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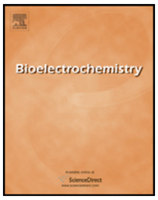

Competition of copper and/or calcium in nickel–pectin interaction by potentiometric and voltammetric techniques

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ABSTRACT

The interaction between nickel and pectin extracted from citrus fruit was studied in 0.10 M KNO_3, at pH 5.5 and 25 °C. Differential pulse and/or square wave polarography were used to determine free nickel. For a high coverage degree (θ) of the pectin by the metal ion a good fitting was observed between experimental results and the model that includes both complex species, ML and ML_2 (M for the metal ion and L for the ligand). In the ML_2 species, Ni(II) interacts with two carboxylate groups of different chains, resulting in an inter-chain association. For low θ values, the formation of ML_2 is hindered due to the repulsion between the negative charges of carboxylic groups in two independent segments of pectin. The influence of calcium or copper ions on the free nickel concentration, in the presence of pectin, may lead to a decrease in free nickel concentration, contrary to what would be expected from direct competition between Ca(II) or Cu(II) and Ni(II) for the pectin binding sites. This is due to the partial neutralisation of the negative carboxylic charges by the positive charges of the divalent cations, which favours NiL_2 formation through the association of independent chains.

1. Introduction

Partially esterified polygalacturonic acid (Fig. 1) is the main component of pectins in higher plants [1], and is an important constituent of the apoplastic transport system.

Polygalacturonic acid (PGA) acts as a selective filter of nutritive elements and regulates the movement of ions through and out of the cells [2,3]. The presence of carboxyl groups and methyl ester has a determinant influence on the physical–chemical properties of PGA, mainly in terms of complexation and gel formation.

To interpret Cu and/or Ni–PGA interaction, some authors assumed only the formation of the ML complex [4,5], and others the ML_2 species [6]. In reference 7 the species ML is proposed for Ni and ML_2 for Cu. Species formation depends on the metal ion, pectin origin and esterified fraction, as well as on the metal ion and pectin concentrations. In the interaction of Ca with pectins a great emphasis is given on the inter-chain association due to stronger cooperative binding of Ca [8].

Although the binding affinity of polygalacturonic acid (PGA) or derivatives with metal ions has been extensively investigated, namely with calcium [3,9–15], copper [4,7,16–18], and nickel [4–7], there is a total lack of information about the competition between different metal ions for the binding sites of PGA. Further investigation should be done in this context. On the other hand, although voltammetric methods have been widely used in metal ion speciation in aqueous solution, in the presence of pectins only very few applications can be found, and none about Ni(II)–pectin interaction, to the best of our knowledge.

The serpentine-derived soils are known to be less favourable to plant growth and productivity due to the large content of Ni(II) present in these type of soils, although in a low dose Ni(II) is an essential trace-nutrient for higher plants [19]. The uptake of Ni(II) by a very common tree in Mediterranean countries, *Quercus ilex*, on highly contaminated serpentine soils of NE Portugal (about 25×10^{-6} mol/g of soil), has been studied by Nabais et al. [20,21]. These authors found Ni concentrations of (3 to 7)$\times10^{-6}$ M in the xylem of *Q. ilex* grown in serpentine soils, which is about nine times higher than the concentration in sandy loam soils. In that range of concentrations, Ni(II) may compete with Ca(II) for the same binding sites of PGA. Since Cu(II) is another heavy metal often present in this type of soils, it is also important to know how its presence affects Ni complexation with pectins. PGA is responsible for important interactions that occur between solutes and cell walls of xylem vessels, since pectins are one of the main constituents of that tissue. The interactions between cations and the negatively charged groups of the cell walls can delay the ion transport in the xylem sap [21]. On the other hand, PGA may be released from the walls of root vascular cells, being present at lower levels in the xylem sap [22]. In this way, cations may be partly transported from roots to shoots of higher plants complexed to PGA.

The knowledge of metal ions interaction with PGA has important implications in the physiology of plants exposed to those metals.

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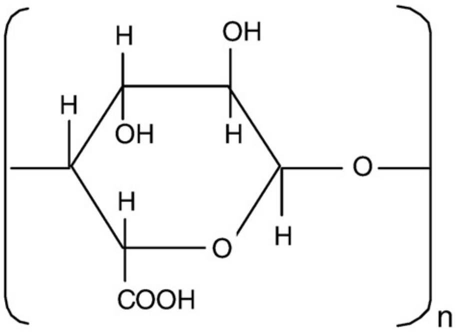

Fig. 1. Polygalacturonic acid (PGA) structure.

Chelating agents as PGA are able to keep free trace metal ions within certain limits, thus reducing their toxicity. On the other hand, data on the interaction of metal ions with pectins allow a better understanding in the transfer of macro and micro metal ion nutrients through the soil–root interface and in the modifications induced by those ions on the fibrillar structure of PGA [23]. If the presence of Ca and/or other divalent ions decrease the distance between adjacent chain-segments, the probability to form more energetic cross-links increases, enhancing the strength of the network [24,25]. Also, due to its metal binding capacity, pectins have been considered a very interesting alternative to remove transition and heavy metals from wastewater and soils [26,27].

In the present work, the interaction of pectin with Ni(II), in the absence or presence of Ca(II) or Cu(II), was investigated at pH 5.5, similar to that of *Q. ilex* xylem sap. Voltammetric techniques were used to determine free nickel and free copper concentrations, for ligand concentrations in the absence of adsorption on the mercury electrode. Potentiometry with specific electrodes was also used in the determination of free Ca(II) or Cu(II). A large range of concentrations were tried to find the best models that fit the experimental results.

2. Experimental

2.1. Reagents

In all solutions, water was distilled and passed through a Millipore Milli-Q system. A potassium salt of esterified (25%) pectin extracted from citrus fruit was purchased from Sigma (P-9311) (www.Sigma-Aldrich.com). Stock solutions of Ca(II), Cu(II), and Ni(II) (as nitrates), with a concentration of 0.10 M, were prepared from analytical grade reagents. The concentration ranges used for pectin and for metal ions are presented in Table 1.

Mercury used in the working voltammetric electrode was suprapure and purchased from Merck.

Dissolved oxygen was removed from solutions in the electrochemical cell (potentiometric and voltammetric experiments) by bubbling nitrogen of 99.995% purity.

2.2. Equipment and experimental conditions

All of the experiments (potentiometric and voltammetric) were carried out in a thermostatic cell under a purified nitrogen atmosphere at 25.0 °C in 0.1 M KNO_3. The experiments in the presence of metal ions were performed either by titration (with a waiting time of 5 min for each point) or using batch solutions (2 h or 1 day).

2.2.1. Potentiometric experiments

For potentiometric measurements, a Sentek-Denver potentiometer was used with a Metrohm Ag/AgCl reference electrode. Free calcium and free copper concentrations were measured using, respectively, Ca(II) (Orion 93-20) and Cu(II) (Orion 94-29) electrodes.

A Metrohm pH electrode (ref. 6.1033.500) with a Metrohm reference electrode (ref. 6.0733.100) and an Orion pH semi-micro electrode (91-03 SCE) were used in the pectin acid/base titration and in the pH control at 5.5, respectively.

2.2.2. Voltammetric experiments

Differential pulse polarography (DPP) and square wave voltammetry (SWV), used to determine free Ni(II) concentration, were performed with an Eco Chemie Autolab-PGST12 attached to a Metrohm 663 VA stand (with a hanging mercury electrode as working electrode (HMDE ref. 6.1246.020), Ag/AgCl reference electrode with double junction and a glassy carbon counter electrode) and to a personal computer using GPES 4.7 software (Eco Chemie). The experimental conditions in DPP were: initial potential of −0.8 V, final potential of −1.3 V, step potential of 2 mV, and amplitude of 50 mV. In SWV, the experimental conditions were: frequency of 10 Hz, initial potential of −0.8 V, final potential of −1.3 V, step potential of 5 mV, and amplitude of 25 mV.

3. Results and discussion

3.1. Working concentration range of pectin

Pectin contains a concentration of $(1.90 \pm 0.02) \times 10^{-3}$ mol of carboxylic groups per gram of pectin. From its dissociation constant (pK_H equal to 3.76 [18]) it can be found that at pH 5.5 the pectin is about 98% de-protonated.

Voltammetric measurements of Cu(II) were limited to a maximum ligand concentration of 0.056 g dm^{-3} due to pectin adsorption on mercury in the potential range of the Cu(II) reduction peak (peak potential $E_p \sim -0.1$ V) [18]. For higher ligand concentrations, free copper was measured by potentiometry with a Cu(II) selective electrode. The highest concentration used in Ni(II)–pectin studies was 1 g of pectin dm^{-3}, since for higher concentrations, the differential pulse base line (obtained in the absence of nickel) began to present two broad peaks in the potential range of −1.0 V, close to the reduction peak potential of Ni(II).

3.2. Voltammetric signal of Ni(II)

The redox couple Ni(II)/Ni^0(Hg) had an irreversible behaviour in the absence or presence of pectin, with a peak width of 103 ± 5 mV. The peak potential was not affected by the presence of pectin, but the peak current decreased with the increase in ligand concentration, i.e., the complex showed an inert behaviour within the timescale of the technique. The lability criteria [28] also predict an inert behaviour for the Ni(II)–pectin system if the Eigen mechanism [29] (ML complex formation is kinetically limited by the dehydration of the metal ion M) is assumed.

For inert complexes, the free metal concentration is directly determined from the peak current:

$$[M] = i_p^{M+L} / i_p^{M} \times [M]_t$$

where i_p^M and i_p^{M+L} are the peak currents in the absence and presence of pectin (M stands for metal ion and L for ligand), respectively, for the same total concentration of Ni(II) in solution, $[M]_t$.

Table 1
Concentration ranges used in this work.

Pectin (g dm^{-3})	Ca(II) (M)	Cu(II) (M)	Ni(II) (M)
0.45–1.0			$(0.02–20) \times 10^{-4}$
0.50–0.80	$(5.0–10) \times 10^{-4}$		$(0.1–0.8) \times 10^{-4}$
0.50–1.0		$(1.0–5.0) \times 10^{-4}$	$(2.0–10) \times 10^{-4}$