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Electrochemistry of copper(II) induced complexes in mycorrhizal maize plant tissues

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ABSTRACT

Aim of the present paper was to study the electrochemical behavior of copper(II) induced complexes in extracts obtained from mycorrhizal and non-mycorrhizal maize (*Zea mays* L.) plants grown at two concentrations of copper(II): physiological (31.7 ng/mL) and toxic (317 μg/mL). Protein content was determined in the plant extracts and, after dilution to proper concentration, various concentrations of copper(II) ions (0, 100, 200 and 400 μg/mL) were added and incubated for 1 h at 37 °C. Further, the extracts were analyzed using flow injection analysis with electrochemical detection. The hydrodynamic voltammogram (HDV), which was obtained for each sample, indicated the complex creation. Steepness of measured dependencies was as follows: control 317 μg/mL of copper < control 31.7 ng/mL of copper < mycorrhizal 31.7 ng/mL of copper < mycorrhizal 317 μg/mL of copper. Based on these results it can be concluded that mycorrhizal fungus actively blocks transport copper(II) ions to upper parts of a plant by means of adsorbing of copper(II) in roots. Rapid complex formation was determined under applied potentials 300, 500 and 600 mV during the measuring HDVs. It was also verified that mycorrhizal colonization reduced root to shoot translocation of Cu(II) ions.

1. Introduction

The majority (~80%) of terrestrial plant species form arbuscular mycorrhizas, a mutualistic symbiosis established with soil-borne fungi belonging to the Glomeromycota. This symbiosis play an important role in plant nutrition by providing access to soil-derived nutrients from sources not necessarily otherwise accessible to roots. The fungi receive a supply of carbohydrates in return. The formation of the arbuscular mycorrhizal symbiosis can improve plant uptake of (especially) phosphorus, and a range of other nutrients including Zn, Cu and K [1]. Although arbuscular mycorrhizas are most often considered important for uptake of immobile nutrients, they also play an important role in reducing uptake of heavy metals, including copper, where soil concentrations are high [2]. Thus, arbuscular mycorrhiza has various roles in terms of plant–copper interactions. Copper is essential for plant's photosynthesis and mitochondrial respiration, carbon and nitrogen metabolism, oxidative stress protection, and is required for cell wall synthesis, to name at least a few of its cellular functions [3]. Existence of copper in two oxidation states (Cu(I) and Cu(II)) allows this element to play a role as a reducing or oxidizing cofactor in various biochemical reactions [3]. But at the same time, this feature makes copper also potentially toxic since copper ions can catalyze the production of free radicals, in particular through Fenton chemistry, thus leading to the damage of proteins, DNA, and other biomolecules [4–7]. Therefore, immediately after its uptake the majority of copper ions are bound by scavenging proteins like metallothioneins to prevent copper from accumulating in a toxic form. However, part of the imported copper escapes this system and becomes captured by small binding proteins, so called copper chaperones [8,9] that spare copper from the detoxification systems and guide it to the target sites in the cell. The genome of *Arabidopsis thaliana* possesses over 30 sequence homologs that might encode for copper chaperones; however, only very few of these proteins are already characterized [10,11]. There are also discussed copper(II) ions complexes with other plant biologically important molecule as phytochelatin2,

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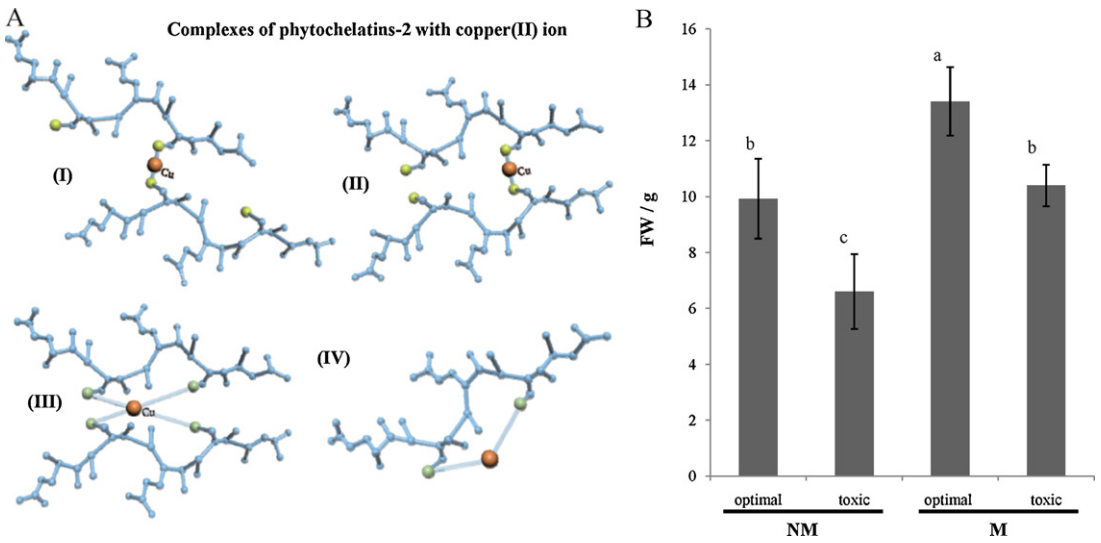

Fig. 1. (A) 3D models of complex between copper(II) ion and one **(IV)** or two **(I, II, III)** molecules of phytochelatin2. (B) Fresh weight (FW) of non-mycorrhizal (NM) and mycorrhizal (M) maize plants fertilized with Hoagland solution containing a physiological (31.7 ng/mL) or a toxic (317 μg/mL) dose of copper(II) ions. Data are means ± SD of six replicates. Data not sharing a letter in common differ significantly ($p < 0.05$).

which are shown in Fig. 1A. Interestingly, copper metabolism is intimately linked to iron metabolism [12]. In particular, the contribution of mugineic acid, nicotianamine, organic acids, histidine and phytate to metal homeostasis and mutual transporting is discussed by Haydon and Cobbett [13] and Curie et al. [14].

To study the fate of copper(II) ions in an organism, various bioanalytical tools with both advantages and disadvantages are used. Various types of spectrometric methods are often used for determination of copper content in real samples of various origins including plant tissues [15]. However, any real sample must be pre-treated before spectrometric analysis which may cause a loss of important information about copper and its complexes. On the other hand, electrochemistry represents a tool by which copper(II) ions content may be determined and also the complexes depending on treatment and type of a sample studied [16–18]. The aim of this paper was to study the effect of the arbuscular mycorrhizal symbiosis and two doses of biologically available copper(II) ions on the content of copper(II) ions and copper(II)-induced thiol-rich complex formation in maize by using flow injection analysis with electrochemical detection.

2. Materials and methods

2.1. Flow injection analysis coupled with electrochemical detection

The instrument for flow injection analysis with amperometric detection (FIA-ED) consisted of solvent delivery pump operating in range of 0.001–9.999 mL/min (Model 582 ESA Inc., Chelmsford, MA, USA), a reaction coil (1 m), and an electrochemical detector. The electrochemical detector includes one low volume (5 μL) flow-through analytical cell (Model 5040, ESA, USA) consisting of glassy carbon working electrode, hydrogen-palladium electrode as reference electrode and auxiliary electrode, and Coulochem III as a control module. The sample (5 μL) was injected manually using 6-way injection valve. The data obtained were processed by Clarity software (Version 3.0.04.444, Data Apex, Czech Republic). The experiments were carried out at room temperature. A glassy carbon electrode was polished mechanically by 0.1 μm of alumina (ESA Inc., USA) and sonicated at room temperature for 5 min using a Sonorex Digital 10 P Sonicator (Bandelin, Berlin, Germany) at 40 W [19,20]. Other experimental parameters were optimized; see in Section 3.

2.2. Chemicals and pH measurements

HPLC-grade methanol (>99.9%; v/v) was from Merck (Dortmund, Germany). Other chemicals were purchased from Sigma–Aldrich (St. Louis, USA) in ACS purity unless noted otherwise. Working standard solutions were prepared daily by dilution of the stock solutions. All solutions were filtered through 0.45 μm Nylon filter discs (Millipore, Billerica, MA, USA) prior to HPLC analysis. The pH value was measured using WTW inoLab Level 3 with terminal Level 3 (Weilheim, Germany) and controlled by software MultiLab Pilot, Weilheim, Germany. The pH-electrode (SenTix H, pH 0.14/0.100 °C/3 M KCl) was regularly calibrated by set of WTW buffers (Weilheim, Germany).

2.3. Biological material

Maize (*Zea mays* L.) seeds were surface-sterilized and sown in wet vermiculite that had been autoclaved at 121 °C for 30 min. Plantlets were transplanted to 500 mL pots containing a sterile mixture of soil/sand (1/3, v/v). The soil was collected from Sierra Nevada (Granada, Spain), sieved through a 2 mm mesh, sterilized by tyndalization for three consecutive days and air dried. The sand was sterilized by autoclaving at 121 °C for 30 min. The soil had the following characteristics: pH 6.58, 10.84% organic matter, 0.47% total N, 0.03% total P, 0.38% Ca, 0.42% K and 65.85 ppm total Cu.

The set of experimental plants was divided into two main groups: (i) maize plants non-inoculated with the mycorrhizal fungus (non-mycorrhizal or control plants), and (ii) maize plants inoculated with the arbuscular mycorrhizal fungus *Glomus intraradices* (mycorrhizal plants). Each main experimental group was halved and treated with a physiological (31.7 ng/mL) or a toxic (31.7 ng/mL) dose of copper (II) ions. Each group consisted of six individual plants. Mycorrhizal plants were inoculated with the arbuscular mycorrhizal fungus *G. intraradices* (DAOM 197198, Smith & Schenck Biosystematic Research Center, Ottawa, Canada) by adding 50 g of a soil–sand-based inoculum to 450 g of growing substrate. The inoculum consisted of thoroughly mixed rhizosphere samples containing spores, hyphae and mycorrhizal root fragments. Control plants received a filtrate (>20 μm) of the inoculum