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Application of Enriched Stable Isotopes in Element Uptake and Translocation in Plant

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1. Introduction

Isotope technique including radioisotopes and stable isotopes is useful and potent tool for various scientific areas. Especially, enriched stable isotopes are indispensable tools for researchers in biological systems (Stürup et al. 2008).

Stable isotope ratios are usually used in examining the biogeochemical cycling of light elements such as carbon(C), oxygen (O), nitrogen (N) and sulphur (S) in the environment. Thermal ionization mass spectrometry (TIMS) for the isotope analysis has been the most standard technique for many years. However, for TIMS analysis, time for sample preparation is needed because sample need to ensure efficient ionization. On the other hand, ICP-MS analysis has some advantages that sample preparation is simple and high sample throughput for isotope experiments where a large amount of samples need to be analyzed (Stürup et al. 2008). The disadvantage to resolve in isotope analysis using ICP-MS is spectroscopic interferences in the process of analysis. It is therefore needed to be resolved these interferences.

When plant physiologists investigate mineral absorption mechanisms in roots of plant, evaluation of symplastic mineral absorption capacity in roots cell in kinetics and time course experiments is very important because mineral translocation in shoots is mainly contributed to capacity of symplastic absorption in roots. In these experiments, radioisotopes methods are mainly used for element uptake in plants. Radioisotopes in solute were the most useful markers used in nutrient uptake and translocation in plants because they are chemically similar to the solute and can be distinguished from non-labeled solutes already contained in the roots (Davenport 2007). However, there are limitations to this method, including radioisotope administrative restriction and the restricted half-life of the radioisotope. Isotope tracer experiments, using a stable isotope, are very similar to those using a radioisotope on element to analyse plant mechanisms (Stürup et al. 2008). Accurate and precise determination of mineral isotope ratios is required for analysis of enriched stable isotopes. Inductive coupled plasma mass spectrometry (ICP-MS) has now become the effective and potent technique for enriched stable isotope tracer experiments due to increased availability. Therefore, the application of enriched stable isotopes in various biological systems increased rapidly.

There are so many research using enriched stable isotopes used as tracers aquatic and terrestrial ecosystems, animals and humans (See review of Stürup et al. 2008). However, there are a few researches using enriched stable isotopes element in plants. Recently, Stürup et al. (2008) reviewed that application of enriched stable isotopes as tracers in biological systems including aquatic ecosystem, terrestrial ecosystem, animals and humans in detail. Therefore, we did not focus on aquatic ecosystem animal and human in this chapter.

In this chapter, we therefore provide a review of some example using isotope technique. Especially, we focus on the application of enriched stable isotopes element uptake and translocation in plants. Our new method for evaluation of symplastic absorption of roots introduced in Section 4 has some merits, compared to radioisotopes techniques. Application of stable isotopes will become a new tool to evaluate element behavior in plants.

2. Application of stable isotopes in plants

The biochemical cycling of light element such as carbon(C), oxygen(O), nitrogen(N) and sulphur(S) have been studying using stable isotopes. The mechanisms of photosynthesis and of element uptake and translocation in plants was clarified by these studies using stable isotopes ratios such as C,O,N and S. Recently, the application of enriched isotopes of such as Mg, Cu, Ca, K and Cd behavior in plants rapidly increased with the development of ICP-MS analysis techniques. There are several studies on element uptake and translocation in plant using enriched stable isotopes (Table1).

Isotopes	Aim of study and method	Reference
^{10}B, ^{11}B	Characterization of boron uptake and translocation in sunflower plant. After preculture under nutrient solution containing ^{11}B, a short time experiment were conducted under nutrient solution containing low or high ^{11}B.	Dannel et al. (2000)
^{10}B	After preculture grown in nutrient solution containing boron, uptake experiment was conducted in solution containing enriched stable isotopes of ^{10}B.	Takano et al. (2002)
^{113}Cd	Intact leaves and cell sap of Cd accumulator plant were subjected to ^{113}Cd-NMR and H-NMR analysis for identification of the form of Cd in leaves.	Ueno et al. (2005)
^{113}Cd and ^{114}Cd	To examine Cd uptake in roots of *solanum* species with different Cd accumulation in shoot, uptake experiments were conducted using ^{113}Cd and ^{114}Cd.	Mori et al. (2009b)
^{113}Cd	Cd accumulation stage in soybean seed was examined in hydroponic solution using enriched isotope of ^{113}Cd.	Yada et al. (2004), Oda et al. (2004)
^{113}Cd	Cd uptake mechanisms in soybean was examined using ^{113}Cd isotopes in pot and field experiment	Kawasaki et al. (2004,2005)

Table 1. Element uptake and translocation in plant using enriched stable isotopes

Dannel et al. (2000) characterized the boron uptake and translocation from roots to shoots in sunflower using the stable isotopes [10] B and [11]B. In the report, after sunflower plant was precultured with high(100 μ M) or low (1 μ M) [11]B supply, plants were treated under differential [10]B supply condition. The results suggested that B uptakes are mediated by two transport mechanisms. First mechanism is passive diffusion which is indicated by the linear components. Second mechanism is energy dependent process which is indicated by the saturated components. Kawasaki et al. (2004, 2005) conducted that an isotope tracer technique with [113]Cd has been used in pot and field experiments. They examined that the most critical stages of soybean in which Cd absorbed via roots was transferred into the seeds. Cd absorbed before the beginning seed stage causes an increase of Cd concentration in seeds. Yada et al.(2004) reported that soybean plants were grown in hydroponic solution and supplied [113]Cd via roots for 48 h at early growth stage to investigate Cd accumulation pathway in soybean seed using enriched isotope of [113]Cd. Cd accumulated in leaves was translocated to seeds at seed beginning maturity stage. Oda et al. (2004) also indicated that the Cd absorbed from full pod to full seed was the most contributive to raise the Cd amount of seeds. Ueno et al. (2005) reported that *Thlaspi caerulescens* which is Cd hyperaccumulator plants have been grown hydroponically with a highly enriched [113]Cd isotope to investigate the form of Cd in the leaves using [113]Cd nuclear magnetic resonance (NMR) spectroscopy. They identified that cadmium binds with malate in the leaves. Several enriched isotopes such as [111]Cd, [113]Cd and [114]Cd will become a new tool to evaluate Cd behavior in plants. Several studies stated above suggest that enriched isotope is a very potent technique for tracking the distribution, uptake, translocation and recycling in biological system. Now, many enriched element stable isotopes except B and Cd are able to purchase in chemical forms such as metallic or oxide. In the future, the benefit of enriched stable isotopes techniques would be paid much attention in plant and environmental science areas.

3. Several methods for evaluating symplastic element uptake in plants

Intensive studies on the absorption mechanisms of various elements by plant roots have been conducted. There are evidence on mineral uptake and translocation in plants. It is well known that ion absorption in plant roots shows a saturated curve in kinetics experiments, indicating that a type of proteinaceous transporter mediates ion absorption (Epstein and Hagen 1952). Plant physiologists examining ion absorption in plant roots have given much attention to ion transport via the symplast across the plasma membrane (Epstein 1973). However, when ion absorption experiments were conducted, it was found that the apoplastically absorbed ions needed to be washed out of the apoplast to determine the symplastically absorbed ions across the plasma membrane or the determination of absorption is overestimated (Glass 2007). Therefore, it is necessary to eliminate the apoplastically bound ions to evaluate the symplastically absorbed ion content in the roots. To evaluate symplastic cadmium(Cd) and other elements absorption in roots, several methods have generally been used in the past: (1) expose the plant material to Cd radioisotopes and subsequent desorption using unlabelled Cd in the root apoplast (Hart et al. 1998, 2002, 2006), (2) plant material is exposed to Cd radioisotopes under conditions at 2°C and 22°C (Zhao et al. 2002, Uraguchi et al. 2009), (3) metabolic inhibitors such as DNP or CCCP (Cataldo et al.1983, Ueno et al. 2009), (4) centrifuge method (Yu et al. 1999, Mitani and Ma 2005, Ma et al. 2004, Ueno et al. 2008), (5)estimation of desorption from roots with time(Lasat et al. 1998)

Regarding evaluation for symplastic element uptake in roots using radioisotopes, this method is used for symplastic element uptake in roots. Hart et al. (1998, 2002, 2006) reported that Cd uptake experiment was conducted in nutrient solution containing ^{109}Cd-labeled $CdSO_4$ and apoplastic ^{109}Cd were desorbed using excessive nonlabelled Cd. As other method, Nakanishi et al. (2006) evaluated that apoplastic Cd in the roots was washed in 0.5 mmol L^{-1} ethylenediaminefetraacetic acid (EDTA) for 1 min. Lasat et al. (1996) evaluated that symplastic Zn uptake in roots of Zn hyperaccumulator and nonaccumulator *Thlaspi* species apoplastic ^{65}Zn in roots desorbed by excessive unlabelled $ZnCl_2$ solution after Zn uptake experiment was conducted using ^{65}Zn raidoisotopes. There is merit that this method is able to detect radioisotope element with high sensitivity. However, there are limitations to this method, including radioisotope administrative restriction and restricted half of the radioisotope. Additionally, the radioisotope technique has toxicological concern. It is required for handling its isotopes to be careful.

Regarding evaluation of symplastic element uptake in roots using differences in the amounts of Cd absorbed at 2°C and 25°C. Uptake of element at 2°Cwas assumed to represent mainly apoplastic binding in the roots whereas the difference in uptake between 22°C and 2°C represented metabolically dependent influx. Zhao et al. (2002) reported that apoplastic and symplastic uptake in two *Thlaspi* species from Cd and Zn depletion in solution using radioisotope tracer. Uraguchi et al.(2009) reported that genotypic variation in cadmium accumulation in rice and evaluated that symplastic Cd uptake in roots of rice using the method of subtraction the Cd content in the roots at 2°C from the Cd content in the roots at 25°C. This method using unlabeled Cd is easy to handle because there is no administrative limitation not using radioisotope elements. However, this method needs double seedlings for evaluation. Additionally, this method cannot be evaluated using same seedling. This method is not easy for dicotyledonous plant such as *Solanum melongena* to handle.

As for methods using metabolic inhibitors, Cataldo et al. (1983) reported that Cd uptake dependent on energy in roots is suppressed by dinitrophenol as metabolic inhibitor. In this study, using dinitrophenol as a metabolic inhibitor, the 'metabolically absorbed' fraction was shown to represent 75 to 80% of the total absorbed fraction at concentration less than 0.5 μ mol, and decreased to 55% at 5 μ mol.

Regarding centrifuge method, tap roots of plants were harvested and 2 cm root tips were excised. Then, cut ends were washed in distilled water and blotted dry. For each sample, 30 roots were used. The cut ends were washed in distilled water quickly and blotted dry. The tips were placed in a 0.45 mM filter unit with the cut ends facing down and centrifuged at 2,000g for 15 min at 4°C to obtain the apoplastic solution. After centrifugation, root segments were frozen at -80°C for 2 h and then thawed at room temperature. The symplastic solution was prepared from frozen-thawed tissues by centrifugation at 2,000g for 15 min at 4°C. Ma et al. (2004) evaluated that symplastic Si uptake of wild type rice and mutant rice using this centrifuge method. Additionally, Mitani and Ma (2005) also evaluated that symplastic Silicon uptake in rice, tomato and cucumber which differ from Si accumulation capacity using this method. Ueno et al.(2009) reported that symplastic Cd uptake is estimated by cell sap obtained from centrifuge method. To check the purity of apoplastic solution, the activity of malic dehydrogenase in apoplastic and symplastic solution was determined. The activity of malic dehydrogenase in apoplastic solution was below one-twentieth and approximately one-fortieth of symplastic solution. This method is valuable for evaluation of symplastic Cd concentration in roots because Cd concentration in roots cell

cap was directly determined. However, evaluation using root tips possibly is not representative of most root tissues. Rain et al.(2006) pointed out that there are the difference of K_m value in kinetics experiment between whole roots and root tips.

As other evaluation method of roots fraction, Lasat et al.(1998) evaluated that each fraction of cell wall, cytoplasm and vacuole by each efflux fraction from roots. They investigated that difference of Zn fraction in roots such as cell wall, cytoplasm and vacuole using this method.

4. Application of enriched stable isotopes in element uptake and translocation in plant

In this section, we introduce that our new method for evaluation of symplastic ion absorption, especially cadmium (Mori et al. 2009a). Several methods stated above is evaluation that apoplastically bound element is desorbed by some elements after element absorption experiment. Our method is that symplastic Cd absorption capacity is evaluated by difference of enriched isotope of ^{113}Cd and ^{114}Cd. Cadmium (Cd) is a hazardous heavy metal with regards to human health and is dispersed in natural and agricultural environments principally through human activities (Wanger, 1993). Arable land contains, to some extent, Cd, reportedly in the range, 0.04–0.32M, even in non-polluted soil (Keller, 1995; Wanger, 1993). This results in Cd accumulation in the edible parts of crops. Recently, the Codex Alimentarius Commission (2005) adopted a maximum concentration of 0.05 mg Cd kg^{-1} (fresh weight) recommended for fruiting vegetables. Approximately 7% of 381 samples of eggplant (*Solanum melongena*), 22% of 165 samples of okra (*Abelmoschus esculentus*), and 10% of 302 samples of taro (*Colocasia esculenta*) contained Cd concentrations above this limit in a field and market-basket study during 1998–2001 in Japan (Ministry of Agriculture Forestry and Fisheries of Japan, 2002); despite the fact that these crops were cultivated in non-polluted fields. Under these circumstances, new technologies for reducing the Cd level in crops are urgently required in Japan. Therefore, it is important to elucidate the mechanisms mediating Cd absorption, accumulation, and translocation in these crops. The crop conditions were represented by low Cd concentration experimental mediums.

4.1 Validity of our method for evaluation of symplastic Cd uptake in roots using enriched isotopes of ^{113}Cd and ^{114}Cd

When ion absorption experiments were conducted, it was found that the apoplastically absorbed ions needed to be washed out of the apoplast to determine the symplastically absorbed ions across the plasma membrane or the determination of absorption is overestimated(Glass 2007). Therefore, it is necessary to eliminate the apoplastically bound ions to evaluate the symplastically absorbed ion content in the roots. There are several methods to eliminate apoplastic ions as stated above. In this section, we introduced our new method for symplastic Cd absorption in roots of *Solanum melongena* using enriched isotopes of ^{113}Cd and ^{114}Cd.

The enriched isotopes of ^{113}Cd (^{106}Cd, 0.16%; ^{108}Cd, 0.135%; ^{110}Cd, 0.81%; ^{111}Cd, 2.53%; ^{112}Cd, 2.61%; ^{113}Cd, 93.29%; ^{114}Cd, 0.46%; ^{116}Cd, 0.01%) and ^{114}Cd (^{106}Cd, 0.05%; ^{108}Cd, 0.05%; ^{110}Cd, 0.05%; ^{111}Cd,0.05%; ^{112}Cd, 0.05%; ^{113}Cd, 5.6%; ^{114}Cd, 93.6%; ^{116}Cd,0.8%) used in the present study were purchased from Isoflex (San Francisco, CA,USA) in metallic form and dissolved in diluted HNO$_3$. The enriched isotopes of ^{114}Cd contained the 5.6 % of ^{113}Cd.
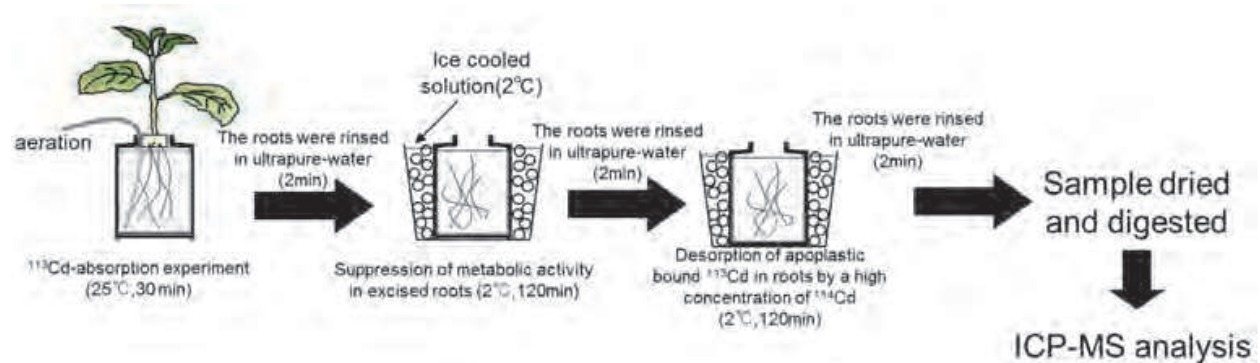
(modified from Mori et al. 2009a)

Fig. 1. Absorption experiment procedure for evaluating symplastic ^{113}Cd absorption in roots

The procedure for evaluating symplastic Cd absorption in the roots, using enriched isotopes ^{113}Cd and ^{114}Cd, is illustrated in Fig. 1. The roots of intact seedlings were rinsed in ultrapure water for 2 min and then exposed to a 500 mL ^{113}Cd solution containing 0.5 mmol L^{-1} $CaCl_2$ and 2 mmol L^{-1} 2-morpholinoethanesulfonic acid monohydrate Tris (hydroxymethyl) aminomethane (MES–Tris) (pH 6.0) at 25°C for 30 min (Fig. 1). The levels of ^{113}Cd were 40 nmol or 400 nmol in the ^{113}Cd treatment. A-B shown in Fig.2 indicates that ^{113}Cd absorbed in roots consists of apoplastic ^{113}Cd and symplastic ^{113}Cd (Fig.2 A, B). To suppress metabolically dependent symplastic absorption from the apoplast, the roots were excised from each seedling and immersed in a cold Cd-free buffer solution (2 mmol L^{-1} MES–Tris [pH 6.0], 0.5 mmol L^{-1} $CaCl_2$) at 2°C for 120 min (Fig. 1, Fig.2 C). The apoplastic-bound ^{113}Cd in the roots from 40 or 400 nmol ^{113}Cd treatment was then desorbed by immersing the roots in the same cold buffer solution at 2°C containing a 50-fold concentration of ^{114}Cd (2 or 20 µmol) for 120 min (Fig.1, Fig.2 D, E, F). The excised roots were then rinsed in ultrapure water for 2 min. Harvested samples were dried in an oven at 75°C for 3 days until dry. After digestion of dried sample, we then determined ^{113}Cd and ^{114}Cd contents in roots by ICP-MS analysis. To confirm the validity of this method, we compared our Cd absorption results with the Cd absorption results obtained at 25°C and 2°C using unlabeled $CdCl_2$ reagent. The experimental procedure was as follows. The Cd-absorption experiments were conducted for 30 min using 500 mL solutions containing 2 mmol L^{-1} MES–Tris (pH 6.0), 0.5 mmol L^{-1} $CaCl_2$ and different concentrations of Cd (40 or 400 nmol) at 25°C. After the absorption experiment, the excised roots from each seedling were rinsed with ultrapure water for 2 min. For the Cd-absorption experiment at 2°C, plants were transferred to an ice-cold pretreatment solution containing 2 mmol L^{-1} MES–Tris (pH 6.0) and 0.5 mmol L^{-1} $CaCl_2$ for 120 min. The Cd-absorption experiment at 2°C was conducted for 30 min. In the unlabeled Cd-absorption experiment at different temperatures, the amount of Cd reportedly absorbed into roots at 2°C was estimated to be apoplastically bound Cd on the assumption that metabolically dependent absorption would be suppressed at low temperature. Therefore, the difference in the amount of Cd absorbed at 2°C and at 25°C represents symplastic Cd absorption depending on metabolic energy. All absorption experiments were replicated three times. Each procedure illustrated in Figure1 signifies a schematic representation shown in Fig. 2.
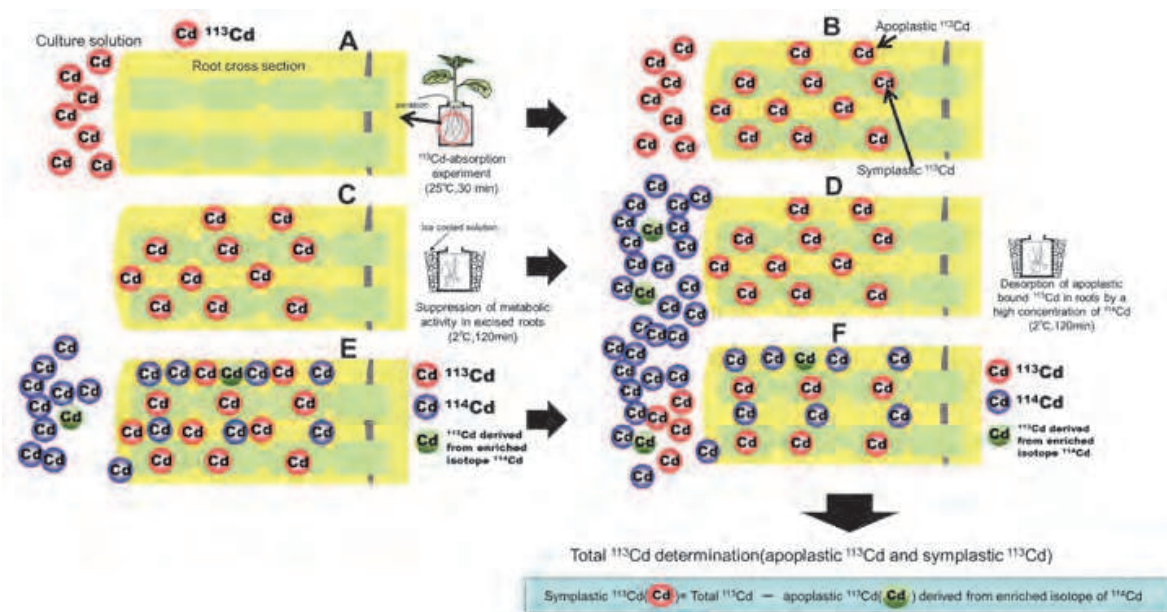
Fig. 2. A schematic representation of Cd absorption and desorption in roots using different enriched isotopes

4.2 Determination of ^{113}Cd, ^{114}Cd and the Cd contents in the roots

Approximately 0.05–0.1 g of dried roots was transferred and digested in a 10 mL Teflon tube containing 3 mL HNO$_3$. After digestion, the digested solution was diluted and 10 ng mL^{-1} of indium (In) was added to each diluted solution as an internal standard for ^{114}Cd determination. For ^{113}Cd determination, 10 ng mL^{-1} of tellurium (Te) was added as an internal standard. The concentrations of ^{113}Cd and ^{114}Cd in the digested solutions were determined by ICP-MS (ELAN DRC-e; Perkin Elmer SCIEX, Concord, ON, Canada). The concentrations of Cd in the digested solutions from the Cd-absorption experiment using unlabeled CdCl$_2$ reagent were determined by ICP atomic emission spectroscopy (VISTA-PRO; Varian, Palo Alto, CA, USA). It is well known that MoO interferes spectroscopically in determining the concentration of Cd in ICP-MS analysis (Kimura et al. 2003; May and Wiedmeyer 1998). In addition, it has been shown that it is necessary to remove Mo from the digested solution to avoid spectroscopic interference by molybdenum oxides (Oda et al. 2004; Yada et al. 2004). Therefore, for the ^{113}Cd and ^{114}Cd count intensities, we monitored the spectroscopic interference of the molybdenum oxides (^{97}Mo^{16}O and ^{98}Mo^{16}O) detected in the 10 ng mL^{-1} Mo standard solution. The contribution rate of spectroscopic interference of the putative ^{97}Mo^{16}O and ^{98}Mo^{16}O for ^{113}Cd and ^{114}Cd contents was negligibly small in both treatments (40 and 400 nmol). Therefore, we considered that we could ignore spectroscopic interference of oxidative molybdenum in determining the ^{113}Cd and ^{114}Cd contents in the ICP-MS analysis.

As shown in Fig. 3, after desorption of apoplastic ^{113}Cd by excessive ^{114}Cd, distribution of ^{113}Cd and ^{114}Cd in roots is as follow. (1) apoplastic bound ^{114}Cd is derived from desorption solution of excessive ^{114}Cd. (2) apoplastic bound ^{113}Cd is derived from desorption solution of excessive ^{114}Cd. (3) symplastic ^{113}Cd is derived from ^{113}Cd- uptake experiment. Therefore, ^{113}Cd content in roots is the sum of (1) and (2). Symplastic ^{113}Cd is the subtraction between total ^{113}Cd and ^{113}Cd derived from an enriched stable of ^{114}Cd. As shown in Fig. 1, the total ^{113}Cd contents in the roots signifies the ^{113}Cd contents in the roots after the desorption

experiment (Fig. 1). The total ^{113}Cd content in the roots at 40 and 400 nmol Cd was 23.0 ± 4.3 and 87.7 ± 5.6 mg kg^{-1} (dry weight), respectively (Table2). In contrast, the ^{114}Cd content at 40 and 400 nmol Cd was 117.3 ± 9.4 and 644.5 ± 33.7 mg kg^{-1} (dry weight), respectively (Table2). The purification rate of the ^{114}Cd-enriched stable isotope used in the present study was 93.60%; whereas, the composition rate of ^{113}Cd in the ^{114}Cd-enriched stable isotope was 5.6%. The total ^{114}Cd content in the roots after desorption of 20 μmol ^{114}Cd was approximately 5.5-fold higher than that using 2 μmol ^{114}Cd (Table 2),suggesting that the apoplastically bound ^{113}Cd content, derived from the enriched isotope ^{114}Cd, increased with an increase in the concentration of ^{114}Cd in the desorption solution. Actually, the apoplastically bound ^{113}Cd contents, derived from the enriched isotope ^{114}Cd (2 and 20 μmol) were 6.6 ± 0.5 and 36.6 ± 1.8 mg kg^{-1}, respectively (Table 2); these values were calculated using equation in Fig.3. The contribution rate of ^{113}Cd content derived from the enriched stable isotope of ^{114}Cd for total ^{113}Cd in the roots was 28.6% for the 40 nmol ^{113}Cd treatment. In contrast, the contribution rate of ^{113}Cd content derived from ^{114}Cd for total ^{113}Cd content in the roots was 41.8% for the 400 nmol ^{113}Cd treatment (Table 2). These results indicate that the ^{113}Cd derived from the enriched stable isotope of ^{114}Cd must be subtracted from the total ^{113}Cd content in the roots to evaluate the symplastic ^{113}Cd in the roots. The symplastic ^{113}Cd contents for the 40 and 400 nmol treatments, calculated using equation in Fig.3, were 16.4 ± 3.7 and 51.0 ± 3.8 mg kg^{-1}, respectively (Table 2). In the present study, we disregarded the contribution of ^{114}Cd derived from the enriched isotope of ^{113}Cd because the composition rate of ^{114}Cd in the enriched isotope of ^{113}Cd was considerably lower than that of ^{113}Cd in the enriched isotope of ^{114}Cd.
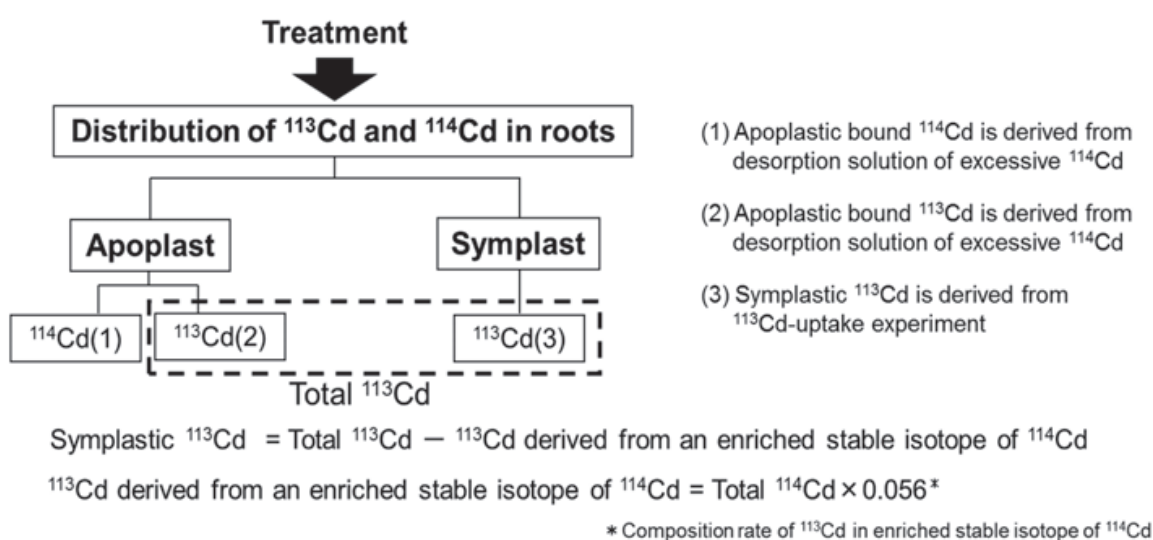

Fig. 3. Calculation of symplastic ^{113}Cd content in roots.

4.3 Comparison of the symplastic Cd contents in the roots between the two methods

To examine the validity of the new method for evaluating the symplastic Cd content in roots using ^{113}Cd and ^{114}Cd enriched isotopes, we compared the symplastic Cd content in roots using differences in the amounts of Cd absorbed at 2°C and 25°C with unlabeled Cd with the results obtained in the present study using the new method. In conventional Cd-absorption experiments, the Cd contents in roots at 40 and 400 nmol Cd in a 25°C treatment were 19.2 ± 1.6 and 84.4 ± 3.4 mg kg^{-1} (dry weight), respectively (Table 3). In contrast, the Cd

contents in roots at 40 and 400 nmol in the 2°C treatment were 4.1 ± 0.3 and 28.1 ± 0.73 mg kg^{-1} (dry weight), respectively.

The symplastic Cd contents at 40 and 400 nmol were estimated to be 15.1 ± 1.3 and 56.4 ± 2.7 mg kg^{-1}, respectively, which was evaluated using the difference in the amount of Cd absorbed at 2°C and at 25°C.

In the ^{113}Cd-absorption experiment, the symplastic ^{113}Cd contents in the roots at the 40 and 400 nmol ^{113}Cd treatments were 16.4 ± 3.7 and 51.0 ± 3.8 mg kg^{-1}, respectively (Table 2, 3). Therefore, the symplastic ^{113}Cd content after using the enriched isotopes was similar to the symplastic Cd content evaluated from the difference between the amount of Cd absorbed at 2°C and at 25°C. These results indicate that it is possible to evaluate the contents of symplastic Cd in roots using ^{113}Cd and ^{114}Cd enriched isotopes using the method proposed in the present study.

There have been many reports on Cd absorption in roots eliminating apoplastic bound Cd in Durum wheat, soybean and hyperaccumulator plants, such as *Thlaspi caerulescens* (Cataldo et al. 1983; Hart et al. 1998, 2002, 2006; Zhao et al. 2002). In these studies, the symplastic Cd content in the roots was determined by subtracting the Cd content in the roots at 2°C from the Cd content in the roots at 25°C; the Cd content was determined using a radioisotope of ^{109}Cd or a metabolic inhibitor. These methods have frequently been used to evaluate nutrient element absorption in roots. Radioisotopes in solute were the most useful markers used in these studies because they are chemically similar to the solute and can be distinguished from non-labeled solutes already contained in the roots (Davenport 2007). However, there are limitations to this method, including radioisotope administrative restriction and the restricted halflife of the radioisotope. Although the method involving a temperature difference between 2 and 25°C that was used in the present study is easy to handle because there is no radioisotope administrative restriction, there is, however, a limitation to this method: the symplastic Cd content in the roots cannot be evaluated using the same seedlings. This method has the advantage of no radioisotope administrative restriction and no restrictive radioisotope half-lives. In addition, this method uses half the number of seedlings that are required for the method using the temperature difference between 2 and 25°C because the symplastically absorbed Cd in the roots can be evaluated using roots from the same seedlings. In addition, the method proposed in the present study is applicable to other plants, not only *S. melongena*. We indicated that it is possible to evaluate symplastic Cd in roots using ^{113}Cd and ^{114}Cd enriched isotopes. The proposed method will contribute to research on symplastic ion absorption in plant roots stated below.

40nM			
Total ^{114}Cd	**Total ^{113}Cd**	**^{113}Cd derived from enriched ^{114}Cd**	**Symplastic ^{113}Cd**
117.3±9.3	23.0±4.3	6.6±0.53	16.4±3.7
400nM			
Total ^{114}Cd	**Total ^{113}Cd**	**^{113}Cd derived from enriched ^{114}Cd**	**Symplastic ^{113}Cd**
644.5±33.7	87.7±5.6	36.6±1.8	51.0±3.8

Table 2. ^{114}Cd and ^{113}Cd content in roots (modified from Mori et al. 2009a)

40nM			
Symplastic [113]Cd	**Cd(25°C-2°C)**	**Cd(25°C)**	**Cd(2°C)**
16.4±3.7	15.1±1.3	19.2±1.6	4.1±0.3
400nM			
Symplastic [113]Cd	**Cd(25°C-2°C)**	**Cd(25°C)**	**Cd(2°C)**
51.0±3.8	56.4±2.7	84.4±3.4	28.1±0.7

Table 3. Comparison of the symplastic Cd content in roots (modified from Mori et al. 2009a)
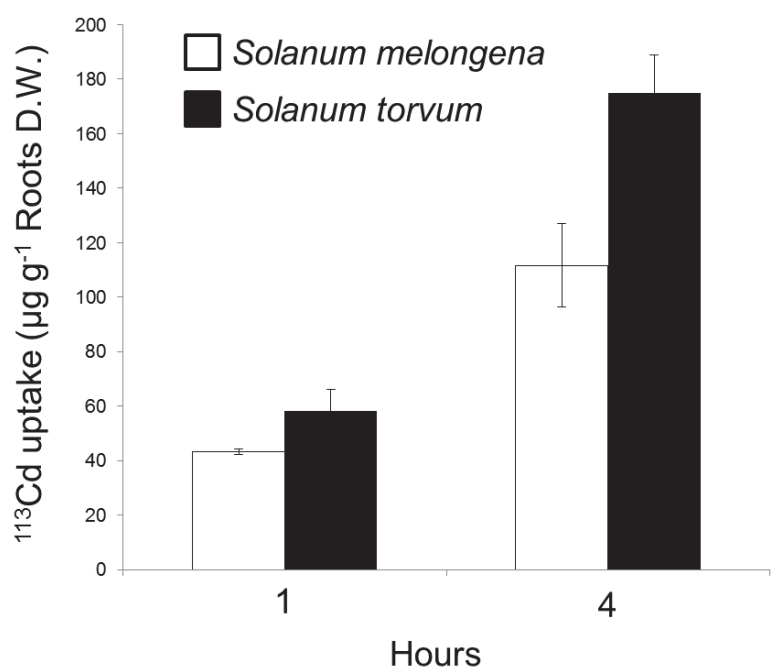

Fig. 4. Symplastic Cd absorption in roots of *Solanum melongena* and *Solanum torvum* with time. Experiment method is followed by the procedure illustrated in Fig.1 (modified from Mori et al.2009b)

We used the new method using enriched stable isotopes for evaluation of symplastic Cd absorption in roots of *solanaceous* plants (*Solanum melongena* and *Solanum torvum*) with contrasting root-to-shoot Cd translocation efficiencies (Mori et al. 2009a,b).
It is well known that efficiency of Cd translocation from roots to shoots is significantly higher in *S. melongena* than *S. torvum* (Arao et al. 2008, Mori et al. 2009a,b, Yamaguchi et al. 2011). Takeda et al.(2007) found that the Cd concentration in eggplant fruits could be reduced by grafting with *Solanum torvum* rootstock. Additionally, Arao et al.(2008) reported that although the Cd accumulation in shoots of *S. torvum* was lower than that found in *S. melongena*, there was no difference in the Cd content in roots of both plants when grown in culture solution. This result suggests that *S. torvum* develops noteworthy physiological mechanisms to suppress Cd translocation from roots to shoots, corresponding to the results observed in previous reports (Arao et al., 2008). Arao et al. (2008) suggested that symplastic Cd absorption and xylem loading capacity might be ascribed to the difference of Cd concentration in the shoots of *S. melongena* and *S. torvum*. We evaluated the symplastic Cd absorption rate in roots using

enriched isotopes [113]Cd and [114]Cd. In time course-dependent experiments, the symplastic [113]Cd absorption rate for both plants increased with time (Fig. 4). In addition, the symplastic [113]Cd absorption rate of *S. melongena* was slightly higher than that of *S. torvum* at 4 h (Fig. 4). We examined kinetics analysis by similar method using enriched stable isotopes of [113]Cd and [114]Cd (Mori et al. 2009b). A kinetic study revealed that the symplastic Cd concentrations in the roots increased with increasing external Cd concentrations, but saturated at a higher concentration. The saturated curve obtained in this study suggests that absorption in both cultivars is mediated by a transporter that exhibits a similar affinity for Cd.. Moreover, the symplastic Cd concentrations slightly differed between the roots of *S. melongena* and *S. torvum*. Based on the reaction curves obtained, the K_m value was estimated to be 380 and 352 nmol L^{-1} for *S. melongena* and *S. torvum*, respectively. The corresponding V_{max} values were 152 and 101.5 μ g root dw^{-1} 0.5 h^{-1}. The V_{max} value of *S. melongena* was approximately 1.5-fold higher than that of *S. torvum*, which suggests that the density of the Cd transporter in the root cell membranes of *S. melongena* is higher than in *S. torvum*. In this experiments, If the symplastic Cd absorption in roots is estimated by the conventional method using the difference of temperature at 2 and 25°C, it is required time consuming and double seedlings for experiment preparation.

5. Conclusion

For biological system analysis, the application of ICP-MS in enriched stable isotope tracer experiments has increased because ICP-MS has now become the preferred technique. An enriched stable isotope technique would be potent and useful tool for biological system experiments including element uptake, distribution and chemical form in plants. In this chapter, we introduced our one example of element uptake system using enriched isotope of [113]Cd and [114]Cd. This method has several merits compared to conventional methods if ICP-MS instrument is able to use. Application of enriched isotopes such as [113]Cd and [114]Cd would attain a new insight for plant biological system and will become a new tool to evaluate element behavior in plants.

6. Acknowledgment

7. References

Arao, T.; Takeda, H. & Nishihara, E. (2008). Reduction of cadmium translocation from roots to shoots in eggplant (Solanum melongena) by grafting onto Solanum torvum rootstock. *Soil Science and Plant Nutrition*, Vol. 54, pp.555–559.

Cataldo, D.A.; Garland, T.R. & Wildung, R.E. (1983). Cadmium uptake kinetics in intact soybean plants. *Plant Physiology*, Vol.73, pp.844–848.

Codex Alimentarius Commission 2005: Joint FAO/WHO Food Standards Programme. Twenty-eighth session, 4–9 July 2005, Rome, Italy. Report of the 37th session of the Codex Committee on Food Additives and Contaminants, 25–29 April 2005. Para. 175, Appendix XXVI. The Hague, the Netherlands. Available at URL: http://www.codexalimentarius. net/web/reports.jsp]ALINORM 05/28/12

Dannel, F.; Pfeffer, H. & RÖmheld, V. (2000). Characterization of root boron pools, boron uptake and boron translocation in sunflower using the stable isotopes ^{10}B and ^{11}B. *Australian Journal of Plant Physiology*, Vol.27, pp.397-405.

Davenport, R.J. (2007). Ion uptake by plant roots. In Plant Solute Transport. Eds AR Yeo and TJ Flowers, pp. 193–213. Blackwell Publishing, Oxford.

Epstein, E. (1973). Mechanisms of ion transport through plant cell membranes. *International Review of Cytology*, Vol.34, pp.123–168.

Epstein, E. & Hagen, C.E. (1952). A kinetics study of the absorption of alkali cations by barley roots. *Plant Physiology*,Vol.27, pp.457–474.

Glass, A.D.M. (2007). The apoplast: a kinetic perspective. In The Apoplast of Higher Plants: Compartment of Storage,Transport, and Reactions. The Significance of the Apoplast for the Mineral Nutrition of Higher Plants. Eds B Sattelmacher and WJ Horst, pp. 87–96. Springer-Verlag, Dordrecht.

Hart, J.J.; Welch, R.M.; Norvell, W.A. & Kochian, L.V. (2002). Transport interactions between cadmium and zinc in roots of bread and durum wheat seedlings. *Physiologia Plantarum*, Vol.116, pp.73–78.

Hart, J.J.; Welch, R.M.; Norvell, W.A. & Kochian, L.V. (2006). Characterization of cadmium uptake, translocation and storage in near-isogenic lines of durum wheat that differ in grain cadmium concentration. *New Phytologist*, Vol.172, pp.261–271.

Hart, J.J.; Welch, R.M.; Norvell, W.A.; Sullivan, L.A. & Kochian, L.V. (1998). Characterization of cadmium binding, uptake, and translocation in intact seedlings of bread and durum wheat cultivars. *Plant Physiology*, Vol.116, pp.1413–1420.

Kawasaki, A. & Oda, H. (2005). Application of ^{113}Cd as a tracer in evaluation of cadmium uptake by soybean under field conditions. Japanese *Journal of Soil Science and Plant Nutrition*, Vol.76, pp.261–267 (in Japanese with English summary).

Kawasaki, A.; Oda, H. & Yamada, M. (2004). Application of enriched ^{113}Cd-tracer to the soil pot experiment of soybean plant. *Japanese Journal of Soil Science and Plant Nutrition*, Vol.75, pp.667–672 (in Japanese with English summary).

Kimura, K.; Yoshida, K.; Sugito, T. & Yamasaki, S. (2003). Correction of interference by Mo oxide on Cd measurement by ICPMS. *Japanese Journal of Soil Science Plant Nutrition*, Vol.74, pp.493–497 (in Japanese with English summary).

Keller, C. (1995). Application of centrifuging to heavy metal studies in soil solutions. *Communication Soil Science and Plant Analysis*, Vol.26, pp.1621–1636.

Lasat, M.M.; Baker,A.J.M. & Kochian L.V. (1996). Physiological Characterization of Root Zn^{2+} Absorption and Translocation to Shoots in Zn Hyperaccumulator and Nonaccumulator Species of *Thlaspi. Plant Physiology*, Vol.112, pp.1715-1722.

Lasat, M.M.; Baker, A.J.M. & Kochian, L.V. (1998). Altered Zn compartmentation in the root symplasm and stimulated Zn absorption into the leaf as mechanisms involved in Zn hyperaccumulation in *Thlaspi caerulescens. Plant Physiology*, Vol.118, pp.875–883.

Ma, J.F.; Mitani, N.; Nagao, S.; Konishi, S.; Tamai, K.; Iwashita, T. & Yano, M. (2004). Characterization of the Silicon Uptake System and Molecular Mapping of the Silicon Transporter Gene in Rice. *Plant Physiology*, Vol. 136, pp. 3284–3289

May, T.W. & Wiedmeryer, R.H. (1998). A table of polyatomic interferences in ICP-MS. *Atomic Spectroscopy*, Vol.19, pp.150–154.

Ministry of Agriculture Forestry and Fisheries of Japan 2002:Survey of the cadmium contained in domestic vegetables. Available at URL:http://www.maff.go.jp/cd/PDF/C12. pdf,p34, p38, p25 (in Japanese)

Mitani, N. & Ma, J.F. (2005). Uptake system of silicon in different plant species. *Journal of Experimental Botany*, Vol. 56, pp. 1255–1261.

Mori, S.; Kawasaki, A.; Ishikawa, S. & Arao, T. (2009a). A new method for evaluating symplastic cadmium absorption in the roots of Solanum melongena using enriched isotopes ^{113}Cd and ^{114}Cd. *Soil Science and Plant Nutrition*, Vol.55, pp. 294–299.

Mori, S.; Uraguchi, S.; Ishikawa, S. & Arao, T. (2009b). Xylem loading process is a critical factor for determining Cd accumulation in the shoots of *Solanum melongena* and *Solanum torvum. Environmental and Experimental Botany*, Vol.67, pp.127-132.

Nakanishi, H.; Ogawa, I.; Ishimaru, Y.; Mori, S. & Nishizawa, K.N. (2006). Iron deficiency enhances cadmium uptake and translocation mediated by the Fe^{2+} transporters OsIRT1 and OsIRT2 in rice. *Soil Science and Plant Nutrition*, Vol.52, pp.464–469

Oda, H.; Yada, S.; Kawasaki, A. (2004). Uptake and transport of Cd supplied at different growth stages in hydroponically cultured soybean plants. *Biomedical Research on Trace Element*, Vol.15, pp.289–291 (in Japanese with English summary).

Rains, D.W.; Epstein, E.; Zasoski, R.J. & Aslam, M. (2006). Active silicon uptake by wheat. *Plant and Soil*, Vol.280, pp.223–228.

Stürup, S.; Hansen, H.R. & Gammelgaard, B. (2008). Application of enriched stable isotopes as tracers in biological systems: a critical review. *Analytical and Bioanalytical and Chemistry*, Vol.390, pp.541–554.

Takano, J.; Noguchi, K.; Yasumori, M.; Kabayashi, M.; Gajdos, Z.; Miwa, K.; Hayashi, H.; Yoneyama, T. & Fujiwara, T. (2002). Arabidopsis boron transporter for xylem loading. *Nature*, Vol.420, pp.337-340.

Takeda, H.; Sato, A.; Nishihara, E. & Arao, T. (2007). Reduction of cadmium concentration in eggplant (*Solanum melongena*) fruits by grafting with solanum torvum rootstock. *Japanese Journal of Soil Science and Plant Nutrition*, Vol.78, pp.581–586 (in Japanese with English summary).

Ueno, D.; Iwashita, T.; Zao, F.J. & Ma, J.F. (2008). Characterization of Cd translocation and identification of the Cd form in xylem sap of the Cd-hyperaccumulator *Arabidopsis halleri. Plant and Cell Physiology*, Vol.49, pp.540–548.

Ueno, D.; Koyama, E.; Kono, I.; Ando, T.; Yano, M. & Ma, J.F. (2009). Identification of a Novel Major Quantitative Trait Locus Controlling Distribution of Cd Between Roots and Shoots in Rice. *Plant and Cell Physiology*, Vol. 50, pp.2223-2233

Ueno, D.; Ma, J.F.; Iwashita, T., Zhao, F.J. & McGrath, S.P. (2005). Identification of the form of Cd in the leaves of a superior Cd-accumulating ecotype of *Thlaspi caerulescens* using ^{113}Cd-NMR. *Planta*, Vol.221, pp.928–936.

Uraguchi, S.; Mori, S.; Kuramata, M.; Kawasaki, A.; Arao, T. & Ishikawa, S. (2009). Root-to-shoot Cd translocation via the xylem is the major process determining shoot and grain cadmium accumulation in rice. *Journal of Experimental Botany*, Vol.60, pp.2677-2688

Wanger, G.J. (1993). Accumulation of cadmium in crop plants and its consequences to human health. *Advanced Agronomy*, Vol.51, pp.173–212.

Yada, S.; Oda, H. & Kawasaki, A. (2004). Uptake and transport of Cd supplied at early growth stage in hydroponically cultured soybean plants. *Biomedical Research on Trace Elements*, Vol.15, pp.292–294 (in Japanese with English summary).

Yamaguchi, N.; Mori, S.; Baba, K.; Yada, S.; Arao, T., Kitajima, N.; Hokura, A. & Terada, Y. (2011). Cadmium distribution in the root tissues of *solanaceous* plants with contrasting root-to-shoot Cd translocation efficiencies. *Environmental and Experimental Botany*, Vol.71, pp.198–206

Yu, Q.; Tang, C.; Chen, Z. & Kuo, J. (1999). Extraction of apoplastic sap from plant roots by centrifugation. *New phytologist*, Vol.143, pp.299-304.

Zhao, F.J.; Hamon, R.E.; Lombi, E.; McLaughlin, M.J. & McGrath, S.P. (2002). Characteristics of cadmium uptake in two contrasting ecotypes of the hyperaccumulator *Thlaspi caerulescens*. *Journal of Experimental Botany*, Vol.53, pp.535–543.
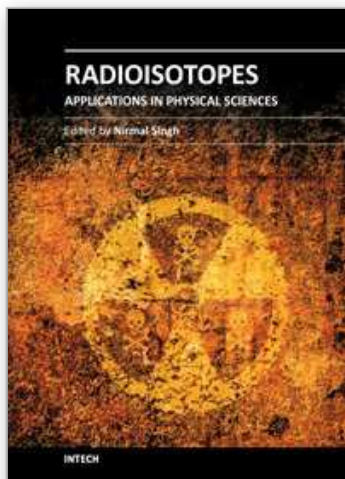
Radioisotopes - Applications in Physical Sciences

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The book Radioisotopes - Applications in Physical Sciences is divided into three sections namely: Radioisotopes and Some Physical Aspects, Radioisotopes in Environment and Radioisotopes in Power System Space Applications. Section I contains nine chapters on radioisotopes and production and their various applications in some physical and chemical processes. In Section II, ten chapters on the applications of radioisotopes in environment have been added. The interesting articles related to soil, water, environmental dosimetry/tracer and composition analyzer etc. are worth reading. Section III has three chapters on the use of radioisotopes in power systems which generate electrical power by converting heat released from the nuclear decay of radioactive isotopes. The system has to be flown in space for space exploration and radioisotopes can be a good alternative for heat-to-electrical energy conversion. The reader will very much benefit from the chapters presented in this section.

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