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Spectroscopic Studies on the Binding of Iron(II) and (III) Ions to Polygalacturonic Acid

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The iron(II) and (III) complexes formed by polygalacturonic acid have been investigated by means of Mössbauer and ESR spectra. It is observed that the binding of iron(II) is dependent on the water content of the samples. In fact, different hexacoordinated species are revealed by Mössbauer spectra in fully hydrated, air-dried and anhydrous samples. On the other hand, iron(III) ions give rise to polynuclear structures, which are not destroyed upon solvation and are stable over a wide pH range. The results provide plausible explanation of the mechanisms governing the iron uptake and transport in plants.

Introduction

Chemical factors involved in the absorption and transport of iron are of biochemical significance for plants, which require a continuous supply of the element to maintain proper growth. Besides, when, due to ion deficiencies, iron stress phenomena occur, the above mechanisms are genetically controlled, becoming essential for the survival of the plant [1].

It is accepted that the main chemical reactions concerning the availability of iron to plants are localized in the roots and are dependent on the mechanisms of iron uptake and transport [1].

On root surfaces polygalacturonic components of the cell-walls are responsible for ion uptake, providing about 90% of cation exchange capacity [2].

We have already reported the ability of such matrices to interact with metal ions by forming complexes of widely differing chemical nature [3].

In view of the role played by interactions with iron mineral forms, we have investigated iron polygalacturonates to provide needed information on the bonding sites, valence states and mobility of iron(II) and (III) ions in these systems. An understanding of the iron(III) binding could be of further theoretical interest as exemplifying the behavior of other trivalent ions, whose interaction mechanisms are still unknown.

The two major methods employed in this investigation were Mössbauer and ESR spectroscopies.

The spectroscopic analysis was performed, as in a previous work [3], on samples with varying hydration degrees.

In this study, also the behavior of the fully hydrated Fe(III)-gels at various pH values was investigated in order to provide information about the ability of the polymeric matrix to protect the coordinated ions against possible hydrolysis and precipitation as oxyhydroxide.

Experimental

Preparation and Analysis of the Complexes

The complexes were prepared, according to described procedures [3], from aqueous suspensions of polyacid (J. T. Baker) in $FeSO_4$ and $FeCl_3$ or $Fe(NO_3)_3$ solutions. Samples with different ion contents were examined. Analyses for iron in the samples were obtained, after oxidizing the organic matter with conc. HNO_3, by using a Spectraspan IV emission spectrometer.

Thermal analyses were performed on a Perkin-Elmer TGS-2 thermogravimetric apparatus under nitrogen stream.

Spectroscopic Measurements

Electronic spectra were recorded on a Beckman Acta M IV spectrophotometer equipped with a

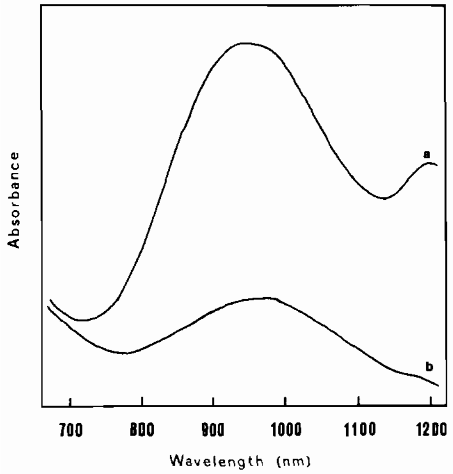

Fig. 1. Reflectance electronic spectra of (a) air-dried (H_2O ~ 15% wt.) and (b) dehydrated Fe(II) polygalacturonate complexes.

reflectance unit. $BaSO_4$ was the reference sample.

First derivative ESR X-band spectra were taken on a Varian E-9 instrument equipped with a standard temperature control unit. DPPH was used as field marker.

Mössbauer spectra were recorded at 77 K with a Laben spectrometer operating in constant acceleration mode. A ^{57}Co/Rh source was employed. The velocity scale was calibrated by using an iron foil as standard. Each spectrum was computer-fitted with Lorentzian components by a least-squares procedure. Isomer shifts are reported with respect to metallic iron.

Mössbauer spectra of hydrated samples were recorded on frozen suspensions. The fully hydrated iron(III) samples were obtained by suspending the air-dried complexes in the minimum volume of water and adjusting the pH to selected values through the addition of either HCl or NaOH solutions. After centrifugation, the remaining slurry was submitted to Mössbauer analysis in frozen state.

Results

Fe(II) polygalacturonates

The electronic spectra of Fe(II) samples in air-dried and dehydrated states respectively, are given in Fig. 1. They are characterized by a broad absorption band typical of $Fe(II)O_6$ octahedral environments [4], corresponding to the $^5T_{2g} \rightarrow {}^5E_g$ transition in O_h symmetry. As previously reported for other metal(II) polygalacturonates [3], small but significant differences distinguish the electronic spectra of air-dried and dehydrated samples.

TABLE I. Mössbauer Parameters for Fe(II) Polygalacturonates at 77 K.[a]

Sample	I.S. (mm s^{-1})	Q.S. (mm s^{-1})	Γ^b (mm s^{-1})
Hydrated	1.32(1)	3.13(1)	0.73(1)
Air-dried	1.364(6)	3.057(7)	0.67(1)
Dehydrated	1.380(5)	3.002(7)	0.69(1)

[a] Estimated standard deviation in the last significant figure of each datum is given in parentheses. [b] Line width.

Indeed, the band shown at about 950 nm by the air-dried samples after dehydration was found shifted towards lower energy values (970 nm). This indicates that in anhydrous samples hexacoordinated ions are still present, which are subjected to increased geometrical distortions, due to weakened σ-donor properties of the ligands, after substitution of water molecules with O-donor groups of the polysaccharid chains.

Mössbauer spectra of Fe(II) samples at various hydration degrees are given in Fig. 2. Clearly evident are peaks constituting quadrupole split pairs. The results obtained for the centre shifts (I.S.) and quadrupole splittings (Q.S.) are listed in Table I. The above values are consistent with high-spin species in octahedral environments, whose distortion extent is dependent on the hydration degree of the samples. In fact, Q.S. values are significantly increased on increasing the water content, as already shown for Fe(II) pectates [5].

In dehydrated samples, divalent ions are subjected to significant geometrical distortions due to the involvement of coordinating O-groups having different donor properties [3]. Thus, the increase of Fe(II) Q.S. values on increasing water content may reflect the progressive formation of predominantly hydrated environments.

The different ligand properties, which affect the symmetry in the electron density on the nuclei of Fe(II) ions, produce also modifications in the *s* electron density, as revealed by the variations of the I.S. values. In fact, in agreement with the electronic spectra, the isomer shifts indicate less covalent bonds as dehydration goes on.

Thus, by analogy with previous results [3], distinct species, associated to different hydration degrees of the ions, are suggested. Most likely, they are the aquo-ion or a largely hydrated species in the hydrated samples and inner-sphere carboxylate complexes after partial or total dehydration.

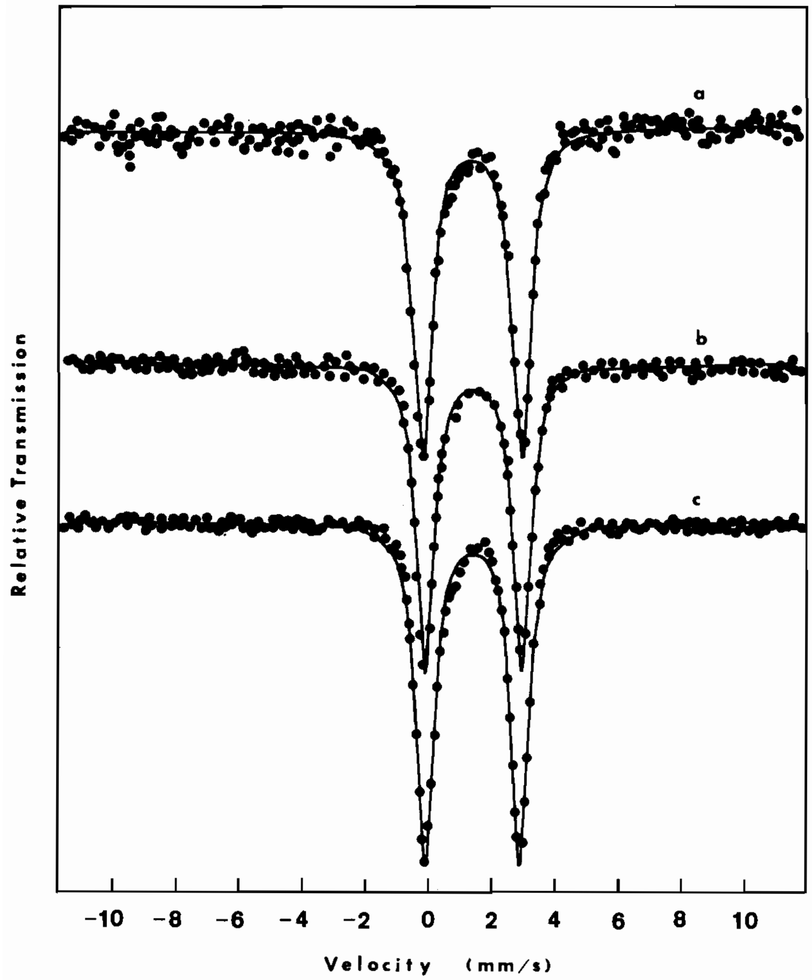

Fig. 2. Mössbauer spectra of Fe(II) polygalacturonates in different hydration conditions: (a) fully hydrated sample; (b) air-dried sample (H_2O ~ 15% wt.); (c) dehydrated sample.

TABLE II. Mössbauer Parameters for Fe(II) Polygalacturonates at 77 K.[a]

Sample	I.S. (mm s^{-1})	Q.S. (mm s^{-1})	Γ^b (mm s^{-1})
Air-dried	0.45(2)	0.87(2)	1.00(4)
Dehydrated	0.44(2)	0.86(2)	0.96(4)
Hydrated pH = 1.0	0.46(6)	0.83(9)	1.41(6)
Hydrated pH = 2.0	0.45(2)	0.84(2)	0.93(4)
Hydrated pH = 3.0	0.43(2)	0.87(2)	1.06(4)
Hydrated pH = 4.5	0.45(3)	0.91(3)	1.00(6)

[a] Estimated standard deviation in the last significant figure of each datum is given in parentheses. [b] Line width.

Fe(III) Polygalacturonates

Fe(III) exchanged samples show Mössbauer spectra with quadrupole doublets characteristic of high-spin ferric complexes (see Fig. 3 and Table II).

In addition, variable amounts of iron(II) ions, exhibiting Mössbauer parameters quite similar to those listed in Table I, are always present in the samples.

The presence of Fe(II) species is attributable to reducing properties of the polysaccharid moiety, revealed also in the interaction with other mineral forms [6].

Both full hydration and dehydration of the samples did not produce remarkable modifications in the Mössbauer parameters of the iron(III) species, suggesting the presence of poorly hydrated complexes with the first coordination sphere of the ions almost completely filled by functional groups of the polysaccharid.

The results point also to stable and covalent Fe(III)–polygalacturonate bonds, the polymeric matrix being able to protect the metal ions from the conversion into oxyhydroxides in the pH range investigated. Indeed, no evidence of magnetically-ordered

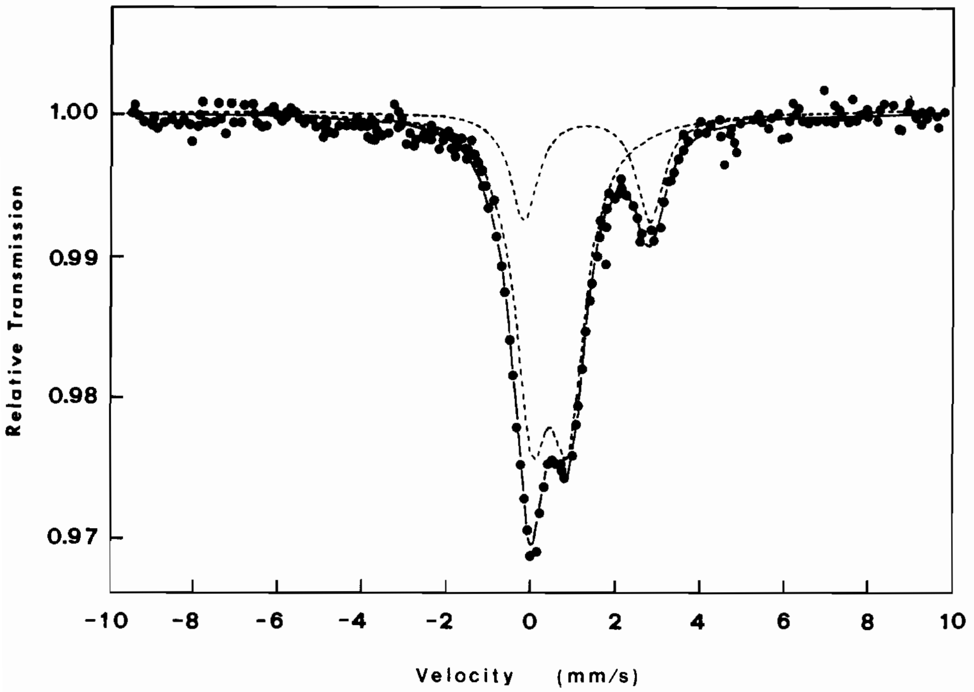

Fig. 3. Mössbauer spectrum of air-dried Fe^{3+}-exchanged polygalacturonate (H_2O ~ 15% wt., Fe = 3.9% wt.).

species was given in the Mössbauer spectra. On the contrary, in the hydrated samples the iron(II) impurity originally present was remarkably decreased at the lowest pH values. The explanation for this behavior is that the ferrous ion is displaced by H^+ more easily than the ferric one, due to the widely different chemical bonds involved in the complexes.

By an examination of the parameters listed in Table II, it is worth nothing that the Q.S. values are particularly high, reflecting a large asymmetry of the electric field gradient on the Fe(III) nuclei. These values show a striking resemblance to those of polynuclear iron complexes, and, particularly, of μ_3-oxo trimers whose structures are well-known. Examples of Fe_3O structures are $[Fe_3(RCOO)_6(OH)_2]X{\cdot}nH_2O$ complexes (Q.S. = 0.43–0.73 mm s^{-1}) [7], $Fe_3O(CH_3COO)_6(H_2O)_3Cl]{\cdot}5H_2O$ (Q.S. = 0.55 mm s^{-1} at 300 K) [8] and $[Fe_3O(C_4O_4)_3(DMSO)_3(H_2O)_3](OH)$ (Q.S. = 0.91 mm s^{-1} at 20 K) [9]. Polynuclear structures on the basis of similar Q.S. values have been proposed also for Fe(III) complexes formed by other natural carboxylic polyelectrolytes as humic and fulvic acids [10, 11]. As for the latter examples, the most likely interpretation of our results is the formation of a relatively discoloured polynuclear structure. Really, the high line widths observed (see Table II) are consistent with the presence of unequivalent Fe(III) sites.

ESR spectra obtained at 9.15 GHz (Fig. 4) substantiate the hypothesis of polynuclear structures.

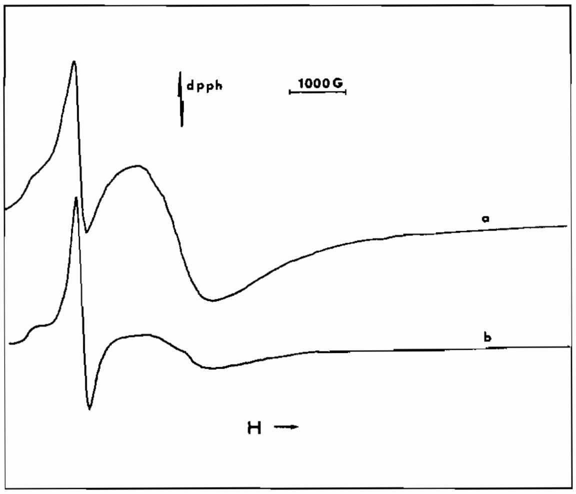

Fig. 4. ESR spectra of air-dried Fe(III) polygalacturonate (H_2O ~ 15 wt.% Fe = 6.8% wt.). Recording temperatures are (a) 295 K and (b) 100 K.

For all ion concentrations the spectra are characterized by a single low field line with a g value of 4.3, always accompanied by a 1350-gauss wide line centered at g ~ 2. As the Fe^{3+} content in the samples was increased, the broad band intensity was enhanced. On lowering the recording temperature, the line widths of the signals did not change but the intensity of the g ~ 2 resonance was notably weakened.

This behavior can be explained by attributing the g = 4.3 signal to isolated Fe(III) species in a rhombic crystal field environment. In such a case, the spin Hamiltonian proposed by Bleaney and Stevens [12] is used to fit the spectra:

$$\mathcal{H}_s = g\beta H\cdot S + D(S_z^2 - 1/3\,S(S+1)) + E(S_x^2 - S_y^2)$$

where the effective spin, S, for the Fe^{3+} ion is 5/2. D and E are energy terms representing the axial and rhombic components of the local crystal field at Fe^{3+} sites. In particular, for $E/D = 1/3$ an isotropic resonance at g = 4.28 is predicted together with resonances at lower magnetic fields down to about g ~ 9 [13].

On the other hand, the broad g ~ 2 signal is accounted for by Fe^{3+}–O–Fe^{3+} superexchange interactions which, for a pair of spins give rise to spin states S = 0–5. In the antiferromagnetic case, the g ~ 2 line is assigned to transitions in the high energy $S > 0$ states, all occupied at room temperature and split by the crystal and magnetic fields [14]. Besides, the intensity dependence of the resonance from the recording temperature, as shown in Fig. 4, confirms such conclusions, in agreement with previous theoretical suggestion and other experimental findings [14]. As the above resonance was produced even by Fe(III) ions doped in Ca- or Fe(II)-samples, the magnetic interactions can be interpreted as arising from specific ion clustering phenomena.

It is noticeable that the detection of two distinct ESR signals is not necessarily indicative that two comparable populations of Fe(III) species are present. Indeed, the different spectroscopic nature of the resonances prevents comparisons and does not exclude that only negligible amounts of isolated ions are present.

Discussion and Conclusions

The reported data support our previous hypotheses made about the interactions between polygalacturonic matrix and coordinated ions [3]. In fact, ions such as Fe(III) interacting with the polysaccharid only through electrostatic bonds retain their aquo-shells after sorption. In this case, as dehydration goes on, significant variations are observed in the ion environment, due to the progressive substitution of water molecules.

The above findings confirm similar results obtained on Fe(II) pectates and alginates [5] and allow to clarify the role played by the polysaccharid components in the binding of nutrient ions on the plant roots. Such ions, being adsorbed through electrostatic forces, are buffered against depletion but, at the same time, retain the mobility essential for the transport into the root cells.

On the contrary, other ions such as Fe(III) are retained through covalent bonds in inner-sphere complexes, which cause immobilization and possible accumulation.

The formation of polynuclear structures in Fe(III) polygalacturonates gives explanation to some biochemical processes occurring in plants. Indeed, ferric ions, by interacting with the polygalacturonic matrix, lose their mobility remaining dispersed in covalent polynuclear arrangements on the external surfaces. Thus ferric species require chemical reduction processes which transform them into divalent ions having a higher mobility due to the involvement of outersphere complexes.

Further investigations in this field will concern the binding of other tervalent ions and the specific mechanisms associated to their biological transport.

Acknowledgements

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