Plant Physiology and Biochemistry 58 (2012) 205–211

Contents lists available at SciVerse ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Significance of the concentration of chelating ligands on Fe^{3+}-solubility, bioavailability, and uptake in rice plant

Hiroshi Hasegawa [a,*], M. Mamunur Rahman [b,c], Kouta Kadohashi [b], Yui Takasugi [b], Yousuke Tate [e], Teruya Maki [a], M. Azizur Rahman [b,d]

[a] Institute of Science and Engineering, Kanazawa University, Kakuma, Kanazawa 920-1192, Japan
[b] Graduate School of Natural Science & Technology, Kanazawa University, Kakuma, Kanazawa 920-1192, Japan
[c] Bangladesh Rice Research Institute (BRRI), Sonagazi, Feni 3930, Bangladesh
[d] Centre for Environmental Sustainability, School of the Environment, Faculty of Science, University of Technology Sydney, P.O. Box 123, Broadway, NSW 2007, Australia
[e] Department of Chemistry and Chemical Engineering, Kanazawa University, Kakuma, Kanazawa 920-1192, Japan

ARTICLE INFO

Article history:
Received 11 May 2012
Accepted 3 July 2012
Available online 13 July 2012

Keywords:
Iron
Bioavailability
Solubility
Chelating ligand
HIDS
EDTA
Rice (*Oryza sativa* L.)

ABSTRACT

Present study investigated the significance of the concentration of chelating ligand on Fe^{3+}-solubility in growth medium and its influence on Fe bioavailability and uptake in rice plant. Rice seedlings were grown in modified Murashige and Skoog (MS) hydroponic growth medium with moderate (250 μM) and high (500 μM) concentrations of ethylenediaminetetraacetate (EDTA) and hydroxyiminodisuccinate (HIDS) under sterile and non-sterile conditions. Concentrations of soluble Fe in the growth medium increased with increasing ligand concentrations, and the growth of rice seedlings was higher at moderate ligand concentration than at control (without chelant) and high ligand concentration. This explains the relationship between Fe solubility and bioavailability in the growth medium, and its effect on Fe uptake in rice plant. Fe exists in the growth medium predominantly as particulate (insoluble) forms at low ligand concentration, and as soluble [$Fe(OH)^{2+}$, $Fe(OH)_2^+$, Fe–L complex] and apparently soluble (colloidal) forms at moderate ligand concentration. At high ligand concentration, most of the Fe^{3+} in the growth medium forms soluble Fe–L complex, however, the bioavailability of Fe from Fe–L complex decreased due to lopsided complex formation equilibrium reaction (CFER) between Fe and the ligands. Also, Fe is solubilized forming stable and soluble Fe–L complex, which is then detached as less stable, but soluble and bioavailable substance(s) after (time-dependent) biodegradation. Therefore- i) ligand concentration and stability constant of Fe–L complex (K_{Fe-L}) influence Fe bioavailability and uptake in rice plant, and ii) the biodegradable ligands (e.g., HIDS) would be more effective Fe fertilizer than the environmentally persistent and less biodegradable ligands (e.g., EDTA).

1. Introduction

Iron (Fe) is the sixth most abundant element in the earth crust, and is an essential nutrient for plants. Despite its abundance in the soil, Fe is only slightly soluble under aerobic conditions, especially in high-pH and calcareous soils [1]. Fe deficiency is one of the most common constraints to the growth of rice plant in acid soils, and together with Zn deficiency, it is the most commonly observed micronutrient disorder in wetland rice [2]. Higher plants have two distinct strategies to acquire insoluble/slightly soluble Fe from the rhizosphere; i) the reduction strategy of nongraminaceous (strategy I) plants, and ii) the chelation strategy of graminaceous (strategy II) plants [3–5]. In a recent review, Kobayashi & Nishizawa [3] discussed the roles of phytosiderophores in Fe uptake, translocation, and regulation in nongraminaceous and graminaceous plants. In addition to the Fe^{3+}-solubilising phytosiderophores, rhizospheric microbes also exude siderophores to solubilize ferric Fe in the rhizosphere, and thus render Fe soluble and bioavailable, resulting in uptake by graminaceous plants via specific membrane receptors [4,5]. Synthetic chelating ligands belonging predominantly to the two groups: aminopolycarboxylates and polyphosphonates, are also effective and have widely been used for increasing Fe bioavailability and uptake [6,7], and for correcting Fe-chlorosis in plants [8].

The synthetic chelating ligands form Fe-ligand (Fe–L) complexes in the rhizosphere that increase Fe solubility and subsequent

* Corresponding author. Tel./fax: +81 76 234 4792.
E-mail addresses: hhiroshi@t.kanazawa-u.ac.jp, rahmanmazizur@gmail.com (H. Hasegawa).

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http://dx.doi.org/10.1016/j.plaphy.2012.07.004

bioavailability and uptake in plants. Aminopolycarboxylates such as ethylenediaminetetraacetic acid (EDTA), nitrilotriacetate (NTA), and diethylenetriaminepentaacetate (DTPA) are popular and widely used chelating agent to achieve this purpose. However, these aminopolycarboxylates are quite persistent in the environment because of its low biodegradability [9]. This, in addition to their high affinity for heavy metal complexation, results in the increased risk of leaching [10]. Therefore, these aminopolycarboxylates are being replaced by new generation biodegradable complexing agents [9,11]. The biodegradability of hydroxyiminodisuccinate (HIDS; Fig. 1A) is higher than EDTA (Fig. 1B) [12], and therefore, HIDS was proposed to be a better choice and substitute to less biodegradable EDTA [7]. It traps various metal ions, particularly Fe^{3+}, over a wide range of pH, shows high stability in harsh conditions and high temperature, is highly soluble in aqueous alkaline solution [13].

In the previous study, we found that the concentrations of chelating ligands (EDTA, EDDS, HIDS, IDS) significantly influence Fe uptake in rice seedlings and subsequent growth of the plant [7]. Fe uptake and growth of rice seedlings were found to be better at 0.25 mM ligand concentration compared to that at lower (0.1 mM) and higher (0.5, 1.0, and 2.5 mM) concentrations of the ligands. From the results of those studies, it was hypothesized that the concentration and stability constant of Fe–L complexes (K_{Fe-L}) are important factors for Fe solubility in the growth medium that influence the bioavailability and uptake of Fe in rice plants. In the present study, we investigated the influence of ligand-induced Fe^{3+}-solubility in the growth medium on Fe bioavailability and uptake in rice plant to justify the effect of ligand concentration and K_{Fe-L} by growing rice seedlings in modified Murashige and Skoog (MS) hydroponic growth medium with moderate (250 μM) and high (500 μM) concentrations of EDTA and HIDS under sterile and non-sterile conditions. Chelating ligands (EDTA and HIDS) and their concentrations (250 and 500 μM) were selected on the basis of the results of previous studies [7] for a scientific contribution, but these concentrations might not be suitable for practical implications since ligands are not used alone for Fe nutrition. Fe concentrations in root surfaces, growth medium, and inside roots of rice were measured at different ligand concentrations using ^{55}Fe radioisotope tracer to investigate the influence of ligand concentrations on Fe bioavailability and uptake in rice plants.

2. Materials and methods

2.1. Seed sterilization and germination

The rice seeds of BRRI Dhan29 were collected from Bangladesh Rice Research Institute (BRRI), Bangladesh. The seeds were surface-sterilized before using them in the experiment. For sterilization, 100 g seeds were soaked in 200 mL of 1% methyl-1-butylcarbamoyl-2-benzimidazole carbonate (Sumitomo Chemical Co. Ltd., Japan) solution for 10 min. After that, the seeds were washed by deionized (DI) water (E-pure® water system, Models D4631, Barnstead, USA) and kept in DI water at 20 °C for 24 h. The seeds were then washed and transferred to DI water of 45 and 52 °C for 2 and 10 min, respectively.

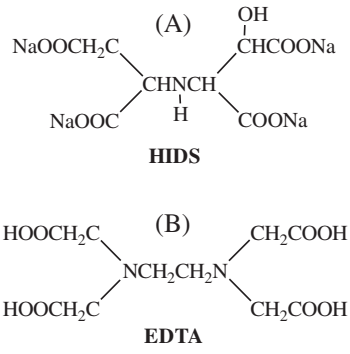

Fig. 1. The chemical structures of ethylenediaminetetraacetate (EDTA) and hydroxyiminodisuccinate (HIDS).

2.2. Chemicals

Stock solutions of EDTA and HIDS were prepared by dissolving ethylenediamine-N,N,N′,N′-tetraacetic acid (Kanto chemical, Japan) in 0.1 M sodium hydroxide and tetrasodium 3-hydroxy-2,2′-iminodisuccinate (Nippon Sokubai, Japan) in DI water, respectively. All chemical reagents used in the experiment were of analytical grade. Glassware and dishes were washed with detergent and 5 M HCl solution, and rinsed with DI water for eight times before use.

2.3. Nutrient solution

Modified Murashige and Skoog (MS) hydroponic solution [14] was used as growth medium for rice seedlings (Table 1). The nutrient salts of MS medium were dissolved in DI water and the pH of the growth medium was adjusted to 5.7 using 0.1 M KOH and HCl. In preparing MS culture solution, $FeCl_3$ was used instead of $FeSO_4 \cdot 7H_2O$ as Fe source. Sodium salt of EDTA ($Na_2EDTA \cdot 2H_2O$) was not used in preparing the growth medium. The chemicals used for the preparation of MS growth medium were of analytical grade and were purchased from Kanto chemical, Japan.

2.4. Experimental setup

Sterilized rice seeds were germinated and allowed to grow for 5 days with DI water on pre-sterilized bloating paper. Rice seedlings were then transferred to the modified MS growth medium (Table 1). The medium of control treatment did not contain chelating ligand, while the other treatment contained 250 and 500 μM of chelating ligands (EDTA or HIDS). To observe if there was any influence of microbial contamination on ligand degradation and Fe bioavailability, the experiment was conducted in sterile (medium with antibiotic) and non-sterile (medium without antibiotic) medium. Medium was sterilized with 50 mg L^{-1} of oxytetracycline hydrochloride (Wako, Japan). The pH of the solution was adjusted to 5.7 using 0.1 M KOH every day though out the

Table 1
Composition of modified Murashige and Skoog (MS) growth medium used for growing rice seedlings hydroponically. Sodium salt of EDTA ($Na_2EDTA \cdot 2H_2O$) was not included in preparing the growth medium. $FeCl_3$ was used as iron source.

Nutrients	Concentration (mg L^{-1})
KNO_3	1900
NH_4NO_3	1650
$CaCl_2 \cdot 2H_2O$	440
$MgSO_4 \cdot 7H_2O$	370
K_2HPO_4	170
$MnSO_4 \cdot 5H_2O$	22.3
$ZnSO_4 \cdot 7H_2O$	8.6
H_3BO_3	6.2
KI	0.83
$Na_2MoO_4 \cdot 2H_2O$	0.25
$CuSO_4 \cdot 5H_2O$	0.025
$CoCl_2 \cdot 6H_2O$	0.025
$FeCl_3$	8.11
pH	5.7