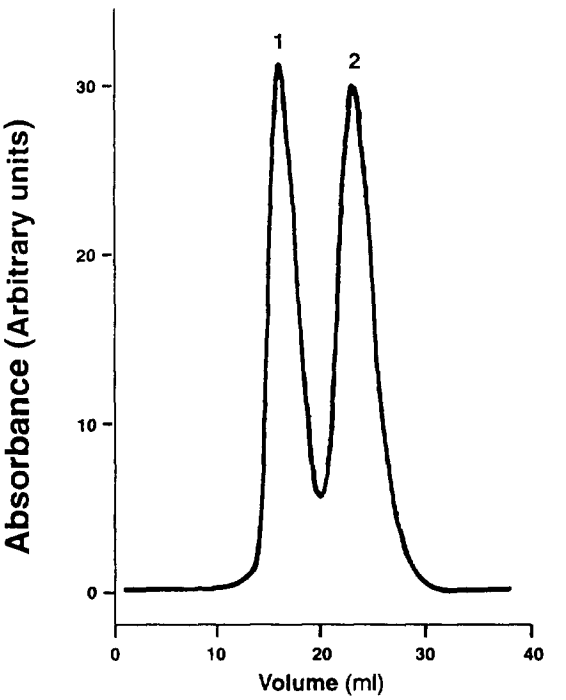

Fig. 2. Separation of (1) *para*- and (2) *ortho*-aminophenyl tetraacetyl β-D-galactoside, on a polymer prepared with the *o*-aminophenyl β-galactoside derivative as the template molecule. Analysis was performed isocratically using acetonitrile–water (2:3, v/v) as the eluent (0.5 ml/min) at room temperature. Detection was at 290 nm. A mixture containing 30 μg of each of the isomers was injected.

derivative. This is in line with previous findings in our laboratory, which show that the amino group is important for the selectivity of imprinted polymers towards enantiomers of amino acid derivatives [1]. A schematic illustration of the proposed cavities that might be formed when the aminophenyl β-galactoside is used as print molecule, is given in Fig. 3. From this model, it can be assumed that if the amino function is

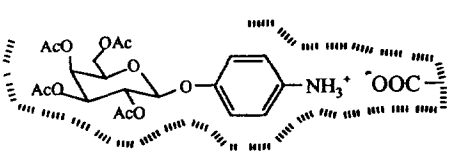

Fig. 3. Schematic illustration of the proposed interaction between the *p*-aminophenyl β-galactoside acetate and the polymer.

missing in the print molecule, induction of polymer cavities with specifically oriented carboxyl groups is not expected. This is in line with the observation that polymers III–VI, obtained with the *p*-nitro- or *p*-acetamido galactosides did not show induced selectivity.

The illustration in Fig. 3 might be used to rationalize the less pronounced selectivities obtained with the *o*-aminophenyl β-galactoside as the print molecule. Thus, comparing the molecular structures of the *p*- and *o*-aminophenyl galactosides, rotation of the *o*-aminophenyl group in the latter type of print molecule leads to a much larger movement of the amino group than in the *p*-aminophenyl β-galactoside, and therefore a much less pronounced chiral recognition might be expected.

In conclusion, the separation of carbohydrates is a challenge because of their complex structures and their potential as pharmaceutically active compounds. The results obtained in this study clearly show induced selectivity in polymers prepared by non-covalent imprinting and the method may be extended to preparation of separation materials which are selective towards other protected carbohydrate derivatives as well as towards materials for the separation of partially protected or non-protected carbohydrates employing other polymerisation conditions.

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