Fractionation Mechanisms of Rare Earth Elements (REEs) in Hydroponic Wheat: An Application for Metal Accumulation by Plants

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Fractionations of rare earth elements (REEs) in wheat (Triticum aestivum L.) were observed through application of exogenous mixed REEs under hydroponic conditions. Middle REE (MREE), light REE (LREE), and heavy REE (HREE) enrichments were found in roots, stems, and leaves, respectively, accompanied by the tetrad effect (an effect that can cause a split of REE patterns into four consecutive segments) in these organs. Investigations into REE speciation in roots and in the xylem sap with X-ray absorption spectroscopy (XAS) and nanometer-sized TiO₂ adsorption techniques, associated with other controlled experiments, demonstrated that REE fractionations in wheat were caused by the combined effects of chemical precipitation, cell wall absorption, and solution complexation by organic ligands in the xylem vessels. REE fractionations in wheat, which were derived from the small differences of chemical properties across REE series, may reflect a sensitive internal chemical environment that influences plant accumulation for REEs and their analogues actinide radionuclides.

Introduction

There is an increasing concern over heavy metal contamination in the environment and its potential threat to public health. In response, the concept of phytoextraction has evoked considerable interest in studies on plant metal accumulation (1). Although there has been recent progress in molecular understanding of plant metal homeostasis (2), the mechanisms at the organismal level, such as the control of metal translocation from roots to shoots, remain to be identified.

The rare earth elements (REEs), mainly including the lanthanides, typically exist as trivalent cations and can be found in a wide variety of mineral phases. They are also found in plants with significant variations in concentration (3–4). After the 1970s, the finding that REEs could improve yield and quality of crops led to large-scale application of REE fertilizers to croplands in China. The increasing use of REEs in a variety of nonnuclear industries also raises the potential that REEs may one day become environmental contaminants, and has spurred research on their behavior in the environment (3–5–7). Some advances have been made in understanding the mechanisms of REE uptake by plant roots (8–12). Even so, little information is available about the distribution and translocation of REEs within plants following uptake by roots.

Over the past few decades, REEs have been used as tracers to elucidate chemical variations in geological systems because of their generally coherent behavior. Furthermore, REEs are considered chemical analogues of the actinides (e.g., Am⁴⁺, Cm³⁺, Cf⁴⁺) because of their identical charge and similar ionic radii (6, 13, 14). Since it is typical for REEs to behave coherently, departure from the expected patterns, i.e., fractionations among the REEs, can provide critical information for understanding the types of processes operating within a system (15). Besides the sensitivity to pH, redox conditions, adsorption/desorption, and precipitation/dissolution reactions for the changes of REE patterns, the property of REEs as tracers is quite useful in hydrogeochemical studies (14, 15).

REEs are a potentially valuable tool for monitoring the chemical environment of analogous metals in plants. The existence of REE fractionations in soil-grown plants has been documented, but without a clear interpretation (16–17). Based on the variations of REE patterns in plants, attempts have been made by Fu et al (18–19) and Fu and Tasuku (20) to explore the mechanisms of REE and inorganic element intake by plants. In addition, in our previous studies, conspicuous fractionations of REEs were observed in hydroponic wheat from which mechanisms of uptake and translocation were inferred (21–22). The aim of this study was to further explore the mechanisms responsible for REE fractionations in wheat with more effective methods, such as X-ray absorption spectroscopy (XAS) and nanometer-sized TiO₂ adsorption techniques (9, 23).

Materials and Methods

Stock Solution of Mixed REEs. Stock solution of 2.1 mM mixed REEs used in this study was composed of 14 lanthanides (La to Lu except Pm), with an identical concentration of each element ([Ln³⁺] = 0.15 mM, Ln represents any lanthanides). Detailed preparation of the solution is presented in Ding et al. (22).

Plant Materials and Growth Conditions. Wheat (Triticum aestivum L. cv Jin-Dong 8) seeds were obtained from the Chinese Academy of Agricultural Sciences (Beijing, China). The wheat was grown in one-fifth strength Hoagland solution (24) until the appearance of the third leaf (in about 2 weeks). To collect the xylem sap of wheat, some of the seedlings were further grown in a one-half strength Hoagland solution for another 8 weeks. Details of plant culture and growth conditions are presented in Ding et al. (22).

Dose-Dependent REE Patterns in Wheat Organs. Wheat seedlings at the third-leaf stage were removed from the nutrient solution, rinsed with deionized water, and then transferred to 1 mM CaCl₂ solutions (pH 5.5) containing 0, 0.2, 1, 5, or 25 μM mixed REEs for 1 day followed by the nutrient solution (one-fifth strength Hoagland solution) for another day. The purpose for this intermittent treatment was to avoid the interactions between REEs and other...
nutrients such as PO₄³⁻. This process was repeated twice again. Afterward, the seedlings were harvested and rinsed with deionized water. REEs in roots, stems, and leaves were prepared for determination of REEs.

Effects of pH on REE Patterns in Roots. Wheat seedlings at the third-leaf stage were exposed to 1 mM CaCl₂ solution (pH 5.5) containing 5 μM mixed REEs with pH values of 3, 4, 5, 6, or 7 adjusted using dilute HCl or NaOH. The solutions were renewed every day and readjusted twice a day to stabilize the pH. After 3 d of exposure, roots were sampled and prepared for determination of REEs.

Temporal Changes of REE Patterns in Leaves. Wheat seedlings at the third-leaf stage were exposed to 1 mM CaCl₂ solution (pH 5.5) containing 5 μM mixed REEs. Leaves were sequentially sampled after 10, 24, 34, 48, 58, and 72 h of exposure and used for determination of REEs.

Effects of EDTA and Ca²⁺ Levels on REE Patterns in Leaves. Wheat seedlings at the third-leaf stage were exposed to 1 mM CaCl₂ solution (pH 5.5) containing 5 μM mixed REEs combined with a range of EDTA (0–4 μM) or to a range of CaCl₂ solution (0–5 mM, pH 5.5) containing 5 μM mixed REEs. The solutions were renewed everyday. After 3 d of exposure, leaves were sampled and used for determination of REEs.

Xylem Sap Collection and Analyses. Nanometer-sized TiO₂ (anatase with an average diameter of 27 nm and specific surface area of 52 m² g⁻¹) was provided by the Laboratory of Inorganic Chemistry, Department of Chemistry, Wuhan University. The synthesis method and characteristics of the nanometer-sized TiO₂ have been presented by Zan et al. (25). The analytical method for the adsorption of REE⁺ on the nanometer-sized TiO₂ has been described by Li et al. (23). An experiment was designed to determine the optimal pH value to separate the organic complexed REE⁺ from REE solutions. Details of the experiment are presented in the Supporting Information (paragraph S1).

Prior to collection of the xylem sap, 10-week-old seedlings were cultivated overnight in a solution containing 1 mM CaCl₂ (pH 5.5), and then exposed to 50 μM mixed REEs in 1 mM CaCl₂ (pH 5.5) solution. After 24 h of culture, stems were severed 2 cm above the roots and the xylem sap was collected for 4 h with a micropipet. The filtrates were diluted to a specific volume with pH adjusted to 4.0 and divided into two parts. One part of the filtrate was used for determination of the total concentration of REEs in the xylem sap and the other was used for the extraction of REEs using the method described in the Supporting Information (S1). Concentrations of free REE ions in the xylem sap were calculated based on the differences between the total and the complexed REEs.

Preparation of Roots and Cell Walls for XAS Measurements. Wheat seedlings at the third-leaf stage were transferred to 1 mM CaCl₂ solutions containing 100 μM La³⁺ over a period of 24 h, with pH values adjusted to 3 and 5 using dilute HCl or NaOH. Afterward, roots were subsequently incubated in an ice-cold 1 mM CaCl₂ solution to remove La³⁺ adhering on root surfaces (9).

Root cell walls were prepared as described earlier (22). A total of 0.5 g of cell walls (fresh weight) was subject to equilibration in a 1 mM CaCl₂ solution (pH 5.0) containing 100 μM LaCl₃ at 25 °C for 24 h, followed by a rinse with deionized water. The fresh wheat root and cell wall samples were immediately ground in an agate mortar and pestle under the protection of liquid N₂. The sample powders were stored in teflonic tempered (diameter of 1 mm and thickness of about 0.15 cm) and care was taken to keep them frozen prior to XAS measurements.

XAS Experiments and Data Analyses. The XAS data at La L₃ edges were recorded on beam line 1 WIB at the EXAFS station of the Beijing Synchrotron Radiation Facility (BSRF) using a Si (111) double crystal monochromator. The electron storage ring operated at 2.2 GeV, with the angle between the monochromator crystal faces being adjusted to misview the incident beam by 30%. The incident beam intensities were measured and recorded using a nitrogen gas flowing ionization chamber. A Lylte-type fluorescence detector with TiO₂ filter was used to obtain the fluorescence signal. The XAS data were scanned from 5.38 to 5.88 keV. Energy resolution was about 0.5 eV for X-ray absorption near-edge structure (XANES) and about 1.3 eV for the extended X-ray absorption fine structure (EXAFS). An average of 2 scans was performed for wheat samples and model compounds in order to improve the signal-to-noise ratio. The data were analyzed by standard methods using the WinXAS 2.3 software (26). Amplitude, phase shifts, and theoretical EXAFS simulations were performed using FEFF V7.0 (Faradayweg 4-6, D-14195 Berlin, Germany).

Determination of REEs. Concentrations of REEs in wheat samples were determined by an ICP-MS (βTurbo, VG) after the samples were digested with a mixture of 5 mL of HNO₃ and 0.5 mL of HClO₄. The detailed analytical procedure is presented in Ding et al. (22).

Quantification of the Fractionation Features of REEs. REE series is normally divided into three subgroups, i.e., LREEs, MREEs, and HREEs, representing the elements of La to Eu, Sm to Gd, and Gd to Lu, respectively (27). Most of the natural chemical fractionations occur among the three subgroups. These fractionations can be generally characterized by LREE enrichment, HREE enrichment, and MREE enrichment or depletion, of which the term of enrichment means a greater abundance of the subgroup compared with the other two. The LREE or HREE enrichment is reflected by concentration ratio of the sum of HREEs to LREEs (i.e., HREE/LREE), and the MREE enrichment or depletion is simply quantified as

\[ \Delta \text{MREE} = \frac{\sum \text{MREE}}{\sqrt{\sum \text{LREE} \times \sum \text{HREE}}} \]

where the ΣLREE, ΣMREE, and ΣHREE refer to the sum of the concentrations (mole) of each subgroup. The level of no enrichment or depletion for MREEs is defined when \( \Delta \text{MREE} = 0.40 \).

A radius-independent fractionation in wheat, called the tetrad effect, was also found in our previous studies (21–22). This effect can cause a split of REE patterns into four segments (called tetrads) (first tetrad, La–Ce–Pr–Nd; second tetrad, Sm–Eu–Gd; third tetrad, Gd–Tb–Dy–Ho; and fourth tetrad, Er–Tm–Yb–Lu) with “breaks” at Gd, between Nd and Pr, and between Ho and Er, respectively (28). There are two types of variations of the tetrad effect, and the overall shapes of the tetrads are either convex or concave and form M-shaped and W-shaped distributions, respectively (29). The W-type tetrad effect generally exists in fluids and the M-type tetrad effect is observed in the corresponding solid materials that react with these fluids (29). A proposed method was adopted to quantify the degree of the tetrad effect according to Irber (30) and Monceke et al. (31) using a T value. The tetrad effect can be simply characterized by an M-type or a W-type for the T value being larger than one or smaller than one, respectively. Detailed information and computations for the tetrad effect are presented in Ding et al. (22).

Results and Discussion

REE Fractionations in Wheat Organs. Wheat seedlings precultured in nutrient solution were exposed to mixed REE solutions with concentrations ranging from 0 to 25 μM. Significant fractionations of REEs occurred when applications...
of mixed REEs reached 5 \(\mu\)M for roots, 1 \(\mu\)M for stems, and 5 \(\mu\)M for leaves of wheat, and MREE, LREE, and HREE enrichments were observed in these organs, respectively (Figure 1). Moreover, REE patterns exhibiting the above fractionation features were simultaneously characterized by an M-shape consisting of four consecutive convex segments in roots, and a W-shape consisting of four consecutive concave segments in stems and leaves, which are defined as the M-type and W-type tetrad effects in REE geochemistry, respectively.

**XAS Investigation of Coordination Environment of La in Roots.** Because REE fractionations in wheat roots are likely linked to their speciation, the coordination environment of REE ions in roots was investigated using the XAS technique, with La used as an example. XANES spectra for the model compounds and La laden wheat samples are shown in the Supporting Information (Figure S1). All data had roughly the same edge energy (varying within a range of 1.0 eV), suggesting that the La(III) initially contained in the solution remains in the same ionic state after being absorbed by the roots.

The observed and best-fit calculated EXAFS and the corresponding Fourier transforms of the model compounds and wheat samples are shown in the Supporting Information (Figure S2). The best-fit parameters are listed in Table 1. The La(III) bound to the root cell walls was found to be coordinated with nine oxygen atoms at a bond length of 2.50 Å, a coordination identical to that of lanthanum acetate. The La(III) from the root pH 3 (the sample was acquired after exposure to La solution at pH 3) has a similar coordination environment with the cell walls and lanthanum acetate. These data show that the La(III) in the root pH 3 is likely bound to the cell walls via their carboxyl functional groups. Similar results were obtained for the absorption of Eu(III) to the roots of water hyacinth (*Eichhornia crassipes*) under pH 3.5 (11). Similarities were also found between the root pH 5 (the sample was prepared after exposure to La solution at pH 5) and lanthanum phosphate. The lower coordination number for oxygen atoms in the first coordination shell compared to other samples and the presence of a second coordination shell (phosphorus atom) appear diagnostic for La(III) in the root pH 5 coordinated with phosphate. These results were also strongly supported by the consistent peaks for the Fourier transformed EXAFS spectra between the wheat samples and the corresponding model compounds (see Supporting Information, Figure S2). Thus, one may conclude that the coordination of REE ions in wheat roots is controlled by cell wall absorption under strongly acidic conditions, and gradually by phosphate precipitation with the increase of pH.

**Fractionation Mechanisms of REEs in Roots.** REE fractionations in wheat roots had been suspected to result exclusively from phosphate precipitation occurring in or on the roots (21). The evidence is that REE phosphate precipitation from solution yields REE fractionation features of MREE enrichment and M-type tetrad effect in the precipitate (32), which are similar to those found in wheat roots (see Supporting Information, Figure S3). The coexistence of REE ions (La\(^{3+}\), Gd\(^{3+}\), and Yb\(^{3+}\)) with phosphate was also verified in root-tip portions of rice and pea (33) and in maize roots (34).

![REE patterns in organs of wheat after exposure to different levels of mixed REEs](image)

**FIGURE 1.** REE patterns in organs of wheat after exposure to different levels of mixed REEs. Wheat seedlings at the third-leaf stage were exposed to 1 mM CaCl\(_2\) solutions (pH 5.5) containing 0, 0.2, 1, 5, or 25 \(\mu\)M mixed REEs and nutrient solution on alternate days for 6 d. Numbers in root and stem indicate the multiplying factors of the original REE concentrations. Values are means ± SD of three replicates.

<table>
<thead>
<tr>
<th>sample</th>
<th>bond</th>
<th>(N)</th>
<th>(R(\text{Å}))</th>
<th>(\sigma^2(\text{Å}^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>aqueous lanthanum chloride</td>
<td>La−O</td>
<td>9</td>
<td>2.57</td>
<td>0.011</td>
</tr>
<tr>
<td>lanthanum acetate</td>
<td>La−O</td>
<td>9</td>
<td>2.50</td>
<td>0.005</td>
</tr>
<tr>
<td>lanthanum phosphate</td>
<td>La−O</td>
<td>4</td>
<td>2.48</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>La−P</td>
<td>4</td>
<td>3.41</td>
<td>0.023</td>
</tr>
<tr>
<td>root cell walls</td>
<td>La−O</td>
<td>9</td>
<td>2.50</td>
<td>0.018</td>
</tr>
<tr>
<td>root pH 3</td>
<td>La−O</td>
<td>8</td>
<td>2.52</td>
<td>0.017</td>
</tr>
<tr>
<td>root pH 5</td>
<td>La−O</td>
<td>4</td>
<td>2.50</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>La−P</td>
<td>2</td>
<td>3.50</td>
<td>0.012</td>
</tr>
</tbody>
</table>

*Errors of approximately 25% in the coordination number and 0.02 Å in the distance.*
XAS investigation shows that not only phosphate precipitation, but also cell wall absorption, might play an important role in REE fractionations in wheat roots. This is consistent with previous work by Ding et al. (22). That study showed that a majority of REEs (72%) in wheat roots were bound to the cell walls after exposure of the roots to 1 mM CaCl₂ solution (pH 5.5) containing 5 μM mixed REEs for 48 h. The MREE enrichment was observed in the cell walls but lower than that in the roots. Meanwhile, the M-type tetrad effect initially observed in the roots almost disappeared. A maximum around Sm and Eu was also observed for the absorption of REEs onto bacterial cell walls (35). The MREE enrichment found in the cell walls illustrates that not only phosphate precipitation, but also cell wall absorption, contributes to formation of the MREE enrichment in wheat roots. However, the tetrad effect should be mostly caused by the phosphate precipitation since this effect was not evident in the cell walls. A weak M-type tetrad effect was observed for the distribution of REEs absorbed onto bacterial cell walls (35). This may be related to the small differences in ionic radius among the lanthanides (including Pm), as a weak tetrad-effect-like distribution of ionic radius exists across the lanthanides. More details are required of REE fractionations based upon their ionic radius.

When the seedlings were exposed to mixed REE solutions, with an increase of pH values from 3 to 7, the degree of the M-type tetrad effect in roots had a nearly linear increase. On the other hand, the MREE enrichment showed a marked increase from pH 3 to 4, followed by a consistent decrease after pH 4 (Figure 2). Results of the pH effects on REE fractionations in roots indicate that the MREE enrichment derived from the cell wall absorption is likely the greatest under strongly acidic conditions (around pH 4), and decreases with increasing pH value. Meanwhile, the progressively stronger phosphate precipitation tends to maintain the MREE enrichment to some extent, and more importantly, to consistently promote the tetrad effect in the roots. These changes of REE fractionations in roots with pH might be a reflection of competition for the removal of REEs from solution between a precipitation mechanism and an absorption mechanism.

**Fractionations Mechanisms of REEs in Aboveground Parts.** A conspicuous HREE enrichment was observed in leaves, combined with the W-type tetrad effect (Figure 1c). The presence of the W-type tetrad effect suggests that REEs must be in a dissolved state before they are transported to the aboveground parts. It is proposed that the W-type tetrad effect in leaves is also likely caused by the phosphate precipitation occurring in or on the roots, since the inverse variation, i.e., the M-type tetrad effect, was observed in roots with REEs in the corresponding solid fractions (bound to the cell walls and/or precipitated by phosphate). According to this hypothesis, REE patterns in leaves should exhibit MREE depletion due to the presence of MREE enrichment in root solids. Thus, a kinetic experiment was conducted through exposure of wheat seedlings to a 5 μM mixed REE solution (Figure 3). Different levels of MREE depletions were first observed in the REE patterns sampled at 24, 34, and 48 h, which likely supports the deduction that MREE depletion has occurred in leaves. However, HREE enrichment was observed in the REE patterns after 48 h exposure, and this feature became evident at 72 h exposure. Obviously, there are other processes leading to these changes apart from phosphate precipitation and cell wall absorption.

Components in nutrient solution may influence REE fractionations in leaves, thereby contributing to the HREE enrichment of leaves. However, applications of both EDTA and Ca²⁺ in mixed REE solution had negative effects on the HREE enrichment in leaves when they were taken as examples to investigate the effects of exogenous cations and anions (Figure 4). Thus, this feature is likely originated from reactions occurring within the plants. Literature data show that chelation with certain ligands in the xylem plays an important role in transition metal transportation within the plants (36). Because the stability constants of REE complexes generally increase with the increase of atomic number of REEs for most ligands (15, 37, 38), solution complexation in the xylem vessels should be an important process causing the REE fractionations in aboveground parts of wheat.

A new method has been developed to determine free REE ions and REE organic complexes in solution using nanometer-sized TiO₂ as a solid-phase extractant (23). Without any other chemical pretreatment, these REE species can be determined respectively based on the differences of their abilities to
absorb onto nanometer-sized TiO$_2$ after the pH condition in solution is optimized. In this study, the nanometer-sized TiO$_2$ was used to extract REE organic complexes in the xylem solution. An experiment was carried out to determine the optimal pH value for this extraction. Satisfactory separations of La$^{3+}$ (the lightest element across the lanthanides) and Lu$^{3+}$ (the heaviest element across the lanthanides) complexes were achieved respectively when the pH values of both solutions were set to 4.0, as shown in the Supporting Information (Figure S4).

The xylem sap was collected after exposure of 10-week-old seedlings to a 50 $\mu$M mixed REEs solution over a period of 3 d. These processes have been successfully simulated by comparison of REE phosphate precipitation from solution with and without addition of citrate (22).

The presence of LREE enrichment in stems (Figure 1b) is evidently the result of the above processes occurring within the plants. There should be a strong complexing environment within the xylem before the transfers of REEs into the leaves, which inhibits the HREEs from chemical precipitation or absorption to the cell walls, and by contrast favors the retention of LREEs in the path. As a consequence, the stems, different from the leaves as the end of the transport path, exhibit a slight enrichment of LREEs.

**Implications.** The conspicuous fractionations of REEs in plants derived from the slight differences of chemical properties across REE series, in fact, reflect a sensitive internal chemical environment influencing their accumulation and distribution processes. Particularly for the root-to-shoot transport, there is a pH-dependent equilibrium inside the xylem among low-molecular-weight chelators in the mobile transpiration stream, chemical precipitators such as PO$_4^{3-}$ and CO$_3^{2-}$, and stationary metal-binding sites in the cell wall material surrounding the xylem vessels. Furthermore, the vital role of solution complexation in the xylem vessels on REE fractionations supports the hypothesis of the so-called histidine response (39) that differences in the root-to-shoot translocation resulting from chelation in the xylem are a potential factor determining at least the accumulation rate of metals.

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**Supporting Information Available**

Analytical method for the adsorption of REE$^{3+}$ on the nanometer-sized TiO$_2$ and effects of pH (paragraph S1, Figure S4); L$\_3$ XANES and EXAFS spectra of La(III) in samples (Figures S1 and S2); REE patterns in phosphate precipitates during a 10 h precipitation of phosphate with mixed REEs (Figure S3). This material is available free of charge via the Internet at http://pubs.acs.org

**Literature Cited**


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