

## A molecular dynamics investigation on the occurrence of helices in polygalacturonic acid

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### Abstract

Partially esterified polygalacturonic acid is the main component of pectin in higher plants. It constitutes the mucilaginous soil–root interface and acts as an accumulation phase for nutrients, being an important media for the diffusion of ions towards the root absorbing cells. The carboxylic groups and their methyl esters markedly affect the ability of the pectin molecules to bind oppositely charged ions and to form gels. Molecular Dynamics was employed to investigate the conformational equilibrium and the intermolecular interactions of a system constituted by two polygalacturonic acid chains, each formed by 24 units of galacturonic acid. The results suggest that, as evidenced for other polysaccharides, a helix based structure could be proposed for the polygalacturonic acid chains. © 1997 Elsevier Science B.V.

*Keywords:* Polygalacturonic acid; Polysaccharides; Molecular dynamics

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### 1. Introduction

Acid sugars play an important role in the biochemical processes involved in plant nutrition. They are found, both in monomeric and polymeric forms, on the root surfaces and cell walls. The polymers are the main constituents of the mucilaginous soil–root interface (mucigel): they behave as an accumulator for the nutrients and are involved in the diffusion process of the ions towards the absorbing cells [1–3]. These properties may be due to the polygalacturonic acid (PGA) chains (Fig. 1), which are the main constituents of the root mucilage [4–6]. Electron microscopy studies provide evidence that these polymers are organised in a fibrillar structure [7–11]. These

structures act as selective filters for the nutritive elements and regulate the movement of ions through and out of the cells [12].

The knowledge of only the primary structure of complex carbohydrates is no longer sufficient to understand and explain their function and specificity [13]. The three-dimensional structures of pectin and polyuronic acid determine their interactions with other ions, molecules and macromolecules and are significant for their function and biological activity [14–16].

In previous work [17], we applied Molecular Dynamics to the study of the motion of  $\text{Ca}^{2+}$  ions around four units PGA chains, and found evidence for the existence of channels where the  $\text{Ca}^{2+}$  ions preferentially move. Computational chemistry methods may greatly help in the determination of the

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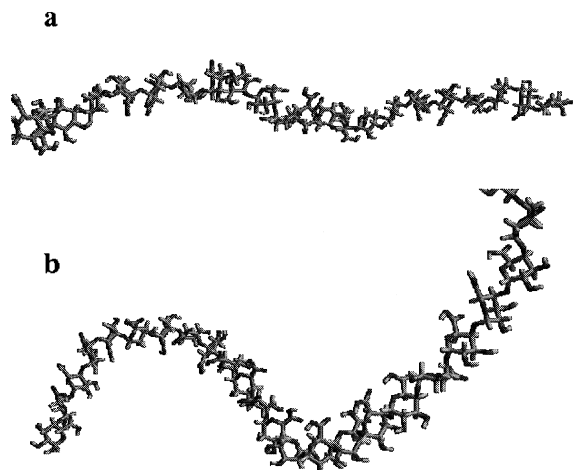


Fig. 1. (a) The MM optimized conformation of the 24 units PGA chain. (b) An equilibrium conformations extracted from the trajectory computed for the one-chain system. Hydrogen atoms are omitted.

three-dimensional structure of polysaccharides allowing us to understand and explain the behaviour of the PGA chains and the diffusion of ions inside. This study reports the results of a Molecular Dynamics survey about the aggregation process between PGA chains counting 24 galacturonic acid monomers. The evidence of helix formation among the chains is reported. The driving force of the aggregation process is the formation of interchain hydrogen bonds.

## 2. Materials and methods

The length of the PGA chains on which the Molecular Dynamics (MD) experiments were performed was 24 units, with an overall molecular weight of 4264 per chain. The conformation of the chain used

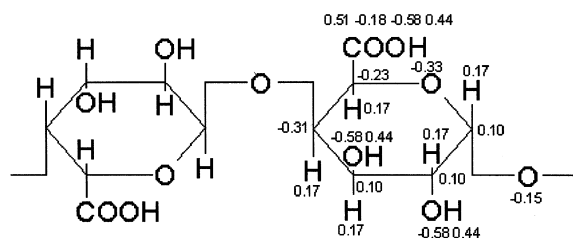


Fig. 2. Schematic representation of the polygalacturonic acid (PGA) chain. Numbers indicate the partial atomic charges fitted on the electrostatic potential computed by ab initio calculation at the 6-31G\* accuracy level.

as input in the MD experiment was previously optimised via a Molecular Mechanics (MM) calculation and is shown in Fig. 2(a).

The DLPOLY2 [18] program was used to carry out the MD experiments. We employed the AMBER plus GLYCAM force field [19]. The partial atomic charges were calculated by fitting the electrostatic potential computed by ab initio HF-SCF calculations at the 6-31G\* accuracy level. The GAMESS program [20] was employed to perform both the ab initio and charge fitting computations. The values of the fitted charges are shown in Fig. 1. A relative dielectric constant value of 1.0 and a spherical cut off of 20 Å for Coulomb and long range forces were adopted in all the simulations.

Several MD 1000 ps trajectories were performed on the systems with one, two, and three PGA chains. The runs were stopped after 1000 ps as no significant variation was observed in the total energy during the last 300 ps.

## 3. Results and discussion

### 3.1. One chain system

A snapshot of an equilibrated conformation is shown in Fig. 2(b). The radial distribution functions ( $g(r)$ ) between the various types of oxygen atoms in the PGA chain are shown in Fig. 3(a).

The peaks at  $r \approx 3\text{Å}$  in Fig. 3(a) are attributable to intramolecular hydrogen bonding between vicinal hydroxyl groups and between the endocyclic oxygen and the nearest hydroxyl group. The remaining peaks are due to the periodic structure of the PGA.

### 3.2. Two chain system

The analysis of the MD trajectory shows that the two chains strongly interact reciprocally by the formation of hydrogen bonds, which involves both the carboxylic and the hydroxyl functions in the PGA chain. The  $g(r)$  between the oxygen and hydrogen atoms are shown in Fig. 3(b). The pronounced peaks at  $r \approx 2\text{Å}$  are representative of hydrogen bond formation among the chains. Intramolecular association between vicinal hydroxyl groups contributes to the  $g(r)$  in the gOH-HO case. The peaks beyond  $r \approx 3\text{Å}$  in both curves are due to the intramolecular association

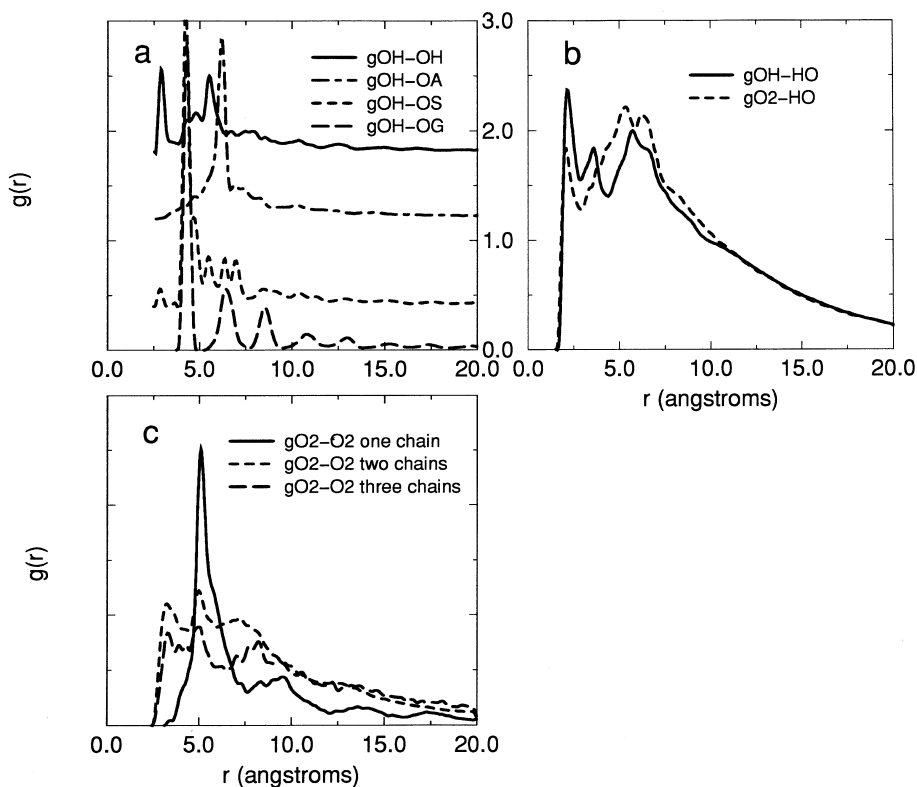


Fig. 3. Radial distribution functions. (a) One-chain system, between the oxygen atoms of the PGA: OH, oxydriol oxygens; OA, sp<sup>3</sup> oxygens in the COOH groups; OS, endocyclic oxygen; OG, glycosidic oxygen. (b) Two-chain system between the hydroxyl (OH) and the carboxyl (O<sub>2</sub>) oxygen atoms and the hydroxyl (HO) hydrogen atoms in the PGA chains. (c) Between the carboxyl (O<sub>2</sub>) oxygen atoms in the three different systems studied.

between the hydroxyl groups. A snapshot of the final conformation after 1000 ps MD trajectory is shown in Fig. 4(b).

The chains are linked together forming a helix with a rough twofold screw symmetry where the stable junction zones consist of hydrogen bridges.

### 3.3. Three chains system

A snapshot from the final step of a trajectory of the 3 chain system is shown in Fig. 5. The oxygen atoms in the same chains have the same colour.

The PGA chains are folded in the middle and exhibit a strong interchain interaction which leads to the folding of the chains onto themselves in a way which closely resembles the so called *egg and box* model [21]. The  $g(r)$  between the carboxylic oxygen atoms are shown in Fig. 3(c) and compared with those

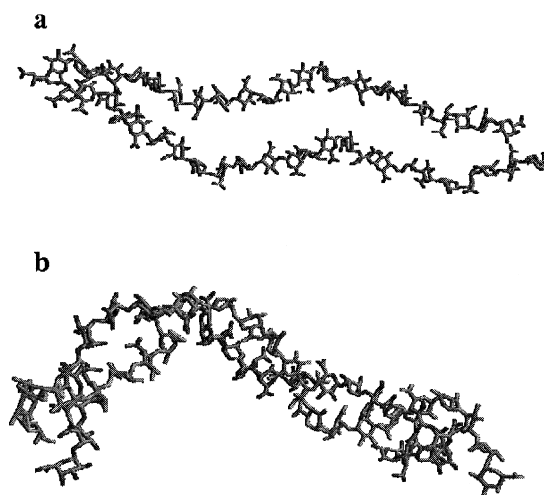


Fig. 4. Snapshot of the MD trajectory for the two-chain system. (a) Initial configuration. (b) After a 1000 ps run. Hydrogen atoms are omitted.

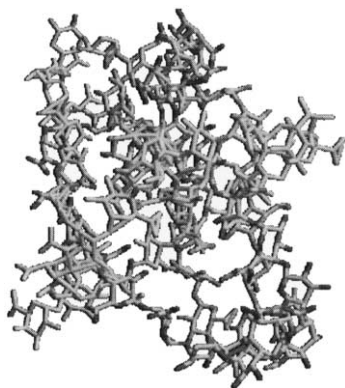


Fig. 5. Snapshot from the MD trajectory of the three-chain system after a 1000 ps run. The oxygen atoms in the same chains have the same colour. Hydrogen atoms are omitted.

computed for the 1 and 2 chain systems. The overall conformation forms a three-dimensional network. These findings agree with the models [22,23] proposed to explain the gel formation in pectic substances.

The pattern of the  $g(r)$  emphasises the importance of the carboxylate groups in the interchain interaction. The peaks, at  $r \approx 5\text{\AA}$ , are due to the carboxyl groups in the neighbouring monomer units, while the peaks at  $r \approx 8\text{--}10\text{\AA}$  originate from the carboxyl groups in the  $n$  and  $n + 2$  monomers. Both the two and three chain systems show a peak at  $r \approx 3\text{\AA}$ , which indicates the formation of the hydrogen bonds between the carboxylate groups of different chains.

The results of the MD experiments show good qualitative agreement with the reports about gel formation by PGA chains in strong acidic media. The analysis of the  $g(r)$  provides evidence that the collapse of the PGA chains is mainly due to the formation of interchain hydrogen bonds.

### Acknowledgements

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