ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 317 (1999) 119–130

CARBOHYDRATE
RESEARCH

Conformational and configurational features of acidic polysaccharides and their interactions with calcium ions: a molecular modeling investigation

Isabelle Braccini [a], Robert P. Grasso [b], Serge Pérez [a,*]

[a] *Centre de Recherches sur les Macromolécules Végétales, CNRS [1], F-38041 Grenoble, France*
[b] *Hercules Incorporated, Research Center, 500 Hercules Road, Wilmington, DE 19808-1599, USA*

Received 9 November 1998; accepted 26 February 1999

Abstract

Modeling simulations have been performed on the four regular glycuronans: α-D-(1 → 4) polygalacturonic, α-L-(1 → 4) polyguluronic, β-D-(1 → 4) polymannuronic, and β-D-(1 → 4) polyglucuronic acids. The goal of this study was to characterize the similarities and differences in conformational and configurational behavior as well as in calcium binding in order to progress in the understanding of the physicochemical properties of the parent polysaccharides of industrial interest, namely pectin, alginate and glucuronan. This required the evaluation of the accessible conformational space for the disaccharide subunits of the four homopolymers, using the flexible residue protocol of the MM3 molecular mechanics procedure. The results were used to access the configurational statistics of representative polysaccharide chains, as well as for the determination of the regular polysaccharide helices and their conformational transitions. The surfaces of all regular helices likely to occur for each polyuronide were explored for cation binding using the GRID procedure. Both α-D-(1 → 4) polygalacturonate and α-L-(1 → 4) polyguluronate chains exhibit a high specificity for calcium binding, and have well-defined chelation sites. In contrast, β-D-(1 → 4) polymannuronate and β-D-(1 → 4) polyglucuronate chains do not display any stereospecificity for calcium binding. The results gathered from molecular modeling lead to a clear understanding of the different structural features that are displayed by the four ionic polymers. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Molecular mechanics; Uronic acids; Pectin; Alginate; Glucuronan; Conformation; MM3

1. Introduction

Polysaccharides are versatile macromolecules that may be neutral or ionic as a result of the presence of anionic groups on the polymeric backbone. The ionic polysaccharides exhibit specific physicochemical properties due to their polyelectrolyte character and to their features resulting from their stereoregularity. For such ionic polysaccharides, the ability to form gels in the presence of divalent cations such as calcium is the key to many applications [1–4]. As the number of industrial uses of the ionic polysaccharides increases, attention has been focused on establishing structure–property relationships in an attempt to explain the observed physical properties, and to control them for particular applications. In an effort to establish structure–property relationships in this area, we decided to investigate the conformational and

---

* Corresponding author. Tel.: + 33-4-76-03-7630; fax: + 33-4-76-03-7629.
E-mail address: perez@cermav.cnrs.fr (S. Pérez)

[1] Associated with Université Joseph Fourier Grenoble.

PII: S0008-6215(99)00062-2

configurational features of the four acidic components of pectin, alginate, and glucuronan, along with their interactions with calcium ions. The four polysaccharides considered here are (1→4)-linked homopolymers consisting of the monosaccharides α-D-galacturonic, α-L-guluronic, β-D-mannuronic, and β-D-glucuronic acids, which are configurational isomers of each other. On the basis of a comparative study, the investigation establishes, via molecular modeling procedures of increasing complexity [5], the different levels of structural organization of these polysaccharides, their relative stiffness in solution and assesses quantitatively their ability to bind Ca^{2+}.

2. Nomenclature

A representation of the disaccharide subunits of the four acidic polymers is given in Fig. 1, along with the labeling of the atoms and the torsion angles of interest. The conformation about the glycosidic linkage is described by the two torsion angles:

$\phi = \text{O-5}'\text{–C-1}'\text{–O-4–C-4}$

$\psi = \text{C-1}'\text{–O-4–C-4–C-5}$

The orientation of the acidic groups is described by the dihedral angles:

$\gamma = \text{O-5–C-5–C-6–O-7}$

$\gamma' = \text{O-5}'\text{–C-5}'\text{–C-6}'\text{–O-7}'$

The signs of the torsion angles are in agreement with the IUPAC–IUB commission of Biochemical Nomenclature [6].

3. Computational methods

Molecular mechanics calculations.—The general molecular mechanics program MM3(92) [7–9] was used in this study to compute the energy of any di- or oligosaccharide conformation. Along with the internal (stretching, bending, dihedral) and external (dipolar and van der Waals) terms, the MM3 force field includes cross term effects such as torsion–stretch, torsion–bend, and bend–bend interactions. This force field has been

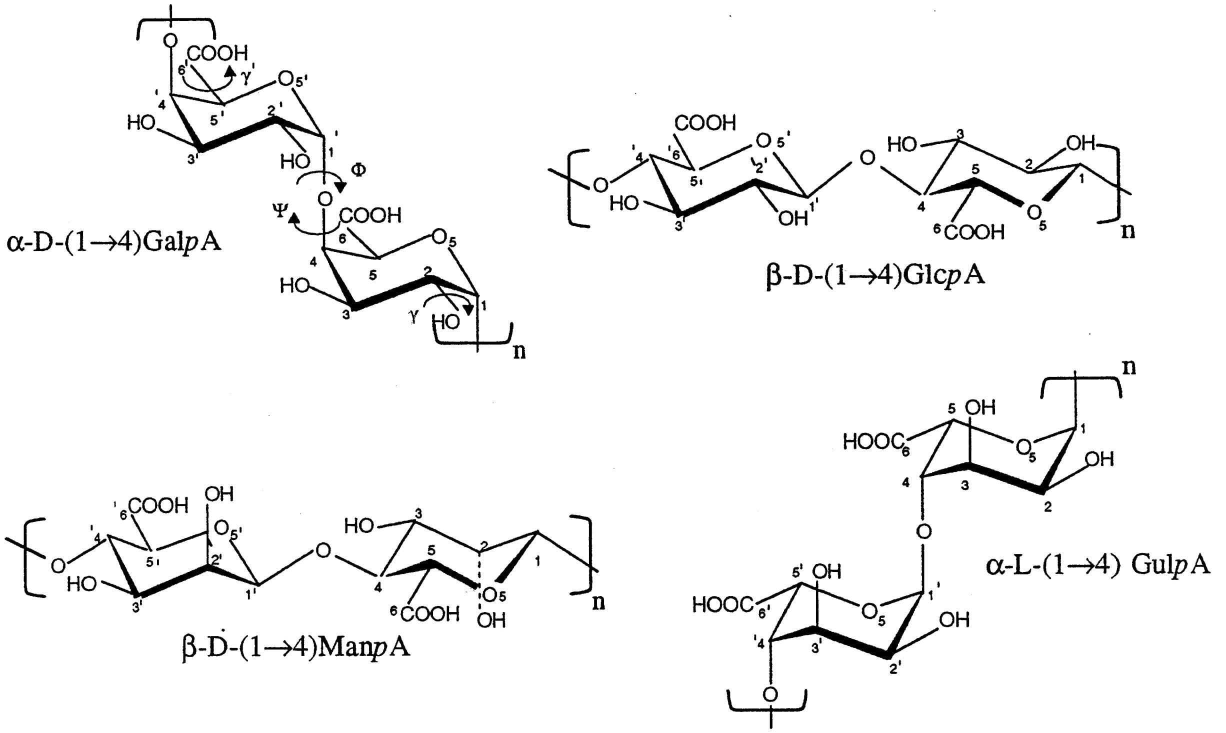

Fig. 1. Schematic representation of the disaccharide repeating units of the four acidic polysaccharides, galacturonan [α-D-(1→4)GalpA], glucuronan [β-D-(1→4)GlcpA], mannuronan [β-D-(1→4)ManpA], and guluronan [α-L-(1→4)GulpA], along with the labelling of the atoms. The torsion angles ϕ, ψ, γ are represented on the GalpA fragment.

extensively used for the conformational study of carbohydrates [10] because it takes into account the anomeric and exo-anomeric effects, and uses an explicit term for hydrogen bonds. The 1992 version includes an angular dependence in the hydrogen bonding function.

Grid search.—Polysaccharide conformations depend mainly on the glycosidic ϕ and ψ torsion angles and can, to a first approximation, be described by the (ϕ, ψ) potential energy maps of the disaccharide subunits. Following this assumption, an adiabatic map was computed for α-D-(1 → 4) galacturonic, α-L-(1 → 4) guluronic, β-D-(1 → 4) mannuronic, and β-D-(1 → 4) glucuronic acid dimers.

As a preliminary study, the conformational behavior of the acidic group was assessed in the reducing residue of the disaccharides and two favored orientations were characterized. These orientations were applied to γ and γ' and were considered in the calculations, leading to four separate starting points for each disaccharide in the calculation of a relaxed map. The starting geometries were built with the POLYS program [11], and then fully optimized. The relaxed maps were computed using rigid rotation in 10° intervals for ϕ and ψ spanning the whole angular range, followed by minimization using the block diagonal method with a termination criterion of n*0.00003 kcal/mol (n: number of atoms). The adiabatic map was finally constructed for each disaccharide by using the lowest-energy conformation from the four relaxed maps for each grid point. In the calculations, the dielectric constant was set to 80, which is appropriate to represent an aqueous environment. The isoenergy contour maps were drawn using the in-house program CARTO.

Generation of statistical chains.—Samples of polymer chains were generated for the four acidic polysaccharides in order to evaluate the dimensions of long chains. Conformations were generated using the Monte Carlo technique as implemented in the METROPOL program [12]. The conformations were selected using the Metropolis algorithm [13] and distributed according to Boltzmann statistics. The detailed description of the procedure is reported elsewhere [12]. Using this program, statistical samples of 5000 polymer chains each containing 2000 residues were constructed for polymannuronic and polyglucuronic acids. These values were optimized in order to give reproducible data with a mean error deviation not exceeding 5%. In the case of polygalacturonic and polyguluronic acids, the same level of reproducibility was reached for samples of 6000 polymer chains each containing 5000 residues. The temperature was set to 298 K and a dielectric constant of 80 was used in the calculations.

The extension and relative stiffness of the chain are described by the characteristic ratio (C_∞) and the persistence length (L_p) [14]. The average dimensions (C_∞ and L_p) were evaluated from the statistical samples generated by METROPOL as follows:

$$C_\infty = \lim_{x \to \infty} Cx = \lim_{x \to \infty} \langle \boldsymbol{r}^2(x) \rangle / x.L_o^2$$

$$\text{with } \boldsymbol{r}(x) = d(\text{O-4}[1] - \text{O-1}[x])$$

The angular brackets indicate an average ensemble over all the polymer chains, L_o is the virtual bond length connecting two adjacent glycosidic oxygen atoms, and x is the number of glycosyl residues. The persistence length (L_p) is defined as the projection of the end-to-end distance vector $\boldsymbol{r}$ on the first bond of the chain. It represents the maximum length over which a straight direction is preserved. No long-range interactions between residues are considered in this kind of calculation. Therefore, the calculated data refer to the unperturbed dimensions of the polymers.

Generation of regular helices.—Regular helical conformations were extrapolated from the low-energy regions of the potential energy maps of the four disaccharides, using the program POLYS [11]. The helices are characterized by the two helical parameters n and h, where n is the number of residues per turn of the helix and h is the projection of one residue on the helical axis. The chirality of the helix is described by a sign attributed to n: a positive value of n corresponds to a right-handed helix and a negative value to a left-handed helix. Starting from the (ϕ, ψ) values of the different energy minima in the low-energy regions, the POLYS procedure slightly modified these values in order to obtain the nearest integral n-fold helical structure. The regular helices were then constructed using the optimized

Table 1
Favored orientations[a] of the carboxylic group for the four uronic acids

Compound	α-D-GalpA	α-L-GulpA	β-D-ManpA	β-D-GlcpA
$\gamma 1$	140	130	130	130
$\gamma 2$	−150	−140	−130	−140
ΔE[b]	0.8	−1.5	2.9	1.3

[a] Described by the torsion angle γ.

[b] Energy difference (kcal/mol) $\gamma 2-\gamma 1$.

(ϕ, ψ) values. Two sets of such regular helices extrapolated from the low-energy regions were generated for polygalacturonic, polyguluronic, polymannuronic, and polyglucuronic acids, respectively. The two sets refer to the two favorable orientations of the acidic group. Finally, all the helical structures were minimized ($\varepsilon = 4$) with a constraint applied on the glycosidic linkage in order to preserve the regular helicity.

Cation binding.—In order to locate favorable binding sites for calcium ions along the ordered conformations of the polyuronide chains, calculations were carried out with the GRID program [15], version 15. This program computes the positions near a molecular system (the target) at which a small chemical moiety or molecule (the probe) would interact favorably. The van der Waals surface of the deprotonated form of the different helical structures, compatible with experimental data, was explored using a calcium probe. As the calcium-charged oxygen interaction is crucial, different orientations of the carboxylate groups were explored. In the procedure, partial charges of the helical oligomer were computed using Saunderson's method [16]. Then the target oligosaccharide was placed in a 3D grid defined by planes in x, y, z directions. A spacing of 2 planes/Å was used in the calculations, and the size of the grid was fitted to the target system. The GRID program computed the interaction energy between the ionic probe and the oligomer at each point in the grid. The three enthalpic components (Lennard–Jones interactions, hydrogen bonds and electrostatic effects) were considered in the calculations. Dielectric constants of 4 and 80 were used for the molecular target and the surrounding (water), respectively. The favorable calcium binding sites were finally interpolated from the previous grid points within a 15 kcal/mol window above the best interaction energy.

4. Results and discussion

Conformational properties of the constituent disaccharides.—Conformational studies at the disaccharide level have been reported for guluronic [17], and glucuronic [18,19] acid dimers. Furthermore, the pectic digalacturonic acid fragment has been extensively studied using different force fields [20–24]. In the present study, the behavior of the acidic group has been evaluated as a function of the γ torsion angle for the reducing end residue of the different disaccharides. The C-5–C-6–O-7–HO-7 torsion angle was not explored but was set to 180° as it has been reported to prefer this value strongly [19,21]. The torsion angle γ presents two favorable orientations, roughly identical for the four disaccharides. These preferred values of γ, named $\gamma 1$ and $\gamma 2$, are reported in Table 1. The $\gamma 1$ orientation (i.e., 130–140°) is the most favorable one, but for the side-chains of the guluronic acid dimer.

The (ϕ, ψ) adiabatic maps of the disaccharide model fragments of the four polyuronides, computed with a dielectric constant set to 80, are displayed in Fig. 2. There are two distinct shapes exhibited by these potential energy surfaces, which indicates that the conformational behavior of these disaccharides is governed mainly by the type of the glycosidic linkage: (1→4) di-axial or (1→4) di-equatorial. Digalacturonic and diguluronic acids, which both possess an axial–axial linkage, have the most restricted accessible conformational space, with only one low-energy region (Fig. 2(a,c)) containing two energy wells, noted A and B. Polygalacturonic and polyguluronic acids are often compared and thought to exhibit similar behavior [25] since they are nearly mirror images [26]. This relationship between the two polymers is well illustrated by the adiabatic maps of the corresponding disaccharide fragments, the shape of which appears antisymmetric even though the conformational preferences, with respect to the (ϕ, ψ, γ) angles, are roughly the same (Tables 1 and 2). The energy minima have buckled conformations due to the type of glycosidic linkage. The minimum from the A and B wells for the guluronic and galacturonic

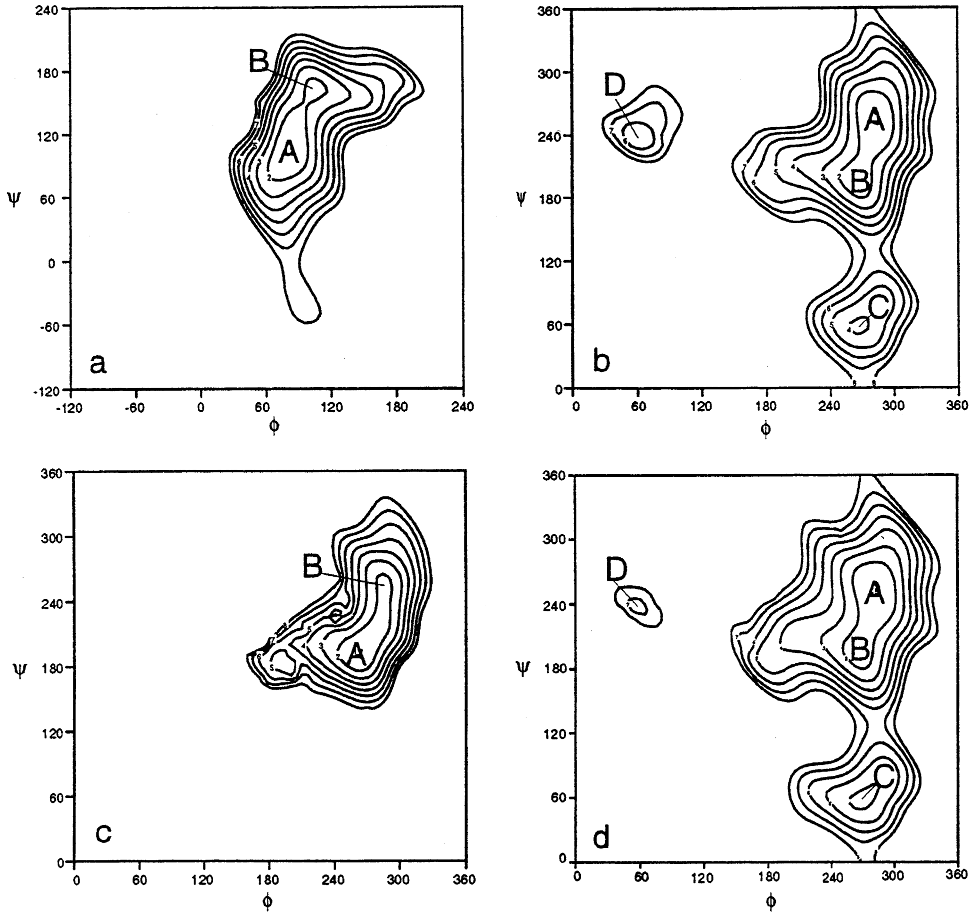

Fig. 2. Adiabatic maps (MM3(92), $\varepsilon = 80$) of (a) digalacturonic acid, (b) diglucuronic acid, (c) diguluronic acid, and (d) dimannuronic acid. Isocontour levels are drawn at 1 kcal/mol increments up to 8 kcal/mol above the global energy minimum.

Table 2
Low-energy conformers of the four disaccharides

Disaccharride	Minimum	ϕ	ψ	γ'	γ	ΔE[a]
α-D-(1→4)-di-Gal*p*A	A	78	102	131	151	0.0
	B	99	161	130	−144	0.3
α-D-(1→4)-di-Gul*p*A	A	−99	−169	−133	−137	0.0
	B	−72	−108	−133	−143	0.8
α-D-(1→4)-di-Man*p*A	A	−80	−101	127	129	0.0
	B	−89	−161	126	127	0.5
	C	−89	59	126	120	2.8
	D	58	−120	127	131	5.7
α-D-(1→4)-di-Glc*p*A	A	−80	−99	126	126	0.0
	B	−90	−159	126	126	0.5
	C	−90	70	123	116	2.9
	D	62	−121	122	131	4.3

[a] Relative energy (kcal/mol).

Table 3
Calculated persistence length (L_p) and characteristic ratio (C_∞) for the four (1→4)-linked acidic polysaccharides

	α-D-Gal*p*A	α-L-Gul*p*A	β-D-Man*p*A	β-D-Glc*p*A
L_p (Å)	166	210	119	105
C_∞	72	91	43	41

acid dimers, respectively, displays a weak O-2···O-6′ hydrogen bond. This interglycosidic hydrogen bond has been reported to stabilize the crystalline structure of both polyguluronic acid [27] and sodium pectate [28]. The adiabatic maps of glucuronic (Fig. 2(b)), and mannuronic (Fig. 2(d)) acid dimers are almost identical. The energy minima are similar with identical (ϕ, ψ) values and comparable relative energies (Table 2). Equatorial–equatorial bonds allow for the greatest freedom with respect to the glycosidic linkage. The main region which contains the A and B wells corresponds to extended conformations, and the isolated C and D wells correspond to folded conformations. The local minimum in region B exhibits the characteristic O-5···O-3′ hydrogen bond observed in the crystal structures of both cellulose [29] and poly-mannuronic acid [30]. The only significant difference between the maps of the mannuronic and glucuronic acid dimers can be found in the D well, which is significantly less populated and deeper for the mannuronic acid case. The disappearance of this well is a direct consequence of the axial position of the hydroxyl group at C-2, which introduces steric conflict thereby destabilizing the conformations in the D region.

Configurational properties of the four glyuronans chains.—In an attempt to improve the understanding of the physical properties of the ionic polysaccharides, numerous experimental studies on the solution behavior of these polymers have been reported. Persistence length, which characterizes the stiffness of the chains, has been evaluated for different alginate samples by electron microscopy [31,32], and for pectin [33,34] and glucuronan [35] samples, light scattering and viscosity measurements have been used. The values, ranging from 75 to 340 Å, characterize semirigid to rigid polymers. Theoretically, configurational characteristics can be calculated from statistical samples of disordered chains using molecular mechanics. Polymer chain models with corresponding dimensions have been presented for different polysaccharides [12,36–40] and this approach has been used in the present study in order to compare the four polyuronides. Experimentally, the dimensions of the acidic polysaccharides were evaluated in high ionic strength conditions, when the charges along the polymer chains were fully screened by the positive ions. The best way to represent charged groups in carbohydrate systems is still a matter of debate with the available current force fields. For example, unrealistic reduced conformational freedom [12] may result. For this reason, we have used the MM3 force field, which is well adapted to carbohydrates, and make the approximation that, in the calculations, the conformational behavior of the acidic form is capable of representing the behavior of the salt form related to experimental conditions. This assumption has led to satisfactory results for glucuronan [19] and hyaluronan [41]. Thus, the disordered chains were generated from the disaccharides using the corresponding (ϕ, ψ) maps and, from the resulting statistical samples, persistence lengths (L_p) and characteristic ratios (C_∞) were calculated. The values are reported in Table 3. The present study shows the results that two classes of configurational behavior can be distinguished, according to the di-axial or di-equatorial nature of the glycosidic linkage. Polyglucuronic and polymannuronic acids behave as semi-rigid polymers whereas polygalacturonic and polyguluronic acids are rather rigid with significant extensions. Snapshots of typical polymer conformations are illustrated in Fig. 3. Polyguluronic acid is well known for its rigidity, illustrated in the present calculations by the highest persistence length, i.e., 210 Å, which represents about 50 residues. As polyguluronic and polygalacturonic acids are mirror images (with the exception of the hydroxyl group at C-3) the calculated dimensions indicate that the position of this group (axial in polyguluronic acid) has a great influence on the stiffness of the chain. Comparatively, the position of the hydroxyl group at C-2 in the (1→4) diequatori-

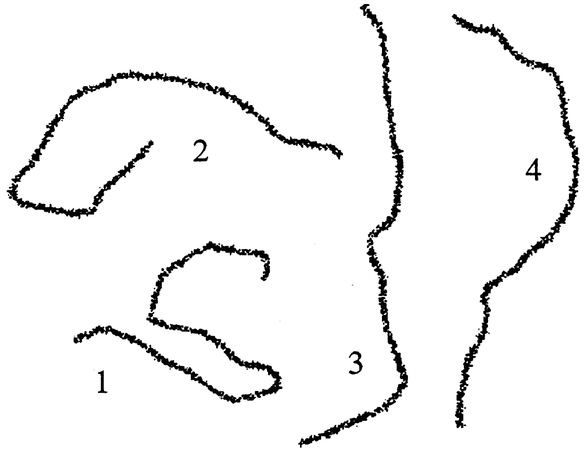

Fig. 3. Snapshots of typical disordered chains containing 100 residues generated with METROPOL (298 K). 1: glucuronan, 2: mannuronan, 3: guluronan, 4: galacturonan.

ally linked polysaccharides has a minor effect (see Table 3).

Direct comparison of the computed dimensions with experimental data is questionable since galacturonan and (guluronan and mannuronan) are the components of pectins and alginates, respectively, and do not occur as pure homopolymers. In pectin, the insertion of rhamnose units in the polygalacturonic acid backbone may induce a more [40] or less [34] pronounced kink. A persistence length of 75 Å has been measured by SANS on a sample containing 0.6% of rhamnose units [34]. In the case of alginates, the polymers consist of alternating blocks of guluronic (G) and mannuronic (M) acid residues. The relative extensions of the three types of blocks decrease in the order: GG > MM > GM [42]. For example, a persistence length of 90 Å has been determined by electron microscopy for an alginate sample rich in mannuronic acid residues (> 90%) [32]. The calculated values seem to be in a qualitative agreement with the above mentioned experimental data.

The ordered chain conformations.—From the lowest-energy wells, A and B, of the potential energy surfaces, regular helices have been extrapolated and minimized for the four polysaccharides. The structural, helical and energetic parameters of the helices generated are reported in Table 4. For the four polyuronides, the most stable conformations from the precursor disaccharides give rise to extended 2- or 3-fold helices. Side and top views of the best helical conformations generated are given in Fig. 4. Most of the present models correspond to the known helical forms of the polysaccharides in the solid or gel state. Fiber diffraction experiments indicate a right-handed 3-fold helical conformation for polygalacturonic acid [28] and a 2-fold screw periodicity for polyguluronic [27], polymannuronic [30], and polyglucuronic [43] acids. The experimental helical parameters (n, h) are in fairly good agreement with those computed in the present study. The calculated difference in energy between the 2- and 3-fold conformation for a given polysaccharide indicates that, with the exception of the guluronan, both types of helical conformation are almost equally favorable. Thus, depending on

Table 4
Structural[a], helical and energetic parameters of the best helical conformations extrapolated from the lowest-energy wells A and B for the four (1 → 4)-linked acidic polymers

Region	α-D-Gal*p*A		α-L-Gul*p*A		β-D-Man*p*A		β-D-Glc*p*A	
	A	B	A	B	A	B	A	B
ϕ	74.6	94.5	−84.2	−64.1	−64.2	−85.5	−67.0	−91.3
ψ	94.8	147.9	−155.3	−104.4	−104.6	−156.4	−107.2	−155.3
γ	154	136	−135	−143	127	126	134	129
n[b]	3	2	2	−3	−3	2	−3	2
h[c]	4.43	4.25	4.27	4.42	5.06	5.14	5.06	5.16
ΔE[d]	0.0	0.8	0.0	1.7	0.0	0.8	0.0	<0.1

[a] Angles in degrees.
[b] Number of residues per turn of the helix.
[c] Projection (Å) of one residue on the helix axis.
[d] Relative energy (kcal/mol/residue).

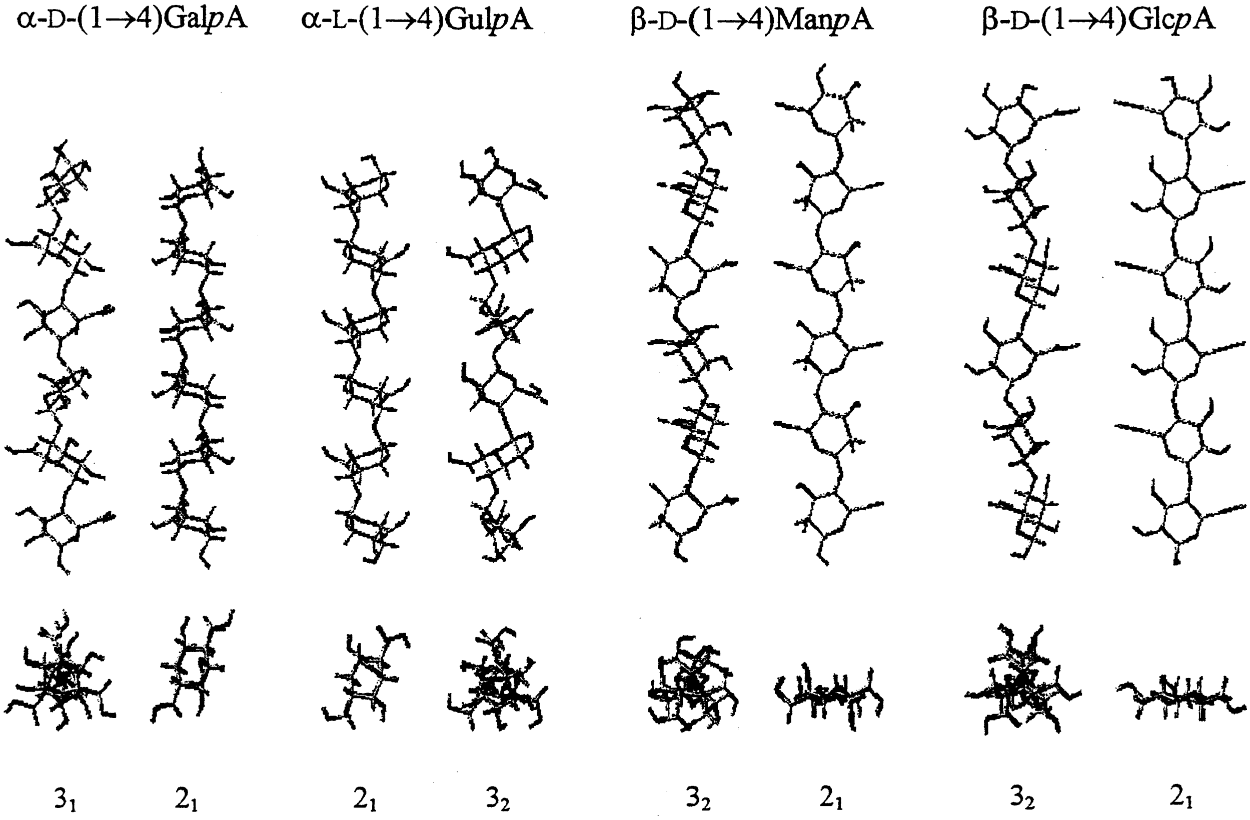

Fig. 4. Stable regular helical conformations of the four acidic polysaccharides. The helices are represented using projection parallel and orthogonal to their axes.

the environment (neighboring ions, solvent, etc.), one or the other form may exist. This conformational flexibility is exploited by the mannuronan, which adopts a 3-fold helical conformation in the solid state when present in the salt form [44]. Besides, based on circular dichroism experiments, a conformational transition between the gel and solid state has been demonstrated for pectin [45]. The ordered segments of polygalacturonic acid in the gel are thought to have a 2-fold screw axis [46]. In contrast, polyguluronic acid maintains a 2-fold helical structure in both gel and solid state. This is observed in both the acid form as well as in the different salt forms [47]. The high calculated energy difference (1.7 kcal/mol/residue) between the two ordered forms, namely 2_1 and 3_2, may explain such a conformational invariability.

Calcium binding.—The ionic polysaccharides alginate, pectin, and glucuronan have a wide range of industrial applications which mainly rest on their ability to form gels in the presence of divalent cations such as calcium. In the case of pectin and alginate, gel formation has been demonstrated to result from a specific interaction between calcium ions and blocks of galacturonate and guluronate residues, respectively. Selectivity coefficients $K_{\mathrm{K}}^{\mathrm{Ca}}$ indicate a strong calcium binding for polygalacturonate and polyguluronate chains compared to that of polymannuronate chains [47]. Furthermore, this interaction for the two former polysaccharides is very specific as the polymers exhibit an ability to bind Ca^{2+} in competition with Na^{+} over a wide range of relative concentrations. Polyglucuronate forms thermally stable gels with Ca^{2+} but the mechanism involved seems to be different [35,48]. In particular, the polymer shows no distinct selectivity since calcium ions are easily removed by sodium ions [35]. The above results demonstrate the importance of the structural features of the polysaccharide chains in calcium binding and indicate that differences between them result in some way from differences in the steric arrangement of the active groups. A structural study of all known single crystals of calcium–carbohydrate complexes reported that calcium interactions display considerable stereospecificity and that the strongest binding is likely to result from sets of oxygen atoms that possess the proper spacing and geometry for substituting water in the calcium coordination shell [49]. In this way, Angyal identified the axial–equatorial–axial sequence of hydroxyl groups on six-membered rings as a very efficient pattern for calcium binding [50]. Guluronic acid residues exhibit this pattern.

In the present study, we screened calcium-chain interactions for the four polyuronides in an attempt to improve our understanding of the experimentally observed differences. For polysaccharide systems, gel formation is thought to result from chain association in which the chains assume an ordered secondary structure [51]. In the case of alginates and pectins, the cooperative nature of the calcium binding is best explained by the presence of binding sites distributed in ordered arrays and formed between chains [26,52]. In agreement with these experimental data, the ordered conformations of the four polyuronides were used to study the calcium–oligouronate interaction. Since the calcium salt of polyguluronate and polymannuronate are known to exhibit, respectively, a 2_1 and a 3_2 helical conformation in the solid state [44], only these forms were explored. The crystal conformation of the salt form of glucuronan is unknown, and the helical conformation of the oligogalacturonate segments involved in the junction zones of pectin gels is debated [53–55]. Consequently, for polygalacturonate and polyglucuronate chains, both 2- and 3-fold helical structures were studied. A calcium ion probe was moved around the van der Waals surface of the different regular uronate chains and the best interaction energies are reported in Table 5.

The interaction energies are significantly lower for the α-(1 → 4)-linked polymers than for the β-(1 → 4) ones. This indicates a strong calcium binding for polyguluronate and polygalacturonate chain, in agreement with experimental data. In the case of the galacturonate chain, the two stable helical conformations provide an efficient binding. In order to evaluate the specificity of the interaction, the number of favorable positions for the calcium ion along the chain has been computed over a 15 kcal/mol window above the lowest interaction energy. These data are reported in Table 5. A representation of the different polymer chains with these favorable binding sites is shown in Fig. 5. As previously suggested [56], the corrugated polygalacturonate and polyguluronate chains offer several oxygen atoms from consecutive residues whose stereochemical arrangement fits into the coordination sphere of the cation. In contrast, the linear conformations of polymannuronate and polyglucuronate chains do not offer such a suitable arrangement. Consequently, the guluronate and galacturonate chains exhibit a high specificity for calcium binding and present well-defined chelation sites (Fig. 6(a,b)). The best interaction positions over the 15 kcal/mol window correspond to periodic sites (perfectly

Table 5

Conformational and energetic features of the most favorable calcium–oligoouronate (DP = 6) interactions derived from GRID calculations

	Pectate		Alginate		Glucuronate	
	α-D-Gal*p*A		α-D-Gul*p*A	β-D-Man*p*A	β-D-Glc*p*A	
n^{a}	3	2	2	−3	−3	2
γ^{b}	154	−120	131	127	134	129
E^{c}	−100	−98	−100	−61	−61	−46
N^{d}	6	3	5	20	36	44

[a] Number of residues per turn of the helix.

[b] Angles in degrees.

[c] Absolute energy (kcal/mol).

[d] Number of binding sites over a 15 kcal/mol window above the lowest interaction energy.

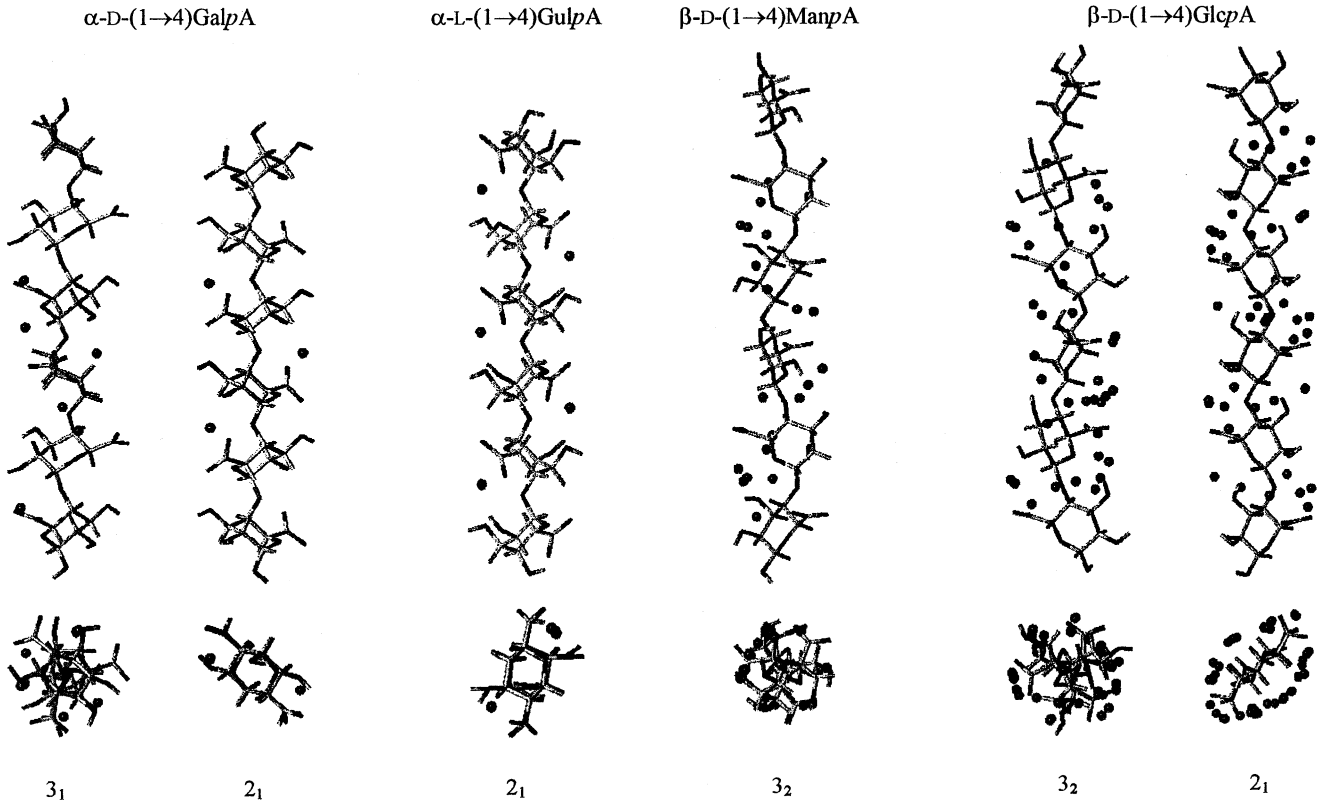

Fig. 5. Favorable positions of calcium ions along the stable ordered conformations of the four polysaccharides over a 15 kcal/mol window above the lowest interaction energy.

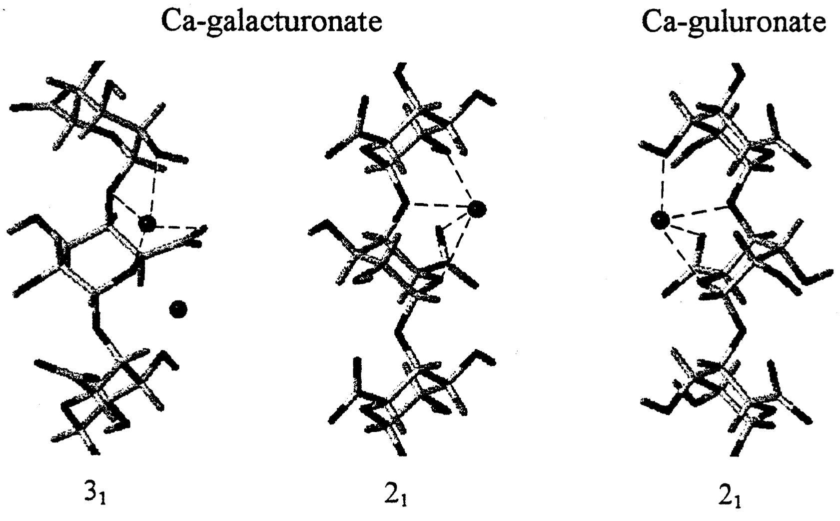

Fig. 6. Predicted chelation sites of calcium ions on the stable helical conformations of galacturonate and guluronate chains.

defined in the case of the guluronate chain with five periodic sites of the hexamer) that refer to an identical chelation site (Fig. 6). It is interesting to note that the 3_1 conformation of the galacturonate chain provides an additional possible binding site. In all cases, the α-(1 → 4)-linked polymers provide tetradentate chelation sites. As for the polygalacturonate chain, the coordination patterns predicted for the two helical conformations are in agreement with those derived from EXAFS experiments for single pectate chains [57]. Nevertheless, as calcium ion dimerizes the pectate and alginate chains in solution, yielding gel formation, the four oxygen atoms predicted as ligands are not all expected to be involved in the coordination sphere of calcium ions at the dimer level. For example, the model derived from fiber diffraction data of concentrated calcium pectate gel [58], mentions that only O-5, O-6, O-2′ from one chain and O-6, O-2′ from the other are coordinated to the calcium ion, compared to the four potential O-5, O-6, O-4, O-2′ ligands.

The highly specific interaction described above clearly indicates that for α-(1 → 4)-linked polyuronides, calcium binding is far from a pure electrostatic interaction between cations and carboxylate oxygen atoms. In contrast, β-(1 → 4)-linked polymers seem to bind calcium essentially through electrostatic interactions. As shown in Fig. 5, many positions for Ca^{2+} are equally favorable, the only necessary condition being the proximity of the negatively charged oxygen atoms. Consequently, polymannuronate and polyglucuronate do not display any specificity for calcium binding. The flat chains do not offer sites for preferential interactions to occur. This is particularly true in the case of polyglucuronate chains which, irrespective of the helical conformation, present a 'cloud' of favorable positions for calcium ions around the chain (Fig. 5, top views). This lack of specificity in calcium binding corroborates the absence of selectivity experimentally observed with respect to sodium ions [35]. Furthermore, the structural features of the modeled calcium–glucuronate interaction, compared with those of guluronate and galacturonate systems, may explain why it is possible to remove calcium ions by sodium ions in the gel [35], while polygalacturonates and polyguluronates form very stable calcium gels where calcium ions are very difficult to displace.

5. Conclusions

The present study provides a detailed description of the different levels of structural organization and interactions that characterize four different (1 → 4)-linked ionic polysaccharides: galacturonan, guluronan, mannuronan, and glucuronan. The application of molecular modeling procedures of increasing complexity has provided: (1) detailed descriptions of the low-energy conformers of the repeating unit of the polymers, (2) insights into the disordered state of the polysaccharide chains, (3) complete characterization of helical structures which are likely to occur in the ordered state as well as in their conformational transitions, and (4) characterization of the calcium binding. The comparative approach used allows for the establishment of the similarities and differences that characterize these polyuronides. The conformational analysis of the disaccharide subunits indicates that besides the type of the glycosidic linkage, which mainly governs the available conformational space, the position of the secondary hydroxyl groups, axial or equatorial, has an influence on the conformational behavior. The axial position may destabilize the zig-zag conformations and favor the extended ones. This effect is particularly important in the case of the polyguluronic acid, which presents a high stiffness compared to that of its quasi mirror image, the polygalacturonic acid, and a characteristic conformational invariability in the ordered state. Configurational statistics derived from samples of disordered chains indicate that the relative stiffness of the chains increases in the order: glucuronan $\sim$ mannuronan $<$ galacturonan $<$ guluronan. Furthermore, the present study establishes quantitative structure–property relationships concerning the gel-forming properties resulting from calcium binding to polyuronides. We do not pretend to model effective junction zones (this is currently under study in our laboratory), but wanted to evaluate the calcium–polysaccharide interaction. The ability of the different uronate chains to bind calcium ions demonstrates the high stereo-specificity of the interaction between Ca^{2+} and the α-(1 → 4)-linked polymers and the presence of well-defined chelation sites along the chains. In contrast, the β-(1 → 4)-linked polysaccharides display a weak calcium interaction with no particularly favorable binding site. In addition to the quantitative results, the calculated models provide an intuitive understanding of the competition between sodium or potassium ions and calcium ions experimentally observed in pectate, alginate and glucuronate gels.

References

[1] J. Courtois, B. Courtois, A. Heyraud, P. Colin-Morel, L. Dantas, T. Stadler, P. David, *Agro-Food-Industry Hi-Tech.*, 6 (1993) 31–34.
[2] B.R. Thakur, R.K. Singh, A.K. Handa, *Crit. Rev. Food Sci. Nutr.*, 37 (1997) 47–73.
[3] E. Onsøyen, *Carbohydr. Eur.*, 14 (1996) 26–31.
[4] K.I. Draget, G. Skjåk-Bræk, O. Smidsrød, *Int. J. Biol. Macromol.*, 21 (1997) 47–55.
[5] S. Pérez, M. Kouwijzer, K. Mazeau, S.B. Engelsen, *J. Mol. Graph.*, 14 (1996) 307–321.
[6] IUPAC–IUB Joint Commission on Biochemical Nomenclature (JCBN), *Arch. Eur. J. Biochem.*, 131 (1983) 5–7.
[7] N.L. Allinger, Y.H. Yuh, J.H. Lii, *J. Am. Chem. Soc.*, 111 (1989) 8551–8566.
[8] N.L. Allinger, Y.H. Yuh, J.H. Lii, *J. Am. Chem. Soc.*, 112 (1990) 8293–8307.
[9] N.L. Allinger, Z.Q.S Zhu, K. Chen, *J. Am. Chem. Soc.*, 114 (1992) 6120–6133.
[10] A.D. French, R.S. Rowland, N.L. Allinger, in A.D. French, J.W. Brady (Eds.), *Computer Modeling of Carbohydrate Molecules*, ACS Symposium Series, 430, American Chemical Society, Washington, DC, 1990, pp. 120–140.
[11] S.B. Engelsen, S. Cros, W. Mackie, S. Pérez, *Biopolymers*, 39 (1996) 417–433.
[12] B. Boutherin, K. Mazeau, I. Tvaroska, *Carbohydr. Polym.*, 32 (1997) 255–266.
[13] N. Metropolis, A.W. Rosenbluth, M.M. Rosenbluth, A.H. Teller, E. Teller, *J. Phys. Chem.*, 21 (1953) 1087–1092.
[14] R. Lapasin, S. Pricl, *Rheology of Industrial Polysaccharides, Theory and Applications*, Blackie Academic and Professional, imprint of Chapman and Hall, Glasgow, 1995, pp. 63–83.
[15] P.J. Goodford, *J. Med. Chem.*, 28 (1985) 849–857.
[16] L.T. Saunderson, *J. Am. Chem. Soc.*, 105 (1983) 2259–2261.
[17] W. Mackie, S. Pérez, R. Rizzo, F.R. Taravel, M. Vignon, *Int. J. Biol. Macromol.*, 5 (1983) 329–341.
[18] S. Miertus, C. Bertocchi, R. Krempaska, S. Paoletti, *Int. J. Biol. Macromol.*, 17 (1995) 183–188.
[19] I. Braccini, A. Heyraud, S. Pérez, *Biopolymers*, 45 (1998) 165–175.
[20] M. Hricovini, S. Bystricky, A. Malovikova, *Carbohydr. Res.*, 220 (1991) 23–31.
[21] S. Cros, C. Hervé du Penhoat, N. Bouchemal, H. Ohassan, A. Imberty, S. Pérez, *Int. J. Biol. Macromol.*, 14 (1992) 313–320.
[22] A. Di Nola, G. Fabrizi, D. Lamba, A.L. Segre, *Biopolymers*, 34 (1994) 457–462.
[23] C. Gouvion, K. Mazeau, A. Heyraud, F.R. Taravel, I. Tvaroska, *Carbohydr. Res.*, 261 (1994) 187–202.
[24] J.R. Ruggiero, R. Urbani, A. Cesàro, *Int. J. Biol. Macromol.*, 17 (1995) 205–212.
[25] D.A. Rees, *Pure Appl. Chem.*, 33 (1981) 1–14.
[26] D.A. Rees, E.J. Welsh, *Angew. Chem., Int. Ed. Engl.*, 16 (1977) 214–224.
[27] E.D.T. Atkins, I.A. Nieduszynski, W. Mackie, K.D. Parker, E.E. Smolko, *Biopolymers*, 12 (1973) 1879–1887.
[28] M.D. Walkinshaw, S. Arnott, *J. Mol. Biol.*, 153 (1981) 1055–1074.
[29] J. Sugiyama, R. Vuong, H. Chanzy, *Macromolecules*, 24 (1991) 4168–4175.
[30] E.D.T. Atkins, I.A. Nieduszynski, W. Mackie, K.D. Parker, E.E. Smolko, *Biopolymers*, 12 (1973) 1865–1978.
[31] B.T. Stokke, A. Elgsaeter, G. Skjåk-Bræk, O. Smidsrød, *Carbohydr. Res.*, 160 (1987) 13–28.
[32] B.T. Stokke, D.A. Brant, *Biopolymers*, 30 (1990) 1161–1181.
[33] I.G. Plashchina, M.G. Semenova, E.E. Braudo, V.B. Tolstoguzov, *Carbohydr. Polym.*, 5 (1985) 159–179.
[34] S. Cros, C. Garnier, M.A.V. Axelos, A. Imberty, S. Pérez, *Biopolymers*, 39 (1996) 339–352.
[35] L. Dantas, A. Heyraud, B. Courtois, J. Courtois, M. Milas, *Carbohydr. Polym.*, 24 (1994) 185–191.
[36] B.A. Burton, D.A. Brant, *Biopolymers*, 22 (1983) 1769–1792.
[37] T.A. Talashek, D.A. Brant, *Carbohydr. Res.*, 160 (1987) 303–316.
[38] G.S. Buliga, D.A. Brant, *Int. J. Biol. Macromol.*, 9 (1987) 77–86.
[39] R. Urbani, A. Di Blas, A. Cesàro, *Int. J. Biol. Macromol.*, 15 (1993) 24–29.
[40] J.R. Ruggiero, R. Urbani, A. Cesàro, *Int. J. Biol. Macromol.*, 17 (1995) 213–218.
[41] Unpublished results.
[42] O. Smidsrød, R.M. Glover, S.T. Whittington, *Carbohydr. Res.*, 27 (1973) 107–118.
[43] A. Heyraud, L. Dantas, J. Courtois, B. Courtois, W. Helbert, H. Chanzy, *Carbohydr. Res.*, 240 (1994) 275–279.
[44] W. Mackie, *Biochem. J.*, 125 (1971) 89P.
[45] E.R. Morris, D.A. Powell, M.J. Gidley, D.A. Rees, *J. Mol. Biol.*, 155 (1982) 507–516.
[46] D.A. Rees, *Carbohydr. Polym.*, 2 (1982) 254–263.
[47] R. Khon, Symp. Carbohydr. Chem., Bratislava, 1974, pp. 371–397.
[48] C. Cantoni, F. Zennaro, C. Bertocchi, P. Mariotti, R. Rizzo, *Biopolymers*, 45 (1998) 157–163.
[49] M.L. Dheu-Andries, S. Pérez, *Carbohydr. Res.*, 124 (1983) 324–332.
[50] S.J. Angyal, *Pure Appl. Chem.*, 35 (1973) 131.
[51] E.R. Morris, I.T. Norton, in E. Wyn-Jones, J. Gormally (Eds.), *Aggregation Processes in Solution*, Elsevier, Amsterdam, 1983, pp. 549–593.
[52] A. Malovikova, M. Rinaudo, M. Milas, *Biopolymers*, 34 (1994) 1059–1064.
[53] E.R. Morris, D.A. Powell, M.J. Gidley, D.A. Rees, *J. Mol. Biol.*, 155 (1982) 507–516.
[54] M.C. Jarvis, *Plant Cell Environ.*, 7 (1884) 153–164.
[55] M.C. Jarvis, D.C. Apperley, *Carbohydr. Res.*, 275 (1995) 131–145.
[56] G.T. Grant, E.R. Morris, D.A. Rees, P.J.C. Smith, D. Thom, *FEBS Lett.*, 32 (1973) 195–198.
[57] L. Alagna, T. Prosperi, A.A.G. Tomlinson, R. Rizzo, *J. Phys. Chem.*, 90 (1986) 6853–6857.
[58] M.D. Walkinshaw, S. Arnott, *J. Mol. Biol.*, 153 (1981) 1075–1085.