

# Study of specific interactions between glucuronic acid and amino acids at the interface using pseudo bioaffinity chromatography and NMR studies

C. Delattre<sup>1,\*</sup>, P. Michaud<sup>2</sup>, J. Courtois<sup>3</sup> and M. A. Vijayalakshmi<sup>1</sup>

<sup>1</sup>Centre for BioSeparation Technology, Vellore Institute of Technology-University, Vellore 632 014, India

Present address: Greentech Biopôle Clermont limagne, 63360 Saint Beauzire, France

<sup>2</sup>Laboratoire de Génie Chimique et Biochimique, Université Blaise-Pascal-Polytech Clermont-Ferrand, 24 Avenue des Landais, 63174 Aubière Cedex, France

<sup>3</sup>EPMV, IUT/GB, UPJV, Avenue des Facultés, Le Bailly, 80025 Amiens Cedex, France

**A new approach to protein–oligosaccharide interaction studies was envisaged using pseudo bioaffinity chromatography with L-histidine amino acid immobilized onto poly(ethylene–vinyl alcohol) hollow-fibre membranes. These interactions have also been investigated by NMR spectroscopy to quantify ionic binding during the interaction between the oligosaccharides and the amino acid. Oligouronides such as tricellouronic oligosaccharides obtained by enzymatic depolymerization of a bacterial poly  $\beta$ -(1,4)-glucuronic acid, were chosen and applied with different derivatives to investigate the specific effects for molecular association.**

**Keywords:** Amino acid, chromatography, NMR spectroscopy, oligosaccharides, pseudo bioaffinity.

CARBOHYDRATE–PROTEIN interactions play a major role in numerous biological processes<sup>1</sup>. In this context, some oligosaccharides acting like signalling molecules (the term hormone is sometime employed) on various organisms arouse great interest<sup>2–7</sup>. Generally, protein–carbohydrate interaction studies are investigated by NMR spectroscopy, X-ray or molecular dynamics (MD) simulation after complexation, as it has been described for lectin–carbohydrate interactions<sup>8</sup>. Lectin–carbohydrate interactions are effectively studied for pharmaceutical applications, since lectins are specific cell-membrane receptors. Therefore, similar studies could be investigated to purify specific oligosaccharides by bioaffinity. During the last decade, pseudo bioaffinity chromatography using L-histidine immobilized onto poly(ethylene–vinyl alcohol) (L-His–PEVA) hollow-fibre membranes was developed to purify proteins like human IgG from serum in large amounts<sup>9,10</sup>. However, few investigations were realized with oligosaccharides. In the earlier works<sup>11,12</sup>, it was shown that oligosaccharides from glucuronan can be retained by pseudo bioaffinity on L-His–PEVA. These oligoglucuronates are  $\beta$ -(1,4)-linked uronic oligosaccharides

obtained by glucuronan depolymerization with a glucuronan lyase from *Trichoderma* sp.<sup>13</sup>. They have been previously described not only as bioactive molecules that activate tissue regeneration on animals<sup>14</sup>, but also as elicitors of defence responses in plants<sup>15</sup>.

The purpose of this article is to study the interaction between amino acid and glucuronic acid using pseudo-affinity chromatography and NMR, in order to propose a new approach for the selective purification of oligosaccharides. By studying the amino acid/glucuronic acid model, the adsorption/desorption aspects were investigated to determine the selective retention properties.

## Experimental

### Glucuronan production

The *S. meliloti* M5N1 CS strain (NCIMB 40472) was cultivated at 30°C in a 20 l reactor (SGI, Toulouse, France) containing 15 l of *Rhizobium* complete (RC) medium<sup>16</sup> supplemented with sucrose (1% wt/v) for the production of mainly 3-*O*-acetylated (standard) glucuronan (PGUac). The inoculum was 1.5 l of *Rhizobium* complete saccharose medium (RCS) inoculated with *S. meliloti* M5N1 CS and incubated for 20 h at 30°C on a rotary shaker at 100 rpm. After 96 h of cultivation, the broth was centrifuged (33,900 *g*, 40 min, 20°C). Polysaccharides in the supernatant were then precipitated by alcoholic treatment (addition of three volumes of isopropanol) and collected by centrifugation (33,900 *g*, 20 min, 4°C). In order to produce deacetylated glucuronan (PGU), a standard glucuronan solution (10 g/l) was treated overnight with KOH (2 M) at 50°C (adjusted at pH 12) and purified as described above.

### Monomer tested

Glucuronic acid (GlcA), galacturonic acid (GalA), guluronic acid (GulA), mannuronic acid (ManA) and histidine (His) were purchased from Sigma (USA).

\*For correspondence. (e-mail: cedric\_delattre@hotmail.com)

### Oligoglucuronates tested

All oligoglucuronates tested were obtained by enzymatic degradation of PGU<sup>13</sup> with glucuronan lyase (GL) from *Trichoderma* sp. GL2. Glucuronan solutions (3% wt/v) in 50 mM phosphate buffer at 20°C were incubated with GL from *Trichoderma* sp. GL2. After incubation, enzymatic  $\beta$ -elimination was stopped by dipping the reaction medium into 95°C water bath for 5 min. The mixture of oligomers was then centrifuged (15,000 *g*, 20 min, 20°C) and the supernatant recovered. Unsaturated oligoglucuronate of degree of polymerization 3 ( $\Delta\text{OGU}_3$ ) and degree of polymerization 4 ( $\Delta\text{OGU}_4$ ) was size-fractionated from the oligoglucuronide ( $\Delta\text{OGUs}$ ) mix by low-pressure gel-permeation chromatography on a Biogel P6 fine (Biorad) column (2.6  $\times$  100 cm, Amersham Bioscience, USA). The  $\Delta\text{OGU}$  mixture was loaded (100–500 mg in 10 ml) and eluted with a 50 mM ammonium formate solution at a flow rate of 0.8 ml/min. Detection was achieved with a UV detector (UA-6 from ISCO) at 254 nm and a RI detector (Melz). Fractions (5 ml) were collected with a Foxy 200 (ISCO) collector. Those belonging to the same peak were pooled and freeze-dried. Purity of  $\Delta\text{OGU}_3$  was confirmed by mass spectrometry analysis, as previously described<sup>13</sup>.

The peracetylated  $\Delta\text{OGU}_3$  ( $\Delta\text{OGU}_{3\text{Ac}}$ ) was obtained by classic acetylation in pyridine/acetic anhydride.  $\Delta\text{OGU}_3$  was dissolved in pyridine and anhydride acetic ( $\text{Ac}_2\text{O}$ ) was added. The mixture was stirred at room temperature for 24 h. Methanol (1/2 volume) was added to stop the reaction and the reactional medium was concentrated under reduced pressure and then precipitated in isopropanol (0–4°C) overnight. The precipitate was washed with isopropanol and freeze-dried. Pure  $\Delta\text{OGU}_{3\text{Ac}}$  was confirmed by <sup>1</sup>H NMR and mass spectrometry analysis (data not shown). The saturated triglucuronate ( $\text{OGU}_3$ ) was manufactured according to the literature<sup>17</sup>, by cleavage of the terminal unsaturated  $\Delta\text{OGU}_4$  residue with mercuric (II) acetate ( $\text{HgOAc}_2$ ; 35 mM at pH 5) by stirring for 10 min at room temperature. The oligosaccharide mixture was purified on Dowex 50W-X8  $\text{H}^+$  column eluted by distilled water and freeze-dried. Production of  $\text{OGU}_3$  was confirmed by <sup>1</sup>H NMR and mass spectrometry analysis.

### Chromatographic procedure on L-His–PEVA hollow-fibre membranes

Pseudo bioaffinity chromatographic modules (surface 0.1 m<sup>2</sup>) composed of L-His–PEVA hollow-fibre membranes were prepared as described in the literature<sup>9</sup>. The schematic representation of immobilized L-histidine ligand is presented in Figure 1.

All chromatographic analyses were carried out at room temperature (20–22°C) and at a specific pH 4.8 (=pK<sub>a</sub><sub>GlcA</sub>). Modules were equilibrated with acetate buffer (50 mM, pH 4.8) at the flow rate of 0.4 ml/min in

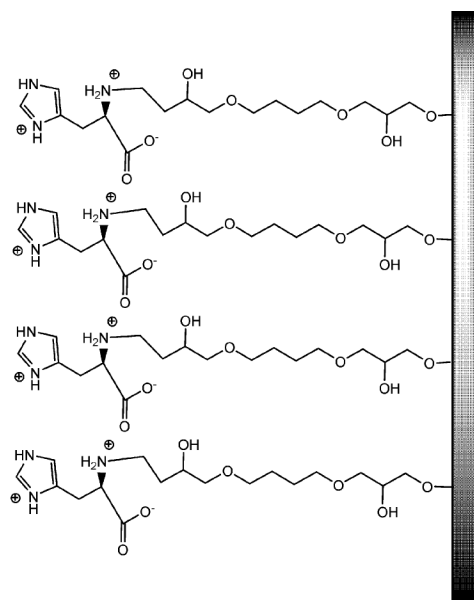
cross-flow mode using two peristaltic pumps, according to the procedure described by Bueno *et al.*<sup>18</sup>. Oligosaccharide samples (4 g/l) were injected on closed circuit during 2 h and the system was then washed with the same buffer, according to the method of Bueno *et al.*<sup>18</sup>. Finally, elutions were performed with a linear gradient of acetate buffer (0.05–2 M). Each fraction collected was assayed by colorimetric method using metahydroxydiphenyl (mHBP).

### Oligosaccharides assay

Monomers and oligosaccharides were quantified by mHBP using microtitration plate<sup>19</sup>. Colorimetric assays were performed. A microplate spectrophotometer (Opsys MR, Dynex Technologies, USA) was used for absorbance measurements.

### <sup>1</sup>H NMR studies

<sup>1</sup>H NMR analyses were performed at 30°C with a Bruker Avance 300 spectrometer of 300 MHz equipped with <sup>13</sup>C/<sup>1</sup>H dual probe. The NMR experiments were recorded with a spectral width of 3000 Hz, acquisition time of 1.36 s, pulse width of 7  $\mu$ s, relaxation time of 1 s and 256 scans. The HOD signal was presaturated by a presaturation sequence. All samples were previously dissolved in D<sub>2</sub>O (99.9% D) and lyophilized to replace exchangeable protons with deuterium. The lyophilized samples were then dissolved in D<sub>2</sub>O at a concentration of 10–20 g/l. The spatial interactions between L-histidine and glucuro-



**Figure 1.** Schematic representation of L-histidine immobilized to PEVA hollow-fibre membrane by bisoxirane activation.

nic acid were analysed at various molar ratios in D<sub>2</sub>O at pH 4.8 and other pHs modulated with DCl and NaOD. The <sup>1</sup>H chemical shifts for free amino acid ( $\delta_{\text{free}}$ ) and histidine in interaction with GlcA ( $\delta_{\text{int}}$ ) were calculated in the absence or presence of GlcA. Ligand-induced chemical shift difference ( $\Delta\delta$ ) was measured using the following equation

$$\Delta\delta = |\delta_{\text{int}} - \delta_{\text{free}}|. \quad (1)$$

## Results and discussion

### Oligouronic acid/histidine model study

In order to analyse the oligouronate/histidine interaction, the pseudo bioaffinity chromatography was investigated using the L-His-PEVA module with large surface membrane of 0.1 m<sup>2</sup>, equilibrated and eluted with AcOH/ACOOH<sub>4</sub> (pH 4.8; 50 mM) buffer.

It is well established that biological properties of carbohydrates are largely associated to their high level of structural diversity and to their dynamic conformation. Consequently, a good knowledge of the dynamical aspect was essential to establish structure/function relationships in order to understand and control binding between carbohydrates and proteins, which are important in a majority of biological phenomena.

During purification processes, enthalpic and entropic energy (driving forces) related to electrostatic and hydrophobic interactions takes place for stabilization of oligoglucuronides and adsorption onto the histidine-PEVA membrane. Van der Waals interactions and hydrogen bonds (2.5–3 Å, 8–34 kJ/mol) are factors influencing the adsorption of oligoglucuronides on the PEVA-histidine system. On the other hand, electrostatic distribution is the phenomenon necessary for the fixation of oligoglucuronides due to specific interactions. Introduction of positive charges at the histidine liganded onto PEVA-hollow fibre by buffer modulation<sup>11</sup> is essential to create the imidazolium cation, which increases stability and adsorption of uronic oligosaccharides.

### Determination of ionic interaction by <sup>1</sup>H NMR studies

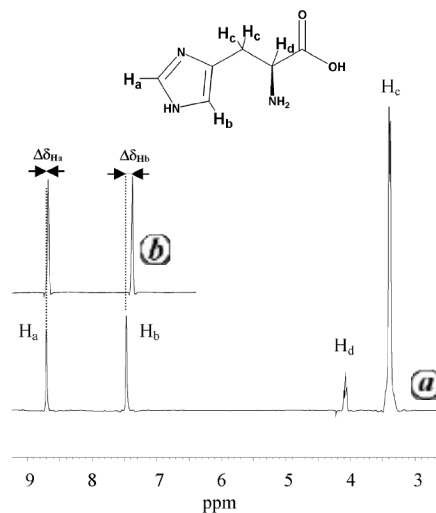
The spatial interactions of amino acid and glucuronic acid were analysed to help us understand the adsorption mechanism between oligouronates and amino acids (amino acid/GlcA model). We focused on the fundamental interaction properties of amino acids such as histidine, tyrosine, alanine and cysteine with GlcA, to quantify by <sup>1</sup>H NMR spectroscopy the specific orientations of bioactive glucuronic oligosaccharides in the 'protein neighbourhood'.

We had neglected the influence of steric effect (for histidine) due to the presence of bisoxirane spacer in this

study, even if it was clear that the degrees of freedom were lower in the L-His-PEVA system. The dynamic interaction between GlcA and amino acids was investigated by measuring the spatial interactions between them using <sup>1</sup>H NMR, according to eq. (1).

Different parameters like molar ratio and pH were applied to envisage the interaction. Since all these amino acids possess different pK<sub>a</sub> values (for example, histidine: pK<sub>a</sub> = 1.8, 6.0, 9.2 and 14.2) following multiple protonation states and tautomeric forms, distinct species of cations, zwitterions and anions were formed in aqueous solution depending on the pH values. The interactions between amino acids and GlcA were evaluated at various pH values in D<sub>2</sub>O by NMR spectroscopy analysis in order to measure the driving forces and adsorption stability. We had chosen four specific pH values (4.8, 7, 10 and 12) to engender cationic charge onto NH<sub>2</sub> and radical groups (imidazole, ...) and anionic charge to GlcA (and ΔOGU), since the carboxyl groups of uronic acid were un-ionized at low pH. A residual spatial interaction was observed ( $\Delta\delta_{\text{ppm}} < 0.001$ ) in RMN analysis with pH lower than the pK<sub>a</sub> of GlcA, due to the inherent hydrodynamic effect.

As shown in Figure 2, we observed a chemical shift modification concerning <sup>1</sup>H from imidazole ring of L-histidine, when 1 molar equivalent of GlcA was added at pH 4.8. Similar observations were obtained with 0.5 and 2 molar equivalent of uronic acid and for each amino acid studied. These parameters induced ionic charges permitting relative orientation (lowest energy) of sugars favouring geometrically GlcA/amino acid interaction between -NH<sub>3</sub><sup>+</sup> ( $\delta^+$ ) and carboxylate function ( $\delta^-$ ) of sugar (Figure 3). This interaction depended on [ $\delta^+ \cdots \delta^-$ ] non-covalent bonds for each system studied, and was higher in the case of the GlcA/His system.



**Figure 2.** Interaction studies between L-histidine and GlcA by <sup>1</sup>H NMR analysis (300 MHz, pH 4.8, 30°C). (a) NMR spectrum of L-histidine; (b) Spectrum resulting from the mixture of L-histidine and GlcA (1 molar equivalent).

The induced changes ( $\Delta\delta$ ) observed (Figure 4), revealed specific modifications of  $^1\text{H}$  chemical shifts of amino acid in  $\text{D}_2\text{O}$  in accordance with solution pH. In fact,  $\Delta\delta$  values of imidazole ring protons (Hc and Hd) from histidine increased as pH decreased. On the contrary, other  $^1\text{H}$  chemical shifts (Ha and Hb in case of histidine) moved slightly at any pH, showing the lower interaction with GlcA. The NMR investigation highlighted the link between ionic charges and adsorption between monomers of the GlcA/amino acid system. This indicated a specific interaction around the imidazole ring of the amino acid, since it was interesting to notice a more specific interaction between histidine and GlcA (and  $\Delta\text{OGU}_3$ ).

In numerous cases, the aromatic and imidazole residues of the amino acid also contributed extensively to the stability of the carbohydrate–amino acid complex and by extension towards the oligosaccharide–protein interaction. This appeared to be a crucial factor to support optimization of pseudo bioaffinity chromatography using immobilized amino acids, leading to the increase of high-affinity binding sites in the resulting hollow-membrane matrix in order to develop more efficient processes. The NMR data obtained now permit a more comprehensive view of the adsorption events in these pseudo bioaffinity systems. The same results were observed with other monouronic acids such as GalA, GulA and ManA (data not shown). Thus various bioactive oligouronate structures of importance could be studied and purified using L-histidine pseudo bioaffinity chromatography, to envisage a large scale-up for biological applications.

These NMR experiments permit us to envisage a more comprehensive view of the interaction events and their potential impacts on the binding properties. On the basis of the experimentation, these NMR spectroscopy analyses reveal a predominant electrostatic interaction between uronates and charged amino acids. Further work on the nature of the interactions described in this study and

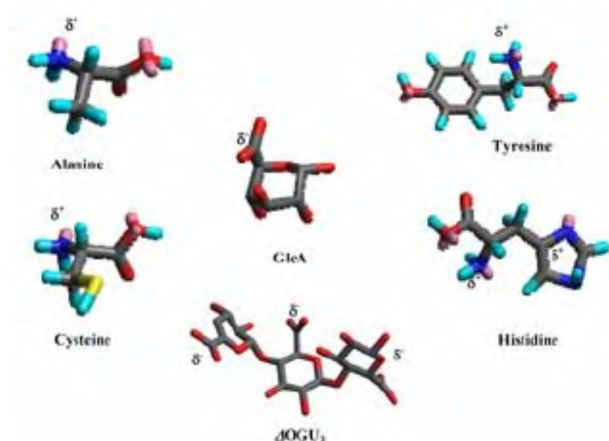
probable binding patterns is in progress. This approach on the mechanistic interaction between oligosaccharides and amino acids suggests that the steric and conformational effects influence non-covalent binding due to charge–charge interactions. These interactions could be extrapolated in future to those between glycosylated proteins and amino acids in light of the biological importance of oligosaccharides, which could be implicated and modulated by non-covalent interaction.

Nevertheless, even if NMR spectroscopy has been used to characterize a predominant electrostatic interaction during adsorption between oligosaccharides and amino acids, it seems interesting to envisage others parameters favouring these specific adsorptions.

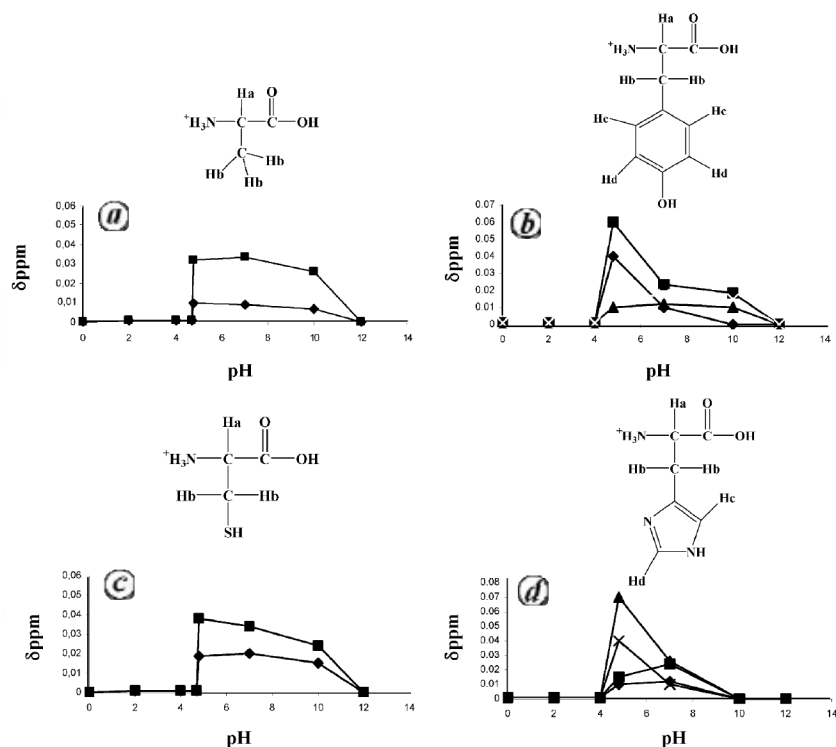
### *Conformational and configurational influence on uronate adsorption*

To examine and characterize the conformational effects of carbohydrates on histidine/sugar interactions, we have investigated two epimeric uronic acids: GalA and GlcA, which are configurational isomers, in order to understand their dynamic behaviour. First, each monouronate (GalA and GlcA) was injected on to the L-His–PEVA module ( $0.1\text{ m}^2$ ) to quantify the maximum of adsorption capacity. After injection and elution, uronates were quantified by colorimetric assay. Table 1 shows better retention of GlcA than GalA, on PEVA–histidine, since  $19.5\text{ }\mu\text{g}/\text{cm}^2$  of GlcA and  $16.8\text{ }\mu\text{g}/\text{cm}^2$  of GalA were adsorbed onto the pseudo bioaffinity chromatographic module. This result indicated a probable conformational effect of uronic residue with a coherent orientation of 3D structure favouring a specific binding recognition, where for example, GalA did not display any stereospecificity for histidine binding.

As predicted, these parameters induced ionic charges permitting relative orientation (lowest energy) of sugars, geometrically favouring GlcA/His interaction between the charged imidazole ring (imidazolium) of histidine and carboxylate function of sugar, depending on the  $[\delta^+ \cdots \delta^-]$  non-covalent bonds. Consequently, the diverse spatial arrangement of the hydroxyl groups leads to specific features for each epimer. This approach suggested that several oligouronates should be adsorbed onto PEVA–histidine hollow fibre with different degrees of retention according to their conformational dynamics and also to the linkages and epimerization of their sugars. In fact, the conformational mobility and dynamics of the bound carbohydrates were largely dependent on conformation of the chain, where the glycosidic linkages were oriented according to their  $\psi$  and  $\phi$  torsion angles<sup>20</sup>. This could modulate the interaction between binding partners with a steric arrangement of the active carboxylate groups, revealing the relationships between structures of uronic acid and adsorption strength. In addition, as a general rule it was well established that  $\alpha$  and  $\beta$  conformers (anomers)



**Figure 3.** Three-dimensional coherent representation according to specific charge of amino acids, GlcA and  $\Delta\text{OGU}_3$  at pH > 5.



**Figure 4.**  $^1\text{H}$  chemical shift modifications of amino acids during their interaction with GlcA (1 equivalent) according to pH modulation. (a) Alanine, (b) tyrosine, (c) cysteine and (d) histidine. (—■—), Ha; (—◆—), Hb; (—▲—), Hc and (—x—), Hd.

**Table 1.** Adsorption capacity ( $\mu\text{g}/\text{cm}^2$ ) of GlcA, GalA,  $\Delta\text{OGU}_3$  and derivatives using PEVA–histidine cartridge ( $0.1\text{ m}^2$ ) at optimum pH (4.8) in acetate buffer system at 50 mM

| Adsorption capacity ( $\mu\text{g}/\text{cm}^2$ ) |      |
|---|------|
| GlcA  | 19.5 |
| $\Delta\text{OGU}_{3\text{Ac}}$                   | 19.0 |
| $\Delta\text{OGU}_3$                              | 17.5 |
| GalA  | 16.8 |
| $\text{OGU}_3$                                    | 14.1 |

were usually in a dynamical equilibrium solution (mutarotation). However, according to solvent polarity, the anomeric effect generally decreased with increasing polarity of the solvent and hence the  $\beta$  conformer was the predominant form because of solvation effects.

#### *O*-acylesters influence on uronate adsorption

The *O*-acetyl effect of the oligouronate fraction was studied with a pure peracetylated triglucuronate ( $\Delta\text{OGU}_{3\text{Ac}}$ ). The *O*-acetyl substitutions probably affect the 3D structure orientation because of the inherent steric effect of acetate substituents that decreases the backbone mobility. As shown in Table 1, the  $\Delta\text{OGU}_{3\text{Ac}}$  sample showed better affinity with the L-His–PEVA module (19% against 17.5% for  $\Delta\text{OGU}_3$ ). The conformational equilibrium of

*O*-acetyl ester substitution seems to modulate the orientation of the carboxylic functions ( $\text{COO}^-$ ) for better interaction with L-histidine. Numerous substitutions onto poly- and oligosaccharides such as sulphatation, acetylation, and phosphorylation are known to modify the levels of structural organization of these poly- and oligomers features. In this case, acetate substitution conferred to oligosaccharides a coherent three-dimensional conformation, which implicated more levels of interaction by a specific orientation toward histidine liganded onto the PEVA module. Moreover, several examples described that substituents, independent of the backbone, play a role on inter-chain and/or intrachain molecular interactions<sup>21</sup>. This phenomenon could be extrapolated to interactions between oligouronides and L-histidine of the support<sup>22,23</sup>.

#### *Influence of unsaturation on adsorption*

In order to study the unsaturation effect ( $\text{C}_4=\text{C}_5$ ) on oligosaccharide adsorption, triglucuronate was applied as a model for chromatographic analysis, where  $\Delta\text{OGU}_3$  and  $\text{OGU}_3$  were injected into the L-His–PEVA module. The adsorption capacity (Table 1) was different with unsaturated oligosaccharide ( $\Delta\text{OGU}_3$ ), which was retained more ( $17.5\text{ }\mu\text{g}/\text{cm}^2$  against  $14.1\text{ }\mu\text{g}/\text{cm}^2$  for  $\text{OGU}_3$ ). This result corroborates with the 3D structure consideration with orientation of specific entities, according to low interaction

energy. In fact, the C–C bond (sp<sup>3</sup> hybridization:  $\sigma$  axial bond) opened the possibility to free rotation and thus according to minimum molecular energy, there are multiple conformations. On the other hand, the C=C bond (sp<sup>2</sup> hybridization:  $\sigma$  axial bond and  $\pi$  lateral bond) imposed a specific plan and conformation for the unsaturated uronic residue. Consequently, specific interaction increased with using unsaturated oligosaccharides.

## Conclusion

The present study has explored the potential of <sup>1</sup>H NMR spectroscopy and pseudo bioaffinity chromatography using L-histidine immobilized for investigating the interaction process occurring in the recognition mechanism in several biological phenomena. According to system buffer pH of 4.8 (=pK<sub>a</sub> GlcA) and ionic strength, electrostatic interactions were predominant and adsorption on L-His-PEVA module depended of the charge density and charge availability generated by conformational effects, since different monomers interact differently with L-histidine, as was observed, suggesting a dependence on chemical properties such as configurational features. The privileged 3D structure (usually lowest energy) showed that the O-acetyl ester substituents favour the adsorption of oligouronates by coherent orientation of carboxyl functions (COO<sup>−</sup>) with L-histidine. There was a relationship between 3D structure and specific oligosaccharides retention onto the L-His-PEVA module. This approach on the mechanistic oligosaccharide–protein interaction suggests that the steric and conformational effects were essential for establishing the biological activities, where the binding was non-specific and was due to charge–charge interaction effect between COO<sup>−</sup> function of anionic oligosaccharides and histidine imidazole ring. The adsorption of oligouronate by pseudo bioaffinity on the L-His-PEVA membrane opens the way for large-scale specific production and purification of bioactive compounds. Consequently, these preliminary studies were necessary to optimize the chromatographic module, where other anionic oligosaccharides could be selectively purified on the L-His-PEVA membrane.

The knowledge of the mechanistic interaction implicated between oligosaccharides and proteins could serve as a basis for the development and designing of novel specific chromatographic processes for the purification of bioactive glycoproteins and oligosaccharides after immobilization of specific receptors.

1. Varki, A., Biological roles of oligosaccharides: All the theories are correct. *Glycobiology*, 1993, **2**, 97–130.
2. Albersheim, P. and Darvill, A. G., Les oligosaccharines. *Pour la Sci.*, 1985, **97**, 18–26.
3. Aldington, S. and Fry, S., Oligosaccharins. *Adv. Bot. Res.*, 1993, **19**, 1–101.
4. Darvill, A. et al., Oligosaccharins–oligosaccharides that regulate growth, development and defence responses in plants. *Glycobiology*, 1992, **2**, 181–198.

5. Natsume, M., Kamo, Y., Hirayama, M. and Adachi, T., Isolation and characterization of alginate-derived oligosaccharides with root growth-promoting activities. *Carbohydr. Res.*, 1994, **258**, 187–197.
6. Pineo, G. F. and Hull, R. D., Low-molecular-weight heparin: Prophylaxis and treatment of venous thromboembolism. *Annu. Rev. Med.*, 1997, **48**, 79–91.
7. Ariyo, B., Tamerler, C., Bucke, C. and Keshavarz, T., Enhanced penicillium production by oligosaccharides from batch cultures of *Penicillium chrysogenum* in stirred-tank reactors. *FEMS Microbiol. Lett.*, 1998, **166**, 165–170.
8. Neuman, D., Lehr, C.-M., Lenhof, H.-P. and Kohlbacher, O., Computational modelling of the sugar–lectin interaction. *Adv. Drug Deliver. Rev.*, 2004, **56**, 437–457.
9. Bueno, S. M. A., Haupt, K. and Vijayalakshmi, M. A., Separation of immunoglobulin G from human serum by pseudo bioaffinity chromatography using immobilized L-histidine in hollow fibre membranes. *J. Chromatogr. B*, 1995, **667**, 57–67.
10. Vijayalakshmi, M. A., *Biochromatography: Theory and Practice*, Taylor & Francis Ltd, London, UK, 2002, p. 526.
11. Pirlet, A.-S., Pitiot, O., Guentas, L., Heyraud, A., Courtois, B., Courtois, J. and Vijayalakshmi, M. A., Separation of Low-molecular-mass acetylated glucuronans on L-histidine immobilized onto poly(ethylene-vinyl alcohol) hollow-fiber membranes. *J. Chromatogr. A*, 1998, **826**, 157–166.
12. Pirlet, A.-S., Guentas, L., Pitiot, O., Heyraud, A., Vijayalakshmi, M. A., Courtois, B. and Courtois, J., Selective affinity of L-histidine immobilized onto poly(ethylene alcohol) hollow-fiber membranes for various oligoglucuronans. Influence of the degree of polymerization and the degree of substitution by acetate. *J. Chromatogr. A*, 1999, **841**, 1–8.
13. Delattre, C., Michaud, P., Lion, J. M., Courtois, J. and Courtois, B., Large-scale production and purification of glucuronan oligosaccharides using a new glucuronan lyase activity. *J. Biotechnol.*, 2005, **118**, 448–457.
14. Petit, E., Papy, D., Muller, G., Caruelle, J. P. and Courtois, J., Controlled sulfatation of natural anionic bacterial polysaccharides can yield agents with specific regenerating activity *in vivo*. *Biomacromolecules*, 2004, **5**, 445–452.
15. Lienart, Y., Heyraud, A. and Sevenou, O., Use of glycuronic polysaccharides and oligosaccharides as phytosanitary product and/or fertilizers, WO 01000025, 2001.
16. Courtois, B., Hornez, J. P., Courtois, J. and Derieux, J. C., Mise en évidence d'une propriété métabolique de *Rhizobium meliloti* utilisable pour sa classification. *Anal. Microbiol.*, 1983, **182**, 141–147.
17. Yang, H. O., Gunay, N. S., Toida, T., Kuberan, B., Yu, G., Shik Kim, Y. and Linhardt, R. J., Preparation and structural determination of dermatan sulfate-derived oligosaccharides. *Glycobiology*, 2000, **10**, 1033–1039.
18. Bueno, S. M. A., Legallais, C., Haupt, K. and Vijayalakshmi, M. A., Experimental kinetic aspects of hollow fiber membranes-based pseudobioaffinity filtration: Process for IgG separation from human plasma. *J. Membr. Sci.*, 1996, **117**, 45–56.
19. Van Den Hoogen, B. M., Van Weeren, P. R., Lopes-Cardozo, M., Van Golde, L. M. G., Barnveld, A. and Van de Lest, C. H. A., A microtiter assay for the determination of uronic acids. *Anal. Biochem.*, 1998, **257**, 107–111.
20. Braccini, I., Heyraud, A. and Perez, S., Three-dimensional features of the polysaccharide (1 → 4)- $\beta$ -D-glucuronan: A molecular modelling study. *Biopolymers*, 1998, **45**, 165–175.
21. Rinaudo, M., Role of substituents on the properties of some polysaccharides. *Biomacromolecules*, 2004, **5**, 1155–1165.
22. Delattre, C., Michaud, P., Hamze, K., Courtois, B., Courtois, J. and Vijayalakshmi, M. A., Purification of oligouronides using hollow-fiber membrane functionalised with L-histidine. *J. Chromatogr. A*, 2005, **1099**, 121–126.
23. Delattre, C., Kamalanathan, A. S., Michaud, P. and Vijayalakshmi, M. A., Purification of oligouronides by immobilized L-histidine pseudo-affinity chromatography. *J. Chromatogr. B*, 2008, **861**, 181–185.

Received 2 February 2007; revised accepted 4 April 2008