



Hydroxyiminodisuccinic acid (HIDS): A novel biodegradable chelating ligand for the increase of iron bioavailability and arsenic phytoextraction

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ABSTRACT

The influence of biodegradable chelating ligands on arsenic and iron uptake by hydroponically grown rice seedlings (*Oryza sativa* L.) was investigated. Even though the growth solution contained sufficient Fe, the growth of rice seedlings gradually decreased up to 76% with the increase of pH of the solution from 7 to 11. Iron forms insoluble ferric hydroxide complexes at neutral or alkaline pH in oxic condition. Chelating ligands produce soluble 'Fe–ligand complex' which assist Fe uptake in plants. The biodegradable chelating ligand hydroxyiminodisuccinic acid (HIDS) was more efficient than those of ethylenediaminetetraacetic acid (EDTA), ethylenediaminedisuccinic acid (EDDS), and iminodisuccinic acid (IDS) in the increase of Fe uptake and growth of rice seedling. A total of 79 ± 20 , 87 ± 6 , 116 ± 15 , and 63 ± 18 mg dry biomass of rice seedlings were produced with the addition of 0.5 mM of EDDS, EDTA, HIDS, and IDS in the nutrient solution, respectively. The Fe concentrations in rice tissues were 117 ± 15 , 82 ± 8 , 167 ± 25 , and 118 ± 22 $\mu\text{mol g}^{-1}$ dry weights when 0.25 mM of EDDS, EDTA, HIDS, and IDS were added to the nutrient solution, respectively. Most of the Fe accumulated in rice tissues was stored in roots after the addition of chelating ligands in the solution. The results indicate that the HIDS would be a potential alternative to environmentally persistent EDTA for the increase of Fe uptake and plant growth. The HIDS also increased As uptake in rice root though its translocation from root to shoot was not augmented. This study reports HIDS for the first time as a promising chelating ligand for the enhancement of Fe bioavailability and As phytoextraction.

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1. Introduction

Iron is an essential micronutrient for plants, which plays important roles in respiration, photosynthesis, and many other cellular functions such as DNA synthesis, nitrogen fixation, and hormone production (Vert et al., 2002). Although abundant in nature it forms insoluble ferric hydroxide complexes (also known as Fe-plaque) at neutral or alkaline pH in oxic condition (Guerinot and Yi, 1994). The Fe-plaque formation in the rhizosphere soils, however, results in the Fe deficiency to plants. In nature, rhizospheric microbes exude siderophores to the root-plaque interface. These siderophores solubilize ferric iron in the rhizosphere, render its bioavailability, and plants uptake the Fe by specific membrane receptors (Romheld, 1987).

Elevated levels of As in soil from natural and anthropogenic sources is a threat to plants' health (Rahman et al., 2008). Remediation of contaminated soil is important to prevent As deposition in food crops and its subsequent transfer into the human body

through the food chains (Rahman et al., 2008). Phytoremediation becomes a promising alternative and environmentally safe technology for the remediation of environmental pollutants (Raskin et al., 1997; Tu et al., 2002). An essential prerequisite for phytoremediation of contaminated soil is solubility and bioavailability of As (Fitz and Wenzel, 2002). But the solubility and bioavailability of As becomes reduced by adsorption to variable charged minerals (Fe and Al) at alkaline pH (Xu et al., 2008). In the past decade, chelant-enhanced phytoremediation has received much attention (Pastor et al., 2007). This technique aims to cleanse polluted soils by solubilizing the toxic metals, allowing it to be accumulated in plants that would subsequently remove toxic metal from the site. Publications on chelant-enhanced phytoremediation have increased steadily to about 15–20 per year in the last few years, indicating that this is a growing and active research field (Nowack et al., 2006).

Research on the interaction of plants with chelating ligands started in the 1950s with a view to reduce the deficiencies of the essential nutrients such as Fe, Mn, Cu, and Zn (Wenger et al., 2005). Among all soil-applied Fe fertilizers, synthetic Fe(III)-chelates, mainly Fe(III)-chelates of polyaminocarboxylic acids with phenolic groups, such as ethylenediamine di(o-hydroxyphenylacetic)

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acid (EDDHA), and ethylenediamine di(2-hydroxy-4-methylphenylacetic) acid, are the most effective and commonly used (Alvarez-Fernandez et al., 2005). On the other hand reports on As phytoextraction by chelating ligands is limited though a number of investigations have been conducted on chelant-enhanced phytoextraction of Pb, Zn, Hg, Cu and some other heavy metals (Luo et al., 2005). Ethylenediaminetetraacetic acid (EDTA) has been very popular to achieve this purpose, but it is quite persistent in the environment because of its low biodegradability. This, in combination with its high affinity for heavy metal complexation, results in an increased risk of leaching. EDTA also impairs plant growth severely, even at low concentrations (Bucheli-Witschel and Egli, 2001).

Biodegradable chelating ligands, such as ethylenediamine-disuccinic acid (EDDS), hydroxyiminodisuccinic acid (HIDS), and iminodisuccinic acid (IDS) would be good choice and alternative to less biodegradable EDTA. The physicochemical properties of EDDS, EDTA, and IDS have already been discussed and tested for the phytoextraction of heavy metals by a number of researchers (Helena et al., 2003; Evangelou et al., 2007). HIDS is a new chelating ligand introduced by Nippon Shokubai Co. Ltd. It is one of the highly biodegradable (biodegradation rate is about 22.4% within 48 h) and safe chelating ligands. It traps and inactivates various kinds of metals ions over a wide range of pH, particularly Fe^{3+} and Cu^{2+} , as well as Ca^{2+} and Mg^{2+} ; shows high stability in harsh conditions and high temperature (80 °C); is highly soluble in aqueous alkaline solution (Sokubai, 2009). Because of high degradation rate and high stability constant with Fe^{3+} ($\text{pK}_a \text{ Fe}^{3+}$ is 12.5) of HIDS, we become interested to investigate the effectiveness of the chelating ligand for the increase of Fe bioavailability and phytoremediation of As. The EDTA, EDDS, and IDS were also used in the present study to compare the results of HIDS. Our research approach was to find a biodegradable and eco-friendly chelating ligand that is more desirable than EDTA or EDDS for Fe bioavailability and As phytoextraction.

2. Materials and methods

2.1. Seed sterilization

Rice seeds of BRRI dhan 29 were collected from Bangladesh Rice Research Institute. The seeds were surface-sterilized before using them in the experiment. For sterilization, about 100 g seeds were soaked in 200 mL of 1% methyl-1-butylcarbamoyl-2-benzimidazole carbonate solution for 10 min. After that, the seeds were washed by deionized (DI) water (using an E-pure system (Barnstead)) and kept in DI water at 20 °C for 24 h. The seeds were then washed and transferred to DI water of 45 °C for 2 min, and of 52 °C for 10 min.

2.2. Chemicals

Stock solutions of EDTA, EDDS, HIDS, and IDS were prepared by dissolving ethylenediamine-N,N,N',N'-tetraacetic acid (Dojindo Molecular Technologies, Japan), ethylenediamine-N,N'-disuccinic acid (Chelest), tetrasodium 3-hydroxy-2,2'-iminodisuccinate (Nippon Syokubai, Japan), and tetrasodium iminodisuccinate (Bayer) in 0.1 M sodium hydroxide, respectively. Other reagents were of analytical grade or better. All solutions were prepared with DI water.

2.3. Nutrient solution

Sterilized rice seeds were germinated on pre-sterilized blotting paper (seed bed) with standard Murashige and Skoog (MS) (Murashige and Skoog, 1962). Iron concentration in the experi-

mental solution was 0.36 mM while its concentration was 27.8 mg L⁻¹ in pre-experimental solution (used for growing rice seedling prior to the experiment). The pH of the pre-experimental solution was adjusted to 6.5 while the pH of experimental solution was 9.0. Rice seedlings were grown on the seed bed for one week. In preparing MS culture solution, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was used as Fe source instead of NaFe(III)-EDTA.

2.4. Experimental setup

Rice seedlings were transferred to the experimental solution after one week of growth in pre-experimental solution. In the experimental solution, rice seedlings were grown in two steps. In the first step, rice seedlings were grown with different concentrations of chelating ligands (up to 2.50 mM) to observe the effect of chelating ligands on Fe uptake. In the second step, 6.0 μM of As ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) was added to the nutrient solutions containing 1.0 mM of chelating ligands to see the effect of chelating ligands on Fe and As uptake. Iron concentration in the experimental solution was 0.36 mM, and the pH of the solution was adjusted to 9 using 0.1 M KOH. About 100 mL of the solution was taken into 250-mL polystyrene bottles with three replications, and three uniform seedlings were cultivated in each bottle. The experiment was performed following randomized design. Rice plants were grown in a plant growth chamber and the conditions in the chamber were set as 14:10 h light/dark schedule, 100–125 μE m⁻² s⁻¹ light intensity, 22(±2) °C temperatures. Rice seedlings were grown in experimental solution for 5 d.

2.5. CBE-extraction of Fe-plaques

At harvest, the shoots were cut from 1 cm above the roots and separated. The Fe-plaques from root surfaces were extracted using citrate–bicarbonate–ethylenediaminetetraacetate (CBE)-technique, a modified method of dithionite–citrate–bicarbonate extraction by Taylor and Crowder (1983) to determine the real amount of Fe and As contents in rice tissues. The CBE solution was prepared from 0.03, 0.125 and 0.050 M of sodium citrate, sodium bicarbonate, and EDTA, respectively. Roots were treated with 30 mL of CBE solution for 60 min at room temperature. The roots were then rinsed with deionized water for three times, and the rinsed water was added to the CBE-extracts to make a total of 30 mL.

2.6. Sample preparation

After rinsing with deionized water for four times, the root samples were kept on clean absorbent paper to remove the water from the root surfaces. Both the root and the shoot samples were dried at 65 °C until they reached in a constant weight. Then the dried samples were weighted and taken into 50-mL polyethylene tubes for digestion. Five mL of 65% HNO_3 were added to the sample and kept for 12 h. The samples were heated on a heating block at 95 °C for 2 h. After cooling to room temperature, 3 mL of 30% hydrogen peroxide were added, and the samples were heated again at 105 °C for 20 min. Then, the digests were diluted to 30 mL with DI and analyzed for As and Fe.

2.7. Chemical analysis

Arsenic and Fe were analyzed using graphite-furnace atomic absorption spectrometer (Z-8100, Hitachi, Japan). Certified standard reference material 1573a (tomato leaf from NIST, USA) was used to check the accuracy of analysis. Arsenic concentration in certified standard reference materials was $0.112 \pm 0.004 \mu\text{g g}^{-1}$ dry weight (all the reported data in this article are expressed as

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