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Separation and analysis of chlorine isotopes in higher plants

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ABSTRACT

A novel chemical mass spectrographic method was used in the determination of chlorine (Cl) isotopes in plant tissues. The procedure includes dry ashing, three-step ion chromatographic separation of Cl isotopes, and isotope ratio determination based on Cs_2Cl^+ ion in positive thermal ionization mass spectrometry. The recovery of the method and the fractionation of Cl isotopes were validated using certified reference standard materials. The pretreatment strongly eliminated the effects of organic impurities and other anionic interferences, especially soluble nitrates and sulfates. The results show that there was severe fractionation of Cl isotopic composition in the tissues of plant samples, which might be caused by different molecular mechanisms of uptake and translocation of Cl within plants. The observed Cl isotopic variation is considered to be a useful isotope signature of living systems, which may be used to understand better the Cl cycling process in the environment.

1. Introduction

Chlorine (Cl) is a critical micronutrient in plants (Broyer et al., 1954). Through its physicochemical behavior, it plays an important role in growth, photosynthesis, and enzyme activation in plants (Churchill and Sze, 1984; White and Broadley, 2001; Kusunoki, 2007; Hänsch and Mendel, 2009). At high concentrations, Cl is toxic to plants but a deficiency of it results in death of leaves and shoots. Cl is also applied in a chemical fertilizer to accelerate the growth and enhance the yields of crops (Whitehead, 1985). However, widespread salinization and fertilization in urban areas would be a severe ecological and environmental issue that affects the growth of plants; it increases the amount of Cl in the food chain.

The mechanism of mobilization of Cl from soil to plants, known as the geochemical pump, retards the release of Cl to aqueous systems. Although the requirements for Cl uptake by plants vary in magnitude, the effect of its application to global Cl cycling is immense. Cycling of Cl by uptake from soil and processing in plant tissues by a series of chemical reactions can lead to Cl isotopic fractionation. Because the reaction does not need the transfer of all of the Cl from one plant to another, the fractionation of Cl isotopes occurs within the plants. Lastly, although in the process of global cycling chlorine can be easily washed by rainwater from the surface of dry plants and is integrated in the migration processes without decomposition of dead plants (Kashparov et al., 2007b), plants return Cl to the soil through leaf litter with a Cl isotopic composition different from that of the growth medium. The transfer, translocation, and mechanism of processing of Cl within plants are another powerful contribution to the global cycling of Cl (Kashparov et al., 2007a; Henner et al., 2013; Hurtevent et al., 2013).

In recent years, the acquisition and availability of Cl in biological systems have been investigated (Johnson et al., 1957; Hänsch and Mendel, 2009; Sharp et al., 2013). However, no evidence of discrimination of biological processes between stable isotopes of Cl has been found, except in the case of the radioisotope $^{36}C1$ (Kashparov et al., 2007a,b), which has been identified as an environmental contaminant (Sheppard et al., 1996). Unlike in the case of the other well-established stable isotopes (C, H, O, N, S) (Grusak, 1997; Kelley et al., 2005), no attempt has been made to determine the use of Cl isotopes in biological matrixes such as higher plants. There are many methods developed for separation of Cl for its isotope determination in environmental samples, such as ion exchange (Xiao et al., 2002a; Sun et al., 2004; Wei et al., 2012), AgCl sediment (Magenheim et al., 1994), and CH_3Cl transformation of chlorinated organic compounds (Tanaka and Rye, 1991; Long et al., 1993). However, large amounts of organic matter present in plants can interfere with the emission of Cs_2Cl^+ ion currents in thermal ionization mass spectrometry (TIMS). Therefore, it is critical to develop an efficient separation method and to eliminate the interferences due to impurities during the enrichment of Cl and isotopic determination of Cl in a complex matrix. In our study, we performed a series of

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chromatographic experiments on Cl extraction in plants, and determined its isotopic composition in the various tissues by positive thermal ionization mass spectrometry (PTIMS). Fractionation of Cl isotopes during separation was estimated using a reference standard. Fractionation variations in the Cl isotopic composition in the various tissues of plants are discussed.

2. Materials and methods

2.1. Plants and soil site

To examine the fractionation in chlorine isotope among different plant species, samples were collected from five different sites at three areas. Plant samples investigated in this study included various tissues of *Swertia mussotii* Franch. and *Halenia elliptica* D. Don collected in the Qinghai–Tibet Plateau area, *Weigela florida* cv. Red Prince and *Echinacoa angustifofia* in Shandong area, and *Cynomorium songaricum* Ruper. in Inner Mongolia area, China. *S. mussotii*, generally called as "Zang Yin Chen" by Tibet in China, as well as *H. elliptica*, is a biennial famous medicinal plant in the family Gentianaceae, and grows mainly in the alpine and subalpine meadow in the Qinghai–Tibet Plateau (Yang, 1991). These two samples were collected during the months of September and October (the flowering and fruiting period), 2012, from Yushu county and Banma county of Qinghai province in China, respectively. The root holoparasite *C. songaricum* (Cynomoriaceae genus, Saxifragales family), known in Chinese herbal medicine as "suoyang", is a classic Mongolian pharmaceutical plant. It usually parasitizes the roots of *Nitraria spp. E. angustifofia*, a herbaceous plant species in the daisy family Asteraceae, which blooms from late spring to mid summer. It is also found growing in dry prairies and barrens with rocky to sandy-clay soils. *W. florida*, belonging to the family of Caprifoliaceae, is a deciduous shrubs and collected in June, 2012. The information about sample site, plant species and soil-climate conditions in the regions are summarized in Table 1.

2.2. Instruments, reagents, and samples

Nitric acid, silver nitrate, barium sulfate, and cesium nitrate were of guaranteed reagent grade. Graphite slurry of 13 mg/g was prepared by adding high-purity graphite to an aqueous ethanol solution (80%). The isotopic reference standard for Cl used in this study was ISL 354 NaCl (Xiao et al., 2002b). Ag^+, Ba^{2+}, and Cs^+ resins were prepared using solutions of $AgNO_3$, $BaSO_4$, and $CsNO_3$, respectively. High-purity water, which was used to prepare stock and working solutions, was redistilled by sub-boiling distillation and then passed through a resin column filled with Ag^+ resin.

2.3. Separation of Cl isotopes in plants

Cl matrix separation was performed on a series of strong cation exchange resin, DOWEX 50 W × 8, purchased from Sigma-Aldrich (Shanghai, China). To eliminate potential exogenous contaminants, only quartz glasses and Teflon containers were used for Cl pretreatments. The process included two steps: the first was dry ashing, and the second was three-step ion-exchange chromatography using three different types of anion resins connected in series.

2.3.1. Dry ashing

Traditionally, plant tissue is decomposed through the wet chemical digestion method using HNO_3/H_2O_2, which can lead to the loss of Cl by formation of HCl. Dry ashing was therefore used to decompose plant tissue. Through this method, almost all organic impurities could be removed (Rosner et al., 2011) and loss of Cl could be avoided, as reported by Kashparov et al. (2005). Approximately 0.5 g of dried plant material was weighed into a quartz crucible. The crucible samples were placed in a closed muffle furnace. To avoid the effect of bubbles in samples due to rapid heating, the temperature was raised to 200 °C for 1 h during the carbonization of organic matter. The temperature was then raised to 550 °C for 4 h until the ash changed color from whitish to black. A 0.5 mol/L HNO_3 solution (1 mL) was used to dissolve the ash. The resulting sample solution was then transferred to a polypropylene tube after filtration.

2.3.2. Ion-exchange chromatography

Because of the presence of SO_4^{2-}, NO_3^-, and other contaminants that could interfere with determination of Cl isotopes in mass spectrometry (Xiao et al., 2002a), three different types of cation resins were used to extract and purify Cl in the aqueous sample solution. Ag^+ resin was used to enrich the Cl in the first step. The columns were twice cleaned with 3 mL HNO_3 followed by three 1 mL portions of ultrapure water. The sample solution was loaded into the conditioned Ag^+ resin column. Cl in the form of AgCl, together with other ions such as SO_4^{2-}, Br^-, and so forth, remained at the top of the resin bed, which prevented the lease of AgCl particles. This step removes nitrate compounds completely and prevents the loss of Cl. The eluate was checked for the loss of Cl by using $AgNO_3$ solution. After storing the sample solution for about 6 h in darkness, it was passed through the resin. The resin was subsequently washed twice with 2 mL of ultrapure water, and then 0.1 mol/mL ammonia hydroxide solution was used to dissolve the deposited AgCl. The collected eluates were then completely transferred to 1 mL of the Ba^{2+} resin column to eliminate the sulfate compounds. The resin was subsequently rinsed three times with 0.5 mL ultrapure water. Finally, the eluates were pooled, transferred to a 20-mL conical beaker, and then evaporated to dryness at 80 °C under a stream of air. Three batches of 50 μL of ultrapure water were then added to the container. The solution was passed through the Cs^+ resin, and the resin was washed with 0.5 mL of ultrapure water three times. The solutions were collected together and evaporated to about 2–4 mg Cl/mL at 80 °C with an air stream. The solution was then stored at 4 °C for isotopic analysis of Cl.

An outline of the general workflow of Cl separation in plant samples mentioned above is shown in Fig. 1.

Table 1
Sampling sites and plant species.

Species	Sampling location	Altitude (m)	Longitude	Latitude	T. range[a]/Av. T[b] (°C)	Precipitation [c] (mm)	Habit type	Soil type
Weigela Florida	Linyi, Shandong	71	118°17′13.21″E	35°6′21.24″N	−2–26 13	830	Sand	Shantung soil
Echinacoa angustifofia	Pingyi, Shandong	242	117°4023.52″E	35°15′56.88″N	−3–27 14	800	Sand	Cinnamon soil
Cynomorium songaricum	Jilantai, Inner Mongolia	1060	105°37′13.83″E	39°34′42.61″N	−10–27 8	110	Sand	Sandy soil
Swertia mussotii	Yushu, Qinghai	3585	97°53′23.28″E	33°20′12.12″N	−18–16 2.9	460	Shrub grassland	Alpine steppe soil
Halenia Elliptica	Banma, Qinghai	3514	100°47′3.48″E	32°46′27.12″N	−11–12 3	640	Bottomland meadow	Meadow soil

[a] Temperature range per year.
[b] Average temperature per year.
[c] Average precipitations per year.