polyuronates, allows plant roots to remove nutrient cations from clay particles [1, 2].

The study of metal binding to polygalacturonic acid (Fig. 1) has drawn attention to the possible role

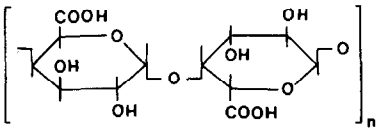
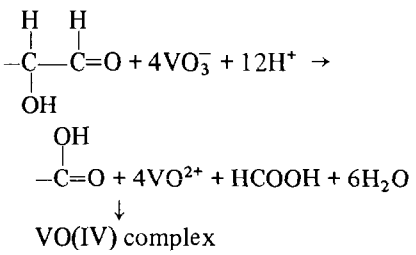


Fig. 1. Polygalacturonic acid.

of root polysaccharides in the mineral nutrition of plants. Evidence was found to show that specific interactions, which could have relevant functional and physiological implications, are established by the polysaccharide with metal ions [3–5]. Metal–polyion interactions of electrostatic nature are possible mechanisms accounting for the uptake and buffering of faster-moving nutrient ions. In this case, metals bound to the polysaccharide moiety retain enough mobility to reach the root cells and translocate into the plant. On the other hand, inner-sphere binding to carboxylate groups could be necessary for the uptake of certain micronutrients which, being in low concentration, are less competitive in the exchange process. With regard to the iron uptake, it has been shown that Fe(III) gives rise to polynuclear structures on the surfaces of the polysaccharide, whereas Fe(II) is more weakly retained as hydrated ion [4].

Recent developments have been concerned with redox processes occurring upon interaction of polygalacturonic acid with soil mineral species. Reduction of Fe(III) to Fe(II), V(V) to V(IV) and Mo(VI) to Mo(V), followed by complexation of reduced ions, has been observed. The reaction is due to the reducing properties of the polysaccharide end-units. For example, the following mechanism has been assessed for the reduction of VO_3^- to VO(IV):



Such processes appear to be highly significant, particularly in relation to iron uptake by plant roots. In fact, ferric species are not available for plants unless reduction to the divalent state occurs [6]. Based on our results, it is suggested that polysaccharides are active also in redox interactions and provide suitable pathways for the adsorption and transport of iron, which is essential for the survival of plants.

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S14

90% of Erythrocyte Copper is Coordinated in Cu_2-Zn_2 Superoxide Dismutase

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37% of the total copper concentration in red blood cells is found in superoxide dismutase as determined by immunochemical assays [1]. Most of the present purification techniques of superoxide dismutase include solvent precipitation of the haemolysate. Using this method 60% of erythrocyte copper cannot be further characterized due to coprecipitation and/or denaturation of possible other copper proteins.

A strictly aqueous preparation technique was employed which allowed the recovery of 90% of intracellular copper in the first step and 50% after the fifth and final step.

Bovine erythrocyte lysate was passed through DEAE-Sephacel. The bonded copper proteins were eluted with a linear NaCl-Gradient. By way of contrast discontinous absorption of the haemolysate resulted in the recovery of limited amounts of superoxide dismutase [1]. Concentration of the DEAE eluate and gel filtration of Sephadex G-75 yielded crude copper proteins of both high and low relative molecular mass. The first Cu-protein was the previously described $M_r = 400\,000$ Cu_2–$(haem_b)_2$–protein. It was purified to homogeneity and characterized as published elsewhere [2, 3]. The latter species turned out to be Cu_2Zn_2 superoxide dismutase which was further purified according to Stansell and Deutsch [1].

Determination of superoxide dismutase activity in the haemolysate employing the nitrotetrazolium blue assay in the presence and absence of EDTA

revealed that 90% of the erythrocyte copper is bound in superoxide dismutase. Only 10% of the copper is found in the high molecular fraction following gel filtration of the haemolysate. All other copper proteins migrate in the region near $M_r = 32\,000$.

The yield of both superoxide dismutase and Cu_2–$(haem_b)_2$–protein clearly shows that the copper of the red blood cell is distributed in these proteins. Taking into account minimal losses of intracellular copper in the course of the fractionation steps no copper proteins other than the preceding ones were detectable. It appears that Cu_2Zn_2 superoxide dismutase or cuprein is the major intracellular copper protein.

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S15

Attempts to Detect and Locate Platinum Metals in Plant Tissues by a Variety of Microscopic Techniques

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Root and leaf samples of *Eichhornia crassipes* (water hyacinth) containing the platinum metals were examined by a number of techniques.

(1) *Light microscopy*. This classical technique is of little use, except where the concentrations are unusually high. However, morphological changes caused by toxic elements can be studied. The palisade mesophyll of the leaf of a Pt-treated plant was found to be distorted in contrast to that in control or Rh (non-toxic) treated plants.

(2) *Scanning electron microscopy with energy dispersive X-ray analysis* (SEM + EDXA). Control root and leaf specimens showed high concentration of Ca and smaller amounts of P and S. Roots treated with $[PtCl_6]^{2-}$ were covered in electron dense deposits. EDXA showed Ca (K_α, 3.96 keV) and Pt (M_α, 2.05 keV; L_α, 9.44 keV; L_β, 11.07 keV). X-ray photo-electron spectra of these deposits showed a corrected Pt 4f binding energy of 76 ± 0.5 eV, indicating Pt^{4+} in the deposits.

(3) *Electron microscope analysis* (EMPA). Examination of Pt-treated roots showed clearly that platinum accumulated in the epidermal region, lesser amounts are in the cortex. Ruthenium however, was distributed more evenly.

(4) *Scanning transmission electron microscopy with energy dispersive X-ray analysis* (STEM + EDXA). Problems arise with Pt-treated root materials, which show widespread electron dense deposits. In order to avoid interference with Pt, sections were viewed unstained, hence cytological detail is absent. EDXA confirmed the presence of Pt.

S16

The Uptake and Accumulation of Platinum Metals by the Water Hyacinth (*Eichhornia crassipes*)

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The biological effects of the platinum group metals on plants have been studied by treating plants with chlorocomplexes of each metal at a range of concentrations. The vascular aquatic plant *Eichhornia crassipes* (water hyacinth) was selected for detailed study because of its remarkable ability to assimilate high levels of transition elements from solution. Water hyacinths are capable of recovering platinum group metals even from dilute solution though to varying degrees depending on the metal:

$$Pt^{2+} > Pd^{2+} > Os^{4+} \approx Ru^{3+} > Ir^{3+} \approx Rh^{3+}$$

decreasing % recoverey at the 0.05 ppm level

When this is compared to the relative order of toxicity at the 10 ppm level for each metal, some similarities emerge:

$$Pt^{2+}(5d^8) \approx Pd^{2+}(4d^8) > Os^{4+}(5d^4) \approx Ru^{3+}(4d^5) > > Ir^{3+}(5d^6) > Pt^{4+}(5d^6) \gg Rh^{3+}(4d^6)$$

decreasing order of toxicity at the 10.0 ppm level

The relationship between phytotoxicity and position in the periodic table is tenuous but appears to be linked with the oxidation state and hence electron configuration of the metal ion. The two least toxic ions, Ir^{3+} and Rh^{3+}, have the electron configuration (d^6); it is significant too that $Pt^{4+}(d^6)$ is far less toxic than $Pt^{2+}(d^8)$. A similar relationship has been found for the phytotoxicity of 1st row elements:

$$Cd^{2+}(4d^{10}) \gg Zn^{2+}(3d^{10}) > Ni^{2+}(3d^8) > Co^{2+}(3d^7)$$

decreasing relative toxicity, decreasing softness

Included in the soft acid classification is Pt^{2+} and Pd^{2+} whilst Rh^{3+} and Ir^{3+} are considered borderline between hard and soft, along with Fe^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+}. This approach goes some way to explaining