Uptake of rare earth elements by *Dryopteris erythrosora* (autumn fern)

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Mechanisms of uptake of rare earth elements (REEs) were investigated, particularly those by REE accumulator species (autumn fern). Rare earth elements are practically insoluble under natural conditions, suggesting some unknown mechanism in REE accumulator species. In the present investigation, two notable phenomena were observed. (I) Concerning the ionic-radius dependence of REE uptake by leaves, nonaccumulator species showed an extremely high uptake for Y compared with the adjacent-ionic-radius REEs in the multitracer, while accumulator species showed no anomaly. (II) REE uptake by autumn fern was influenced by the addition of chelating chemical reagents in the uptake solution, while no effect was observed for nonaccumulator species.

Introduction

Rare earth elements (REEs) are members of the Group IIIA in the periodic table and possess nearly identical chemical and physical properties.[1)] Although there are several different views regarding the definition of REEs, Y and lanthanide elements are defined as REEs for the sake of convenience in this article. The most stable oxidation state of REEs is trivalent, and because of this, REEs are adsorbed strongly on surfaces of rocks or bound to organic substances such as humic acid, which results in their low availability to plants from soil.[2–4)] Plants usually absorb very small amounts of REEs under natural conditions,[5)] and no beneficial biological function of the elements has been clearly demonstrated.

About 60 years ago, extremely high concentrations of total REEs (2,300 ppm) were found in hickory leaves (*Carya* sp.).[6)] Recently, however, more accumulator species were discovered with the increase of researchers' interest in the elements.[7)] For instance, some fern species, such as *Asplenium trichomanes*,[8,9)] are reported to accumulate REEs under natural conditions. Another accumulator species, *Dryopteris erythrosora* (autumn fern), exhibited enhanced growth following addition of REEs in a culture medium.[10)] In spite of a large number of investigations on the determination of REEs, however, no particular attention has been paid to the mechanisms underlying their uptake. Considering the low availability of the elements under natural conditions, some unknown mechanism is expected to be involved in the uptake of REEs, particularly in accumulator species.

In geological and geochemical research, the Masuda-Coryell diagram is often used to understand the long-term diagenesis of REEs.[11,12)] This approach is to normalize REE concentrations in samples with those in the chondrite meteorite as a reference. This method has been applied to many investigations for the elucidation of origins, such as, of a meteorite or a sedimentary rock.[13,14)] In the field of trace element research on plants, a large number of investigations have been reported in which concentrations of REEs in plant bodies are also normalized to those in the chondrite meteorite.[15,16)] Likewise, normalization with those in host soils is performed, as well.[8,17)] These approaches provide us with a possibility of evaluating the difference in uptake behavior of REEs among different plant species, and the difference in the property of soils in which plants grow. In the present work, a similar approach has been undertaken by normalizing the radioactivities of REEs in leaves and roots to those added to an uptake solution.

The multitracer technique was developed by Ambe *et al.* using the RIKEN Ring Cyclotron, which opened a new field in radiochemistry, namely, the simultaneous tracing of the behavior of various elements.[18–20)] By virtue of the high performance of the RIKEN Ring Cyclotron, ion beams, such as ^{12}C or ^{14}N, can be accelerated up to 135 MeV/nucleon, which is sufficiently strong to cause fragmentation reactions in target metals. Application of this technique allows us to observe the behavior of various elements at the same time. This is a great advantage, particularly for biological samples from the point of view that biological samples are always accompanied by relatively large individual differences, and metal toxicity is not necessary to be taken into consideration because the multitracers are in a carrier-free state. The applicability of this technique has been demonstrated in plant research.[21–27)]

For the purpose of examining the mechanism underlying the uptake of REEs in accumulator species, we performed two types of multitracer experiments. The first one was to compare the uptake of REEs by 11 plant species including nonaccumulator species under a hydroponic condition. The second one was to investigate the influence of chelating reagents added to the uptake solution on the uptake of REEs in three out of the 11 plant species used in the first experiment.

Materials and methods

Plant samples

Eleven plant species listed in Table 1 were grown on soil under the controlled climatic conditions (day/night 16/8 hr; light intensity 150 μE m^{-2} s^{-1}; temperature 25 $\pm$ 2°C; and relative humidity 70–80%) until the shoot length became approximately 7 cm. Before the uptake experiment, they were kept in ultrapurified water with constant bubbling of air for one week to reduce the influence of adhering soil. During this

Table 1. Plant species used in the study.

	Plant species
(a)	*Poa pratensis*
(b)	*Antirrhium majus*
(c)	*Pisum sativum*
(d)	*Abelmoschus esculentus* Moench
(e)	*Nicotiana tabaccum*
(f)	*Cucumis melo*
(g)	*Amaranthus mangostanus*
(h)	*Luffa cylindrica* Roem.
(i)	*Colysis pothifolia*
(j)	*Cyrtomium fortunei*
(k)	*Dryopteris erythrosora*

period, the water was changed every several hours.

Preparation of a multitracer solution

A gold plate was irradiated with a 135 MeV/nucleon ^{14}N ion beam accelerated by the RIKEN Ring Cyclotron. The Au target was dissolved in aqua regia and this acid solution was evaporated to dryness. After the residue was dissolved in 1.5 mol dm^{-3} HCl, a multitracer solution was obtained by extracting Au with ethyl acetate. In this investigation, radioactive nuclides other than REEs were removed by passing the entire amount of multitracer through a cation exchange column, which allowed us to acquire a group multitracer containing Y, Ce, Pm, Eu, Gd, Yb, and Lu. Additional details are described in the literature.[28)]

Uptake experiment

Two types of uptake experiments were conducted using an uptake solution containing radioactive REEs. Experiment 1 was performed to compare the uptake behavior of REEs for the 11 plant species listed in Table 1. The pH of the uptake solution, which had a final volume of 0.05 dm^{-3}, was adjusted to 5.8±0.1 with 0.01 mol dm^{-3} HCl. The roots of plants were maintained for one week in the multitracer solution without any nutrients. After this period, the roots were taken out of the solution and washed with 0.1 mol dm^{-3} HCl to remove the REEs adhering to the surface of the roots. The plant bodies were divided into roots and leaves.

Experiment 2 was conducted to examine the effect of coexisting chelating reagents in the uptake solution on the uptake of REEs by three plant species (*Dryopteris erythrosora*, *Poa pratensis* and *Nicotiana tabaccum*), which were chosen as representatives of the 11 plant species listed in Table 1. In this experiment, five kinds of chemical reagents were used, nitrilotriacetic acid (NTA), lactic acid, succinic acid, ascorbic acid and tris(hydroxymethyl)aminomethane (Tris). Plant samples were allowed to absorb REEs from 0.05 dm^{-3} of uptake solution without any nutrients for one week in 2.0 mmol dm^{-3} of either of the chelating reagents for one week. After this uptake period, the roots were washed with 0.1 mol dm^{-3} HCl. The roots and leaves were dried at 30°C for gamma-ray measurement. Gamma-rays from the samples were counted with a germanium detector. For the calculation of percentage uptake of the elements, a specanal '94 computer was employed.

Results and discussion

On analyzing and characterizing the distribution of REEs, experimental values for each REE are connected, in some cases, by a smooth curve against the ionic radius. For example, the concentration ratio of REEs typically observed in plants (REEs in plant / REEs in soil) increases with the decrease in ionic radius.[29,30)] It is reported that *Phytolacca americana* (pokeweed) shows a smooth uptake curve with a maximum concentration ratio situated around the REEs in the middle-ionic-radius range.[31)] This approach could be a basis for the evaluation of the difference in uptake behavior of REEs for different plant species. Here, the uptake ratios of REEs were plotted against the ionic radii of Y, Ce, Pm, Eu, Gd, Yb, and Lu whose radioactivities were detected in most cases.

The dependence of the REE uptake ratio on the ionic radius of REE in roots and leaves is shown in Figs. 1 (A) and (B), respectively. The uptake ratio was calculated by dividing the radioactivity in each part of the plant by the total radioactivity added to the uptake solution. In the figures, the uptake ratios for each REE are plotted as an arbitrary unit against the ionic radii of REEs. The values connected by lines in the figures represent a single sample with statistical errors resulting from gamma-ray counting. It should be noted that the same trend was always observed for three replications for each species, although the uptake ratio was not identical even for the same plant species. The dotted lines in the figures indicate the ionic radius of Y (0.89 Å).

The patterns of ionic-radius dependence of REE uptake ratios by roots can be classified into four types: (I) increase in the uptake ratios with the decrease in ionic radius (plant species (a), (c), (d), (f), (h), and (k)); (II) an uptake pattern of the opposite trend to the one observed in type (I) (plant

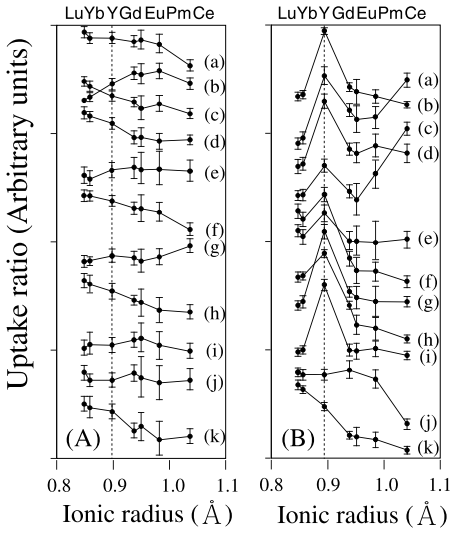

Fig. 1. Uptake of REEs in roots (A) and leaves (B) of the plant species listed in Table 1. ((a) *Poa pratensis*, (b) *Antirrhium majus*, (c) *Pisum sativum*, (d) *Abelmoschus esculentus* Moench, (e) *Nicotiana tabaccum*, (f) *Cucumis melo*, (g) *Amaranthus mangostanus*, (h) *Luffa cylindrica* Roem., (i) *Colysis pothifolia*, (j) *Cyrtomium fortunei*, (k) *Dryopteris erythrosora*) Dotted lines correspond to the ionic radius of yttrium.

species (g)); (III) an uptake pattern with a maximum ratio around the middle ionic radius range (plant species (b)); and (IV) almost equivalent uptake ratio for all the REEs examined (plant species (e), (i), and (j)).

The uptake of REEs by the leaves of the 11 species is shown in Fig. 1(B). As shown in the figure, the uptake patterns can be obviously divided into two patterns according to the Y uptake ratio. Plant species (a)–(i) showed anomalously high Y uptake ratios, compared with the adjacent REEs in the multitracer (Gd and Yb). On the other hand, plant species (j) and (k) showed no anomalies regarding Y uptake. *Dryopteris erythrosora* (k) is reported to absorb high amounts of REEs (about 30 ppm) under natural conditions and *Cyrtomium fortunei* (j) is also known to contain relatively high REE concentrations (about 5.0 ppm).[8] Both species can be regarded as REE accumulators. Based on these observations, some special uptake mechanism, which is common to REE accumulator species, is expected.

Yttrium and lanthanides have very similar physicochemical properties. At the same time, however, Y is in the fifth period while lanthanides are in the sixth of the periodic table, indicating some differences, such as that in terms of mass. Considering this, the results acquired here suggest that the uptake mechanisms of the former group ((a)–(i)) reflect the differences in the mass between Y and the other REEs, while the ones of the latter group ((j) and (k)) do not.

Rare earth elements are considered to be transported into cells *via* carriers for Ca, because of their physicochemical similarities, such as ionic radius.[32,33] Accordingly, if REEs are transported inside the cytoplasms, Ce, which has the closest ionic radius to that of Ca among REEs in the multitracer solution, is expected to be absorbed the most. In this investigation, however, the high uptake ratio for Ce was observed in only a few plant species. In the roots, plant species (g) had the highest uptake ratio for Ce among REEs, and plant species in group (I) had the lowest. In the case of leaves, the uptake ratio for Ce was the highest in plant species (c) and the second highest in plant species (a), whereas it was the lowest in plant species (j). The other species showed uptake ratios for Ce that are similar to those of other REEs. This suggests that factors other than the ionic radius are involved in the uptake of REEs.

The effects of chelating reagents on the uptake ratio for REEs in the leaves of *Poa pratensis*, *Nicotiana tabaccum*, and *Dryopteris erythrosora* are shown in Figs. 2 (A)–(C). Concerning *Poa pratensis*, the uptake pattern of REEs in the case of the control (f) was characterized by having the highest uptake ratio for Y, followed by Ce (Fig. 2 (A)). The addition of any chelating reagent to the uptake solution resulted in no significant change in the uptake pattern for the control (f). Namely, the uptake pattern for *Poa pratensis* was not affected by the addition of chelating reagents. Regarding *Nicotiana tabaccum*, the uptake pattern of REEs in the case of the control (f) showed the highest uptake ratio for Y and a decrease in the uptake ratio with increasing ionic radii except for Y (Fig. 2 (B)). No marked changes in the uptake patterns for the samples in uptake solutions (a), (b), (c), (d), and (e) compared to that for the control (f) were observed, indicating no influence of chelating reagents on the uptake of REEs by *Nicotiana tabaccum*.

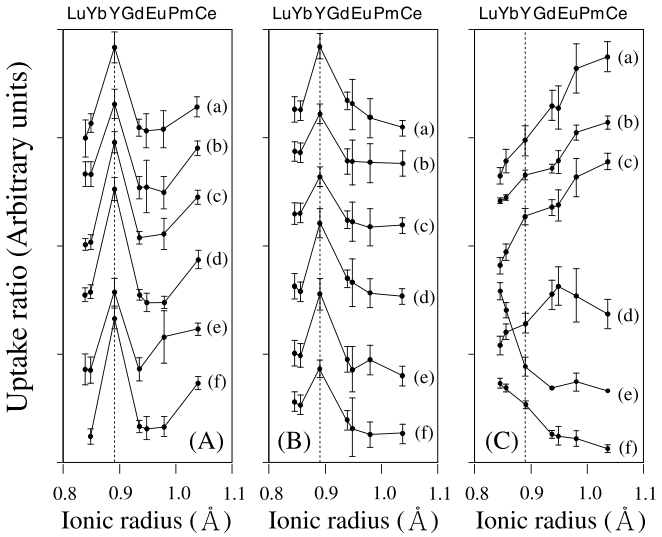

Fig. 2. Uptake of REEs in leaves of *Poa pratensis* (A), *Nicotiana tabaccum* (B), and *Dryopteris erythrosora* (C) with a chelating agent in the uptake solution. (a) nitrilotriacetic acid, (b) lactic acid, (c) succinic acid, (d) ascorbic acid, (e) tris (hydroxymethyl) aminomethane, and (f) control. Dotted lines correspond to the ionic radius of yttrium.

Yttrium anomaly was observed for *Poa pratensis* and *Nicotiana tabaccum*. From this, it is assumed that Y was absorbed as a free ion or a complex chelated with substances excreted from these species which were different from the reagents added to the solution, because the anomaly was also observed under the condition in which no chelating reagent was added in the uptake solution, as described above.

Concerning *Dryopteris erythrosora*, the uptake ratio of REEs by the control (f) decreased with increasing ionic radius (Fig. 2 (C)). The addition of chelating reagents to the uptake solution revealed marked changes in the uptake patterns depending on the chelating reagents. The same trend as that in the control (f) was observed for tris(hydroxymethyl)aminomethane with a very weak binding ability to REEs. On the other hand, under the experimental conditions where the chelating reagents used have high binding ability, such as NTA, lactic acid, or succinic acid, the opposite was observed. With ascorbic acid in the solution, whose binding ability is stronger than that of Tris and less than those of the other reagents, a positive peak in the uptake appeared for REEs around the middle ionic radius range. It should be noted that no anomaly in the Y uptake was observed for all the conditions.

In the present investigation, *Dryopteris erythrosora* showed a characteristic uptake pattern for REEs, that is, (I) no anomaly in Y uptake and (II) variation in the uptake curve caused by the addition of chelating reagents. In general, the binding ability of REEs to chelating reagents increases with a decrease in the ionic radius. Namely, Lu forms the most stable chelate complexes. The relationship between the uptake ratio and ionic radius obtained in the presence of NTA, lactic acid, and succinic acid suggests that the plant cannot absorb REEs in a form chelated with the reagents and thus requires the initial dissociation of these complexes prior to REE absorption.

It is reported that some plant species have special mechanisms to facilitate the uptake of nutrient elements. For example, barley has characteristic mechanisms for Fe uptake. It is known that the roots of barley release chelating substances (phytosiderophore) in response to Fe deficiency, which are highly effective in the solubilization of sparingly soluble inorganic Fe(III) compounds by formation of Fe(III)-phytosiderophores and the compounds are absorbed into plant bodies as an Fe(III)-phytosiderophore complex.[34,35)]

Dryopteris erythrosora is also expected to possess such a mechanism for the uptake of REEs. From the results in Fig. 2 (C), the binding ability of compounds released from *Dryopteris erythrosora* is considered to be weaker than that of NTA, lactic acid, and succinic acid. The compounds facilitate dissolution of REEs which otherwise are highly insoluble under natural conditions and conceal the differences in physicochemical properties among Y and lanthanides to some extent.

The plant species showed its characteristic uptake behavior of REEs. *Dryopteris erythrosora* and possibly *Cyrtomium fortunei* are expected to possess unique uptake mechanisms for absorbing REEs from the soil, presumably for the purpose of utilizing the elements for their growth.

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