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Interaction of Ca^{2+} and Na^{+} ions with polygalacturonate chains: a molecular dynamics study

B. Manunza*[1], S. Deiana[1], M. Pintore[1] and C. Gessa[2]

[1]*DISAABA, Università di Sassari, V.le Italia 39. 07100 Sassari, Italy*
[2]*Istituto di Chimica Agraria, V.le Berti Pichat 10. 40127 Bologna, Italy*

Partially esterified polygalacturonic acid is the main component of pectin in higher plants. The carboxylic groups and their methyl esters markedly affect the ability of the pectin molecules to bind oppositely charged ions and to form gels. In order to make a contribution to the understanding of the mechanisms which regulate the ionic transfer at the soil–root interface and in the apoplast, we report the results of a set of molecular dynamics experiments in which the interactions of four fully deprotonated fragments of polygalacturonic acid, each counting 12 units, 300 water molecules and 48 or 24 Na^{+} and Ca^{2+} ions were studied.

We observed the formation of Ca^{2+} bridges between the polygalacturonate chains. The forces driving the aggregation processes are characterized by the formation of strong coulombic interactions between the metal ions and the carboxylate groups. The results are consistent with experiment evidence of the formation of Ca–polygalacturonate organized gels. The Ca–polygalacturonate complex exhibits a lower energy compared to that of Na–polygalacturonate. The ratio of the Na^{+} and Ca^{2+} diffusion coefficients agree well with experimental reports.

***Keywords*: polygalacturonic acid, molecular dynamics, soil–root interface**

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 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 which are the main constituents of the root mucilage [4–6]. Transmission and scanning electron microscopy have been used to show that these polymers are organized in a fibrillar structure [7–10]. In previous work [11], we used molecular dynamics (MD) to the study of the movement of Ca^{2+} ions around PGA chains each formed by four units, and suggested the existence of channels where the Ca^{2+} ions move. Computational chemistry methods may greatly help to understand and explain the gel formation by PGA chains and the diffusion of ions inside it. We report here the results of an MD survey about the effect of the Ca^{2+} and Na^{+} ions on the aggregation between charged PGA chains in aqueous solution.

Materials and methods

The MD experiments were performed by using the DLPOLY2 [12] program. The AMBER plus GLYCAM [13] force field was used with the necessary adaptations, while the PGA 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 [14] was used to perform both the *ab initio* and charge-fitting computations. The values of the fitted charges are shown in Figure 1. The DLPOLY utility ‘wateradd’ was used to add 300 rigid simple point charge water molecules [15].

The Ewald summation method was used to evaluate the coulombic interactions with a dielectric constant value of 1.0. A time step of 0.001 ps was adopted in all the simulations. Calculations were carried out on an IBM RS6000 and on CRAY T3D at CINECA (Italy).

Preliminary MD runs were carried out *in vacuo* on a system composed of four PGA chains (twelve units), with an overall molecular weight of 2132 per chain, and 48 or 24 Na^{+} and Ca^{2+} ions (PGA–Na and PGA–Ca, respectively).

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*To whom correspondence should be addressed. E-mail: bruno@antas.agraria.uniss.it.

An orthogonal cell was adopted with a = b = 40 Å and c = 60 Å. The conformation of the PGA chains used as input for the MD experiment, was previously determined by performing a molecular mechanics search for the minimum energy conformation. To allow for a relaxation of the density of the systems, both the PGA–Na and PGA–Ca systems were equilibrated for 150 ps at constant pressure of 1 atm (=101 325 Pa). The pressure relaxation time was $\tau p = 0.5$ ps. The temperature was kept constant by coupling the system to a thermal bath of $T_0 = 298$ K [16] using a temperature relaxation time of 0.1 ps. This value makes the temperature coupling weak enough to avoid any significant effect on the atomic properties of the system [17]. A spherical cut–off was adopted for the non-bonding interactions with an initial radius values of 15 Å which was progressively reduced as the cell dimension decreased. At the end of the 150 ps equilibration period, 300 water molecules were added to each system, allowing them to relax their density for a further 200 ps at $P = 1$ atm and $T = 298$ K. As the total energy changed by less than 1% during the last 100 ps we considered the systems to be at equilibrium. The final cell dimensions are shown in Table 1.

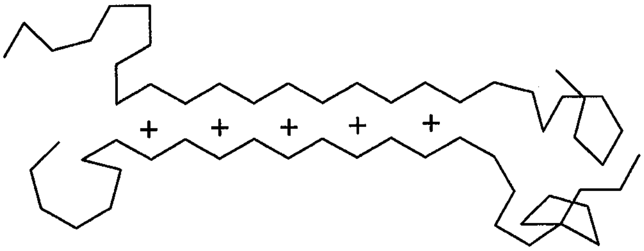

Figure 1. The ‘egg-box’ model represents the interchain association in alginate gels by dimerization of the chain sequences in a regular, buckled, two-fold conformation, with interchain chelation of cations on specific binding sites along each chain.

The final configuration was used as the starting point for a 500 ps NVT (number of molecules, volume and temperature constants) run at 298 K and the trajectory data were collected. No significant variation was observed in the total energy during the last 300 ps.

Results and discussion

The difference in the total energy between the PGA–Ca and PGA–Na system was found to be − 8.15 Kcal/moles of carboxylate group, a value which accounts for the major stability of the PGA–Ca aggregates [18]. The ratio between the Ca^{2+} and Na^{+} computed diffusion coefficients was about 2.0, which agrees with experimental data reported for pectic acid [19].

Table 1. Dimensions (Å) of the molecular dynamics cells after a 200 ps NPT (number of particles, pressure and temperature constant) run

	a	*b*	*c*
PGA–Ca–300H_2O	22.17	22.17	40.46
PGA–Na–300H_2O	20.37	20.37	37.83

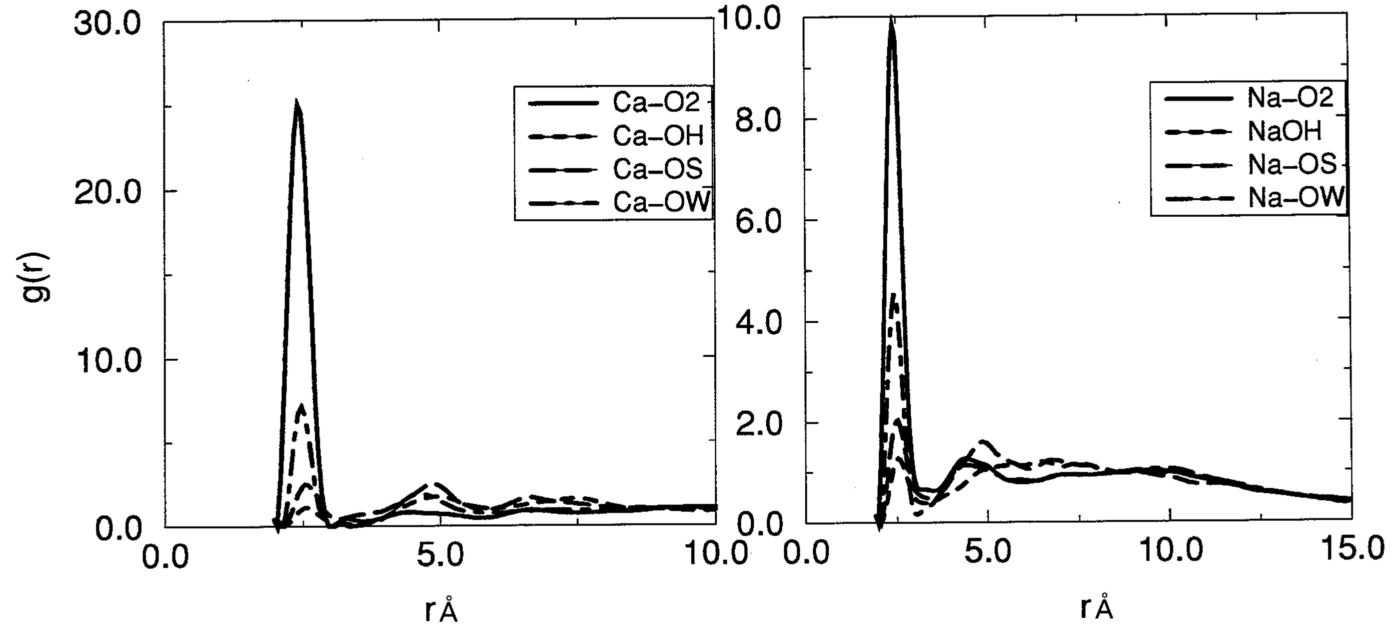

Figure 2. Radial distribution function between the metal ions and the oxygen atoms. (O2, OH, OS and OW oxygen atoms in the carboxylic, alcoholic, ester groups and water, respectively.)

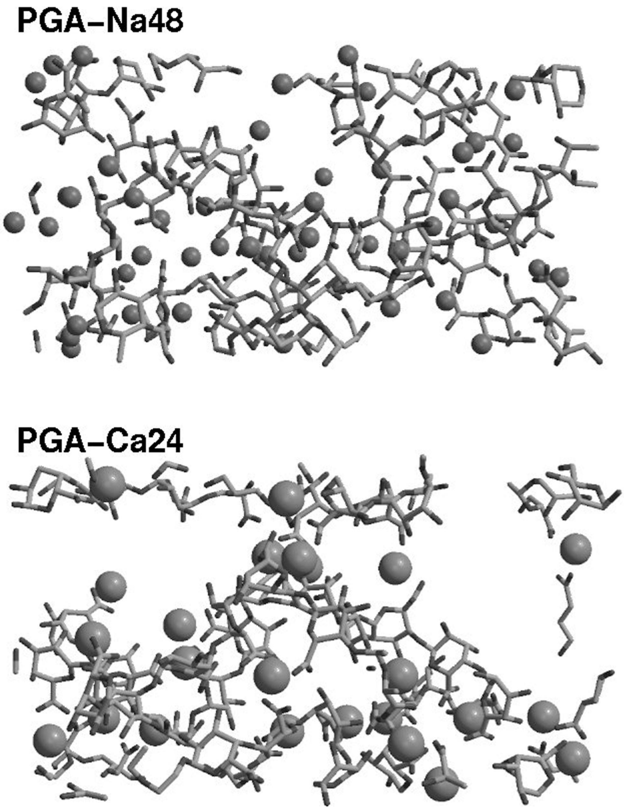

Figure 3. Schematic diagrams of the equilibrium configurations of the PGA–Ca and PGA–Na systems. The PGA chains are arranged in a fibre structure which exhibits a rough two-fold symmetry with the Ca^{2+} ions forming bridges between the chains. Hydrogen atoms and water molecules are omitted.

The radial distribution functions are shown in Figure 2. The Ca^{2+} ions form bridges between carboxylic groups belonging to the same or to different chains while the Na^+ ions interact with only one carboxylic group, with less inter- and intrachain bridging. This fact is evidenced by the relative height of the first peak in the g(Na-O2) and g(Ca-O2) radial distribution functions (O2 indicates the carboxylic oxygen atoms), the latter being double the height of the former. The plots indicate that, even at a lesser extent, the alcoholic (OH) and the esteric (OS) functions enter the coordination sphere of the metal ions [17, 20].

The pronounced peaks in the g(C–C) calculated for the PGA–Ca system, as well as the relative height of the first peak, suggest that the PGA–Ca chains aggregate in a more regular way.

The PGA chains are arranged in a fibre structure which exhibits a rough two-fold symmetry with the Ca^{2+} ions forming bridges between the chains as shown by the plots in Figure 3. The Ca^{2+} ions are disposed along rows bridging intra- or intermolecular carboxylic groups. This arrangement is consistent with the egg-box model proposed for alginates and pectates [19, 20] (Figure 1).

Conclusions

The results of the MD experiments described above show a good qualitative agreement with the reports on aggregation, gel formation and precipitation of PGA–salt chains. The analysis of the MD trajectories, moreover, allows confirmation that the aggregation of the PGA chains is due to the formation of inter- and/or intrachain bridges between the polygalacturonate chains. The carboxylic groups are those mainly involved in such a mechanism. Moreover, the results account for the higher affinity of Ca^{2+} ions towards the PGA matrix, in comparison with the Na^+ ions. We mantain that these findings suggest that more effort should be devoted to calculate the effect of the concentration of both the PGA and the metal ions on the interactions and the conformation of the PGA chains involved in the metal chelation processes. A further approach should be the study of competition among different ions for the binding sites of the PGA chains. The knowledge and the understanding of such phenomena have a basic importance in the study of the ionic transfer at the soil–root interface, which is the first state of the plant nutrition mechanism.

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References

1 Deiana S, Erre L, Micera G, Piu P, Gessa C (1980) *Inorg Chim Acta* **46**: 249–53.
2 Deiana S, Micera G, Muggiolu G, Gessa C, Pusino A (1983) *Colloids Surfaces* **6**: 17–25.
3 Morel JL, Mench M, Guckert A (1986) *Can J Bot* **53**: 1729–35.
4 Floyd RA, Ohlrogge AJ (1970) *Plant and Soil* **33**: 341–3.
5 Leppard GG (1974) *Science* **185**: 1066–7.
6 Paull RE, Johnson CM, Jones RL (1975) *Plant Physiol* **56**: 300–6.
7 Gessa C, Deiana S, Marceddu S (1989) In Plant membrane transport: the current position (Dainty J, De Michelis MI, Marrè E, Rasi Caldogno F, eds) pp 615–16. Amsterdam: Elsevier.
8 Guckert A, Breish H, Reisinger O (1975) *Soil Biol Biochem* **7**: 241–50.
9 Jenny H, Grossenbacher K (1963) *Soil Sci Amer Proc* **27**: 273–7
10 Gessa C, Deiana S (1992) *Plant and Soil* **140**: 1–13.
11 B. Manunza, S. Deiana, C. Gesa (1996) *Theochem* **368**: 27–9.
12 DLPOLY2 is a package of molecular simulation routines written by W. Smith and T.R. Forester, copyright the Council for the Central Laboratory of the Research Councils, Daresbury Laboratory, Nr Warrington (1994–1996).
13 Woods RJ, Dwek RA, Edge CJ, Fraser-Reid B (1995) *J Phys Chem* **99**: 3832–46.

14 Schmidt MW, Baldridge KK, Boatz JA, Elbert ST, Gordon MS, Jensen JH, Koseki S, Matsunaga N, Nguyen KA, Su SJ, Windus TL, Dupuis M, Montgomery JA (1993) *J Comput Chem* **14**: 1347–63.

15. Berendsen HJC, Postma JPM, van Gunsteren WF, Hermans J (1984) *J Phys Chem* **81**: 3684–90.

16 Gidley MJ, Morris ER, Murray EJ, Powell DA, Rees DA (1979) *J Chem Soc Chem Comm* 990–1.

17 Kohler J (1991) In *Molecular Dynamics. Applications in Molecular Biology* (Goodfellow JM, ed). London: Macmillan Press.

18 Ravanat G, Rianudo M (1980) *Biopolymers* **19**: 2209–22.

19 Rinaudo M, Milas M (1974) *J Polym Sci* **12**: 2073–81.

20 Walkinshaw MD, Arnot S (1981) *J Mol Biol* **153**: 1075–85.

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