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ORIGINAL ARTICLE

Vital effects of K isotope fractionation in organisms: observations and a hypothesis

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Abstract Compared with the measureable but limited K isotope variation in geological samples, biological samples have much larger variations in $\delta^{41}K$ values: from $-1.3‰$ to $+1.1‰$ relative to the international K standard NIST SRM 3141a. Notably, higher plants generally have $\delta^{41}K$ values that are lower than igneous rocks, whereas sea plants (algae) have $\delta^{41}K$ values that are higher than seawater; the range in $\delta^{41}K$ values of plants encompasses the $\delta^{41}K$ values of both igneous rocks and seawater. Plant cells utilize different K uptake mechanisms in response to high- and low-K conditions. In a low-K environment, plant cells use energy-consuming ion pumps for active uptake of K; plant cells in high-K environments use non-energy-consuming ion channels. Based on these facts and on K isotope data from sea and land plants, it is hypothesized that the different K uptake mechanisms are accompanied by distinct K isotope fractionation behaviors or vital effects. The enrichment of light K isotopes in terrestrial plants could be attributed to preferential transport of isotopically light K in the energy-consuming active uptake process by K ion pumps in the membranes of plant root cells. On the other hand, the enrichment of heavy K isotopes in algae may be caused by a combination of the lack of K isotope fractionation during K uptake from seawater via ion channels and the preferential efflux of light K isotopes across the cell membrane back to the seawater. The large variation of K isotope compositions in biological samples therefore may reflect the diversity of isotopic vital effects for K in organisms, which implies the great potential of K isotopes in biogeochemical studies.

Keywords K isotopes · Isotope fractionation · Vital effects

1 Introduction

Potassium has three naturally occurring isotopes: ^{39}K (93.258%), ^{40}K (0.012%, radioactive, half-life 1.248 billion years), and ^{41}K (6.730%). $^{41}K/^{39}K$ ratios have long been employed to study geological and cosmochemical processes. Early K isotope analyses used thermal ionization mass spectrometry (TIMS) and secondary ionization mass spectrometry (SIMS). Precision of TIMS and SIMS methods for K isotopes was at the level of $\pm0.5‰$ to $\pm1‰$, which allowed distinction of large (several ‰ in $^{41}K/^{39}K$ ratio), non-equilibrium K isotope fractionation in high-temperature processes including K evaporation/condensation as recorded in lunar soils, meteorites, and microtektites. The precision of TIMS and SIMS methods for K isotopes, however, are not sufficient to discern the more subtle K isotopic partitioning between most terrestrial samples.

Recently, progress in multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS) has led to significant improvements in the precision of $^{41}K/^{39}K$ ratio measurements. The issue of argon hydride ($^{40}ArH^{+}$) interference with ^{41}K, the most serious challenge for $^{41}K/^{39}K$ ratio measurements using Ar-burning ICP-MS, has been overcome either by a combination of high mass-resolution and cool plasma techniques (Morgan et al. 2014), or by collision cell technology (Li et al. 2016; Wang and

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Jacobsen 2016), achieving external precision of ±0.2‰ or better for $^{41}K/^{39}K$ ratio measurements. With the improved precision, the $^{41}K/^{39}K$ ratio of modern seawater has been determined to be about 0.6‰ higher than a variety of igneous rocks (e.g., BHVO, BCR, AVG and GSP; Li et al. 2016; Wang and Jacobsen 2016).

Besides the fact that K is a major element that is involved in numerous geological processes, it is also a critical nutrient; particularly, it is the most abundant metal cation in plant cells (Sardans and Peñuelas 2015). Potassium plays an important role in a plethora of biological processes such as photosynthesis, osmotic pressure balancing, and the famous "sodium–potassium pump" that together regulate many aspects of cell physiology (Szczerba et al. 2009). To date, the biogeochemistry of K isotopes has been little explored, although preliminary results imply that very large K isotope fractionation is associated with biological activities of plants (Li et al. 2016). In this study, we report high-precision K isotope data for a wider range of biological samples and propose a hypothesis of vital effects for K isotopes in organisms to account for the large K isotope variations across biological samples.

2 Materials and methods

2.1 Samples and preparation

A number of biological samples, including corn seeds, beef, seaweeds (*Laminaria japonica,* a type of brown algae, and *Porphyra,* a type of red algae), sea clams, jelly fish, and small-boned fish (*Stolephorus*), were analyzed for K isotope composition. All these edible goods were purchased in July 2014 from a Chinese supermarket. The corn seeds and beef were fresh domestic products and unprocessed when bought; the rest were pre-washed and -dried sea products from the western Pacific coastal seas. No treatment other than washing and drying was noticed to have been applied to these products.

Sample preparation was undertaken at Nanjing University, where all chemical procedures were performed in a clean room with laminar flow hoods and HEPA filtered air. Deionized (18.2 MΩ) water, Teflon-coated hot plates, Teflon beakers, and double distilled reagents were used throughout the experiments; other labware was prewashed in 6 M HCl and deionized water before use. Biological samples were contained in quartz crucibles and ashed in a muffle furnace at 600 °C before leaching with 0.5 M HNO_3 and filtration. An aliquot of the dissolved sample, which typically contained 50 to 200 μg of K, was dried and dissolved in 0.5 mL 1.5 M HNO_3 in preparation for chemical purification using ion exchange chromatography.

Separation of K from matrix elements followed a two-stage ion exchange protocol described in Li et al. (2016). This two-stage column procedure balances versatility, quality of separation, robustness, acid consumption, and efficiency for chemical purification of K. Potassium recovery of the method was 99.4‰ ± 2.1‰ (2SD, n = 54), and the total procedural blank of K was 3 to 8 ng (n = 5), which is negligible compared with the > 50 μg mass of K in samples.

2.2 Mass spectrometry

$^{41}K/^{39}K$ isotope ratio measurements were performed on a Micromass IsoProbe MC-ICP-MS at the University of Wisconsin–Madison, using instrument settings that have been detailed in Li et al. (2016). The IsoProbe MC-ICP-MS was run with standard 1350 W forward RF power, using high-purity He (flow rate: 10 mL/min) as the collision gas and high-purity D_2 (flow rate: 6 mL/min) as the reaction gas. Argon hydride was nearly quantitatively suppressed via proton transfer and atom transfer reactions with D_2 in the collision cell (Li et al. 2016). Potassium solutions were introduced into the plasma using a self-aspirating nebulizer with an uptake rate of ~0.1 mL/min. Typical sensitivity for 1 ppm K solution under standard mass resolution (~400 resolving power) was 7–11 V for ^{39}K and 0.6–1 V for ^{41}K.

A standard-sample-standard bracketing routine was applied for K isotope ratio measurement, with a 1 ppm standard. Sample solutions were diluted to match the concentration of the standard solution better than ±10%. A 60-s on-peak acid blank was measured prior to each isotopic analysis of K solution, and was subtracted from the analyte signal. Each K isotopic analysis consisted of 40 5-s integrations.

2.3 Data reporting, precision, and accuracy

Potassium isotope compositions are reported using the standard per mil (‰) notation of $\delta^{41}K$ for a $^{41}K/^{39}K$ ratio, where

$$\delta^{41}K = \left[\left(^{41}K/^{39}K_{sample}\right)/\left(^{41}K/^{39}K_{standard}\right) - 1\right] \times 1000$$

All K isotope data are reported relative to the international K standard NIST SRM 3141a. The in-house K stock solution (UW-K) has a $\delta^{41}K$ value of −0.12‰ ± 0.03‰ (2 standard error, or 2SE, n = 43) relative to NIST SRM 3141a; seawater has a $\delta^{41}K$ value of 0.06‰ ± 0.10‰ (2 standard deviation, or 2SD, n = 3) (Li et al. 2016).

Internal precision for $^{41}K/^{39}K$ ratio measurement was better than ±0.07‰ (2SE), with most better than ±0.04‰ (2SE). Accuracy of the method was checked by analyzing

pure NIST 3141a K and synthetic samples that were subjected to the two-stage ion exchange columns. The synthetic samples were made by mixing UW-K solution with matrix elements separated from different natural samples during ion exchange column chemistry. The measured δ^{41}K values for the five processed NIST 3141a K samples clustered around 0‰ (−0.03‰ ± 0.13‰, 2SD, n = 5), and the measured δ^{41}K values for the seven synthetic samples that were doped with UW-K clustered around −0.12‰ (−0.10‰ ± 0.08‰, 2 SD, n = 7) (Li et al. 2016). An additional check of accuracy was the consistency of the δ^{41}K value difference between seawater and igneous rocks (e.g., BHVO-1 and BHVO-2), which has been reported as 0.58‰ in Wang and Jacobsen (2016) and 0.56‰ in Li et al. (2016).

3 Results

The measured K isotope compositions of the biological samples are tabulated in Table 1 and plotted in Fig. 1. Among the samples analyzed, daisy flower had the lowest δ^{41}K value of −1.3‰, consistent with the previous observation that light K isotopes are preferentially enriched in tissues of higher plants (Li et al. 2016). The two seaweed samples had high δ^{41}K values: 0.57‰ for green algae (*Laminaria japonica*) and 1.11‰ for red algae (*Porphyra*), both significantly higher than that of seawater (0.06‰). The δ^{41}K value of sea clam was also distinctly higher than seawater (0.97‰), but two other sea animals (fish and jellyfish) had δ^{41}K values lower than seawater. Corn seeds and beef had δ^{41}K values of −0.34‰ and −0.19‰, respectively, both between the δ^{41}K values of igneous rocks and seawater. Overall, biological samples covered a span of 2.4‰ in δ^{41}K, representing the greatest K isotope

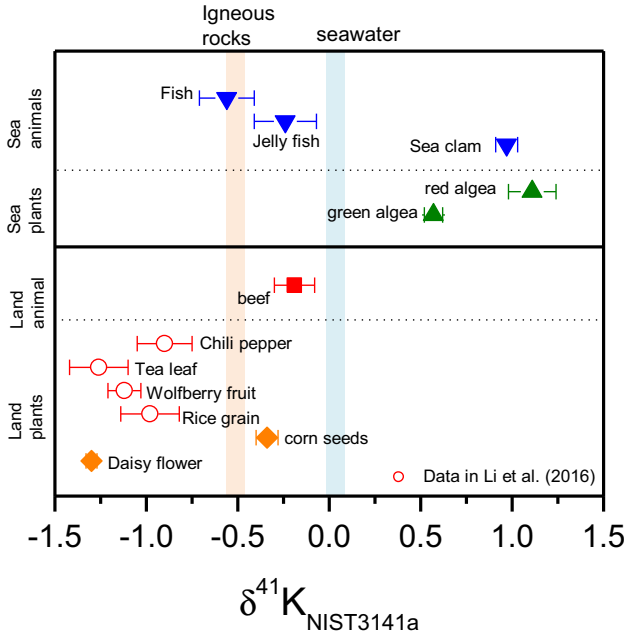

Fig. 1 Comparison of K isotope compositions of different biological samples. K isotope compositions of igneous rocks and seawater data are from Li et al. (2016) and Wang and Jacobsen (2016)

Table 1 K isotope data of biological samples in $\delta^{41/39}$K relative to NIST SRM 3141a

Sample	$\delta^{41/39}$K	2SD	No. of analysis	Source
Higher plant samples				
Daisy flower	−1.30	0.03	2	This study
Corn seeds	−0.34	0.06	2	This study
Rice grains	−0.98	0.16	2	Li et al. (2016)
Wolfberry fruit (*Lycium barbarum*)	−1.12	0.09	8	Li et al. (2016)
Tea leaves	−1.26	0.16	8	Li et al. (2016)
Chili pepper	−0.90	0.15	8	Li et al. (2016)
Sea plant samples				
Sea weed (*Laminaria japonica*)	0.57	0.05	3	This study
Sea weed (*Porphyra*)	1.11	0.13	2	This study
Sea animal samples				
Fish	−0.56	0.15	2	This study
Sea clam	0.97	0.06	2	This study
Jelly fish	−0.24	0.17	3	This study
Land animal samples				
Beef	−0.19	0.11	2	This study

variation that has been observed in natural samples on Earth.

4 Discussions

4.1 K isotope fractionation during K uptake by plants

Potassium is an important vital element, and K is significantly enriched in the biomass of both terrestrial and sea plants relative to the environment (Sardans and Peñuelas 2015). Terrestrial plants have developed sophisticated mechanisms of K uptake and redistribution involving several families of membrane proteins (Szczerba et al. 2009). A number of proteins on cell walls have been indentified to actively or passively uptake K^+ into cells across their membrane. The active K-uptake process is energy consuming, involving an ATP-powered molecular mechanism (pump), which is turned on in low-concentration environments (<40 ppm K) (Britto and Kronzucker 2008; Szczerba et al. 2009). Terrestrial plants rely on root cells to uptake K from soils and weathered rocks. Because K is a mobile element, K deficiency is likely and the active K-uptake mechanism is needed. As this process is energy consuming, it is hypothesized here that the K transporters preferentially uptake light K isotopes to reduce the energy cost of active K pumping. Therefore, the higher plants have $\delta^{41}K$ values lower than that of igneous rock. This is consistent with the observation that five out of six plants measured had $\delta^{41}K$ values distinctly lower than those of igneous rocks (Fig. 1; Table 1).

Biochemistry studies have also revealed that in a high-K environment (>40 ppm K), passive K uptake is used by cells, and this process is considered to be facilitated by ion channels that are not energy consuming (Szczerba et al. 2009). Complementary to the prior hypothesis about preferential uptake of light K isotopes through an energy-consuming mechanism, it is hypothesized that no preferential uptake of light or heavy K isotopes occurs when a cell utilizes the non-energy-consuming ion channel mechanism. It should be noted that the measured $\delta^{41}K$ value for corn seeds was not only higher than other plants, but also higher than igneous rocks (Fig. 1; Table 1). This may reflect that the corn was grown in different conditions in terms of K availability and K source. In well-fertilized farm soils, the root cell of the corn may be in contact with high-K aqueous solutions (e.g., >40 ppm), and the K isotope composition of the K in soil solutions may be controlled by the fertilizer. Potassium-rich marine evaporites are the most common K source for fertilizers, and KCl in evaporites has a K isotope composition that is similar to seawater (Wang and Jacobsen 2016). Therefore, the corn may have grown in an environment where there was abundant K with $\delta^{41}K$ similar to seawater. In this case, the corn sample may have had a K isotope composition that was not fractionated relative to the abundant K available to root cells, under the vital effect hypothesis for K isotopes under high- and low-K concentrations.

4.2 K isotope fractionation during K efflux from cells

Seawater has a K concentration of 399 ppm, and according to the hypothesis proposed above, algae likely utilizes a non-energy-consuming passive uptake mechanism for K, which does not result in K isotope fractionation. However, the $\delta^{41}K$ values of the two algae samples were significantly higher than that of seawater (Fig. 1; Table 1). This can be explained by preferential removal of light K isotopes from the algae cells. It has been suggested that efflux of K across membrane may occur via diffusion, or transport via ion channels (Gaymard et al. 1998) and cation-proton exchangers (Pardo et al. 2006). It is possible that light K isotopes are preferentially transported out of cell membranes, either by faster diffusion of light K isotopes or by favorable selectivity for light K isotopes by related transporters or exchangers on cell membranes. Both processes would result in enrichment of heavy K isotopes in the cell.

4.3 K isotope fractionation related to metabolism in animals

Like plants, samples of animal bodies showed large K isotope variability (Fig. 1; Table 1). In contrast with the plant samples, however, the (albeit very limited) data from the animal samples do not show any apparent pattern. The sea clam sample had a $\delta^{41}K$ value higher than seawater, indicating that the cells in sea clam retain heavy K isotopes within their membranes. However, fish and jellyfish had significantly lower $\delta^{41}K$ values than the seawater. Potassium is a critical element for osmoregulation and it is interesting to explore whether such a process has a K isotopic response. Among the three sea animals, fish should have developed the most sophisticated mechanism for an internal osmotic pressure balancing mechanism, whereas the osmotic pressure balancing mechanism for jellyfish may be the least sophisticated (Willmer et al. 2005). A correlation seems to be lacking between the sophistication level of osmoregulation and K isotope composition in the three marine animals that were investigated. Since trophic level has been proposed to affect the metal stable isotope composition in animals (Clementz et al. 2003), it would be

interesting to investigate whether K isotopes also respond to trophic level. However, further discussion on K isotope variability in animals is currently limited by the scarcity of systematic data.

5 Conclusions

A span of 2.4‰ in $\delta^{41}K$ values was observed in selected biological samples, marking the largest K isotope variation in natural samples on Earth. The fact that K isotope compositions in biological samples were much more variable than those in geological samples implies the existence of kinetic isotope effects in organisms. Some terrestrial plants enrich light K isotopes in their organs, which could be attributed to preferential utilization of isotopically light K in the energy-consuming active K uptake process in low-K conditions. By contrast, some algae are enriched in heavy K isotopes, perhaps due to the lack of K isotope fractionation during K uptake from seawater, in combination with preferential efflux of light K isotopes from the cell. Large K isotope variation was also observed in animals, which may be linked to K isotope effects related to osmoregulation or trophic level differences. In summary, the large K isotope fractionations imply a sensitivity of K isotopes to specific biological processes or physiological mechanisms. The biogeochemistry of K isotopes is a promising research field worthy of further exploration.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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