NOTE

New organic reference materials for carbon- and nitrogen-stable isotope ratio measurements provided by Center for Ecological Research, Kyoto University, and Institute of Biogeosciences, Japan Agency for Marine-Earth Science and Technology

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Abstract

We determined both carbon and nitrogen isotope ratios of ten organic reference materials (CERKU-01 to CERKU-10) in the Center for Ecological Research (CER), Kyoto University, and three organic reference materials (BG-A, BG-P and BG-T) in the Institute of Biogeosciences (BioGeos), JAMSTEC, using an internationally recommended calibration method of two-point anchoring. The reference materials cover $\delta^{13}C_{VPDB}$ range of -34.92 to -9.45‰ and $\delta^{15}N_{Air}$ range of -5.22 to 22.71‰, and can be used to measure isotope ratios of naturally occurring substances.

Key words: Stable isotope, Carbon, Nitrogen, Calibration, Reference materials

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Introduction

Carbon and nitrogen isotope ratios have recently been used to study various kinds of research fields, such as biogeochemistry and ecology (Fry 2006; Michener and Lajtha 2007; Wada 2009; Ohkouchi et al. 2010). In limnology, stable isotope technique has been applied to both nutrient dynamics and food web research in rivers (e.g. Yamada et al. 1996; Finlay 2004; Doi et al. 2007; Kohzu et al. 2009) and lakes (e.g. Cabana and Rasmussen 1996; Yamada et al. 1998; Vander Zanden et al. 1999; Post et al. 2000; Ogawa et al. 2001; Post 2002; Maki et al. 2010). The natural abundances of carbon-13 (^{13}C) and nitrogen-15 (^{15}N) are expressed as per mil (‰) deviations from international standards: $\delta^{13}C$ (‰) or $\delta^{15}N$ (‰) $= (R_{sample} / R_{standard} - 1) \times 10^3$, where R is $^{13}C/^{12}C$ for $\delta^{13}C$ or $^{15}N/^{14}N$ for $\delta^{15}N$, respectively. Vienna Peedee belemnite (VPDB) and atmospheric nitrogen (AIR) were used as international standards for carbon and nitrogen, respectively (Coplen 1994; Böhlke and Coplen 1995). A short history of selecting standards is described in Coplen et al. (2002). Recently, Coplen et al. (2006) recommended that $\delta^{13}C$ values of both organic and inorganic materials measured by an isotope ratio mass spectrometer (IRMS) should be corrected and expressed relative to VPDB on a scale normalized by assigning consensus values of -46.6‰ to L-SVEC lithium carbonate and +1.95‰ to NBS19 calcium carbonate. For nitrogen, two-point calibration with IAEA-N-1 ammonium sulfate (+0.43‰) and IAEA-N-2 ammonium sulfate (+20.41‰) or IAEA-N-1 ammonium sulfate and USGS32 potassium nitrate (+180‰) should be applied (Böhlke and Coplen 1995).

The aim of this paper is to prepare certified reference materials of organic compounds in the Japanese community of stable isotope science based on the currently approved correction method of two-point anchoring. The multiple certified reference materials are useful in comparing carbon and nitrogen isotope ratios measured by different laboratories. The Center for Ecological Research, Kyoto University is approved as a Joint Usage / Research Center by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan, and the reference materials (CERKU-01 to CERKU-10) are available on request. BG-A, BG-P and BG-T are also available from the Institute of Biogeosciences (BioGeos), JAMSTEC on request.

Materials and Methods

In the CER, Kyoto University, special-grade reagents (Wako Co. Ltd.) of DL-Alanine ($C_3H_7NO_2$), L-Alanine ($C_3H_7NO_2$), Glycine ($C_2H_5NO_2$), L-Leucine ($C_6H_{13}NO_2$), L-Threonine ($C_4H_9NO_3$), starch-C3 (($C_6H_{10}O_5)_n$), starch-C4 (($C_6H_{10}O_5)_n$), DL-Alanine ($C_3H_7NO_2$) and L-Tyrosine ($C_9H_{11}NO_3$), and regent for peptide (Kokusan Chemical Co. Ltd.) of L-Alanine ($C_3H_7NO_2$) were purchased, and called as CERKU-01 to CERKU-10, respectively. CERKU-8 and CERKU-9 have previously been used as internal standards in the CER. They were purchased in 500-g bottle and manually well mixed before isotope measurement. CERKU-02 was prepared by dissolving 500 g of L-Alanine (Special grade reagents, Wako Co. Ltd.) and 40 mg ^{15}N-98%-L-Alanine (NLM-454, Cambridge Isotope Labs, USA) in 5 l deionized water. The solution was lyophilized and well mixed with a large bowl and pestle to obtain fine powder. In the BioGeos, JAMSTEC, reference materials called as BG-A, BG-P, and BG-T also represents analytical grade reagents (Aldrich Chemical Co., Inc) of L-Alanine ($C_3H_7NO_2$), L-Proline ($C_5H_9NO_2$) and L-Tyrosine ($C_9H_{11}NO_3$), respectively.

Materials of BG-A and BG-P were carefully pulverized before use.

In this study, to calibrate $\delta^{13}C$ values, international isotope standards NBS19 and L-SVEC were used, while IAEA-N-1, IAEA-N-2 and USGS32 were used for calibrating $\delta^{15}N$ values. In addition, IAEA-CH-3 cellulose (($C_6H_{10}O_5$)$_n$), IAEA-CH-6 sucrose ($C_{12}H_{22}O_{11}$), IAEA-CH-7 polyethylene ((CH_2)$_n$) and USGS40, USGS41 L-Glutamic acid ($C_5H_9NO_4$) (Qi et al. 2003) were prepared.

Stable isotope measurements were preformed in three laboratories, CER, Kyoto University, BioGeos, JAMSTEC, and Tokyo Institute of Technology. In the CER, samples were analyzed using an isotope ratio mass spectrometer (Finnigan MAT Delta S, Germany) coupled with an elemental analyzer (Fisons EA1108, Italy) *via* Conflo II as an interface. The combustion temperature was set to 1020°C and the reduction furnace was kept at 650°C (Qi et al. 2003). One set of measurements was performed after introducing new combustion and reduction tubes until the exhaustion. Reference materials were folded into tin capsules containing carbon and nitrogen equivalent to 1mg alanine (404 µgC for $\delta^{13}C$ analysis and 157 µgN for $\delta^{15}N$ analysis) within 10% variation of each amount. They were supplied to sequential dual isotope analysis with dilution mode for $\delta^{13}C$ but undiluted mode for $\delta^{15}N$. Samples for $\delta^{13}C$ and $\delta^{15}N$ analysis were prepared separately except for alanine.

Samples were analyzed sequentially in the order CERKU-01, CERKU-02, CERKU-03 for C, CERKU-03 for N, CERKU-04 for C, CERKU-04 for N, CERKU-05 for C, CERKU-05 for N, CERKU-06, CERKU-07, IAEA-N-1, USGS40 for C, USGS40 for N, USGS41 for C, USGS41 for N, IAEA-CH-3, IAEA-CH-6, IAEA-N-1, IAEA-N-2, L-SVEC, NBS19, USGS32, CERKU-08, CERKU-09 for C, CERKU-09 for N, CERKU-10, BG-A, BG-P for C, BG-P for N, BG-T for C and BG-T for N. This order was then repeated backward. Additional replicated analyses were performed forward and backward of the sequence by turns. In some sequences, several kinds of reference materials were not analyzed. A blank consisting of an empty 8 x 5 mm tin capsule was analyzed before the sequence, however, the amount of blank generally was so small compared to the sample peak that blank correction was not applied in the CER. All data were averaged and two-point linear calibration of NBS19 ($\delta^{13}C$ = 1.95‰) and L-SVEC ($\delta^{13}C$ = -46.6‰) (#1-#5) was applied for $\delta^{13}C$ (Coplen et al. 2006) and two-point linear calibration of IAEA-N-1 ($\delta^{15}N$ = 0.43‰) and IAEA-N-2 ($\delta^{15}N$ = 20.41‰) (#1-#3) or that of IAEA-N-1 ($\delta^{15}N$ = 0.43‰) and USGS32 ($\delta^{15}N$ = 180‰) (#4, 5) was applied for $\delta^{15}N$ (Böhlke and Coplen 1995). The slope of linear regression of measured and trued-values of two certified reference standards were calculated in the sequences #1-#5.

At BioGeos JAMSTEC, we used a Delta-plus XP isotope ratio mass spectrometer (Thermo Finnigan, Germany) coupled to an elemental analyzer (Flash EA 1112, Thermo Finnigan, Germany) *via* ConFlo III interface with modifications to improve sensitivity (Ogawa et al., 2010). Temperatures of combustion and reduction furnaces were set to 1050°C and 750°C, respectively. Sample was carefully prepared to contain 12 ± 1 µgC for $\delta^{13}C$ or 3.1 ± 0.3 µgN for $\delta^{15}N$, that are equivalent to IRMS intensity of 15 ± 2 V (*m/z* 44) or 3.0 ± 0.5 V (*m/z* 28), respectively. Blank collection was performed to each raw data to offset isotope effect from background contamination of carbon and nitrogen (Ogawa et al., 2010) before further calculation and calibration. All data were averaged and two-point linear calibration of NBS19 ($\delta^{13}C$ = 1.95‰) and L-SVEC ($\delta^{13}C$ = -46.6‰)

was applied for $\delta^{13}C$ (Coplen et al. 2006) and two-point linear calibration of IAEA-N-1 ($\delta^{15}N$ = 0.43‰) and IAEA-N-2 ($\delta^{15}N$ = 20.41‰) for $\delta^{15}N$.

In the Tokyo Institute of Technology (TokyoTech), samples were analyzed using a conventional off-line method consisted of sealed tube combustion followed by cryogenic separation of the resulting CO_2 and N_2 using a high vacuum line system and IRMS measurement. Briefly, ~2.5 mg of sample was combusted in an evacuated and sealed quartz tube with 1.8 g CuO as the oxidant and 0.8 g Cu as the reductant at 850°C for 2 h. The sample tube was attached to the line system with a glass tube cracker and then cooled to −196°C for 5 min. The sample tube was then broken and the released N_2 was transferred to a 6-mm i.d. Pyrex® glass tube containing three pellets of molecular sieves 5A (1/16 Pellets, Wako Co. Ltd.) attached to the line system at −196°C. The Pyrex® glass tube was then flame sealed. Subsequently, the sample tube was heated to −78°C for 5 min. The released CO_2 was transferred to a 6-mm i.d. Pyrex® glass tube attached to the line system at −196°C. The Pyrex® glass tube was then flame sealed. Measurements of $\delta^{13}C$ and $\delta^{15}N$ for the resulting CO_2 and N_2 gases in the Pyrex® glass tubes were made using an isotope ratio mass spectrometer (Finnigan MAT 252, Germany, and Delta-plus XP, USA) with a dual inlet system. In the TokyoTech, isotopic standardization for $\delta^{13}C$ against VPDB was accomplished by a two-point linear calibration of IAEA-CH-6 ($\delta^{13}C$ = -10.45‰) and IAEA-CH-7 ($\delta^{13}C$ = -32.15‰) instead of NBS19 and L-SVEC. For $\delta^{15}N$, isotopic standardization was accomplished by comparison with reference N_2 gas (99.9999%; Taiyo Nippon Sanso Corp.), which was calibrated against AIR using the international nitrogen isotope standard N-SVEC ($\delta^{15}N$ = -2.78‰).

Results and Discussion

Table 1 shows the $\delta^{13}C$ values of reference materials based on the two-point linear calibration. Two-point anchoring has repeatedly been recommended by many authors (e.g. Coplen et al. 2006; Paul et al. 2007). In this study, $\delta^{13}C$ values of 13 analytical grade reagents were carefully determined by the two-point linear calibration of NBS19 and L-SVEC. The calculated $\delta^{13}C$ values of other international standards including IAEA-CH-6, IAEA-CH-3, and USGS40 were well fitted to the literature values within 0.04‰, although USGS41 was slightly (0.19‰) lower than the accepted value. This is consistent with Skrzypek et al. (2010), who have shown that a higher mean error was observed outside the range bracketed by the δ-values of the anchor standards (L-SVEC and NBS19).

Table 2 shows the $\delta^{15}N$ values of reference materials based of the two-point linear calibration. $\delta^{15}N$ values of 13 analytical grade reagents were determined by the two-point linear calibration of IAEA-N-1 and IAEA-N-2 (#1-#3), or IAEA-N-1 and USGS32 (#4). Similar to the $\delta^{13}C$ values, the calculated $\delta^{15}N$ values of additional international standards (USGS41, USGS40 and IAEA-N-2 (#4)) were well fitted to the literature values within 0.03‰.

In the studies of limnology and ecology, stable isotope ratios are often used for investigating nutrient dynamics and food web structure. In such cases, stable isotopic comparisons among study subjects are appropriate, whether they are within-ecosystem or between-ecosystem, if their calculated values are based on the analysis with high analytical precision. However, the isotopic composition is expressed as a relative value to the international standard, so that accuracy is

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important for the quality of inter-laboratory comparisons, such as meta-analysis based on many papers. Coplen et al. (2006) recommended that $\delta^{13}C$ values measured by an IRMS should be anchored at two-point linear calibration of NBS19 ($\delta^{13}C = 1.95‰$) and L-SVEC ($\delta^{13}C = -46.6‰$). They concluded that $\delta^{13}C$ value of the international standard NBS22 oil should be replaced by -30.03‰ (Coplen et al. 2006). In this paper, we determined $\delta^{13}C$ and $\delta^{15}N$ values of reference materials by two-point anchoring and confirmed with several international standards. The slope of correlation between measured and true values of two-point anchors in carbon and nitrogen isotope ratio analysis of #1-#5 was 1.0061 ± 0.0009 (1.0047 − 1.0071) and 1.0006 ± 0.0014 (0.9988 − 1.0022) [mean ± SD with the ranges in parenthesis], respectively. However, the range of slope values varied within 1% of 1:1 line in the delta S system in the CER, concluding that single-point anchoring standardization causes little error in the natural abundance level in this system.

In summary, we determined carbon and nitrogen isotope ratios of 13 reference materials based on the two-point anchoring method. The reference materials cover $\delta^{13}C_{VPDB}$ range of -34.92 to -9.45‰ and $\delta^{15}N_{Air}$ range of -5.22 to 22.71‰, thus can be useful for measuring isotope ratios of naturally occurring substances.

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References

Böhlke JK, Coplen TB (1995) Interlaboratory comparison of reference materials for nitrogen-isotope-ratio measurements. In: International Atomic Energy Agency (ed) Reference and intercomparison materials for stable isotopes of light elements (IAEATECDOC-825), IAEA, Vienna, pp.51-66

Cabana G, Rasmussen JB (1996) Comparison of aquatic food chains using nitrogen isotopes. Proc Nat Acad Sci USA 93: 10844-10847

Coplen TB (1994) Reporting of stable hydrogen, carbon, and oxygen isotopic abundances. Pure Appl Chem 66: 273-276

Coplen TB, Böhlke JK, De Bièvre P, Ding T, Holden NE, Hopple JA, Krouse HR, Lamberty A, Peiser HS, Révész K, Rieder SE, Rosman KJR, Roth E, Taylor PDP, Vocke Jr RD Xiao YK (2002) Isotope-abundance variations of selected elements (IUPAC Technical Report). Pure Appl Chem 74:1987–2017

Coplen TB, Brand WA, Gehre M, Gröning M, Meijer HAJ, Toman B, Verkouteren RM (2006) New guidelines for $\delta^{13}C$ measurements. Anal Chem 78: 2439-2441

Doi H, Takemon Y, Ohta, T, Ishida Y, Kikuchi E (2007) Effects of reach-scale canopy cover on trophic pathways of caddisfly larvae in a Japanese mountain stream. Mar Freshwater Res 58: 811-817

Finlay JC (2004) Patterns and controls of lotic algal stable carbon isotope ratios. Limnol Oceanogr

49: 850-861

Fry B (2006) Stable isotope ecology. Springer, New York

Kohzu A, Tayasu I, Yoshimizu C, Maruyama A, Kohmatsu Y, Hyodo F, Onoda Y, Igeta A, Matsui K, Nakano T, Wada E, Nagata T, Takemon Y (2009) Nitrogen-stable isotopic signatures of basal food items, primary consumers and omnivores in rivers with different levels of human impact. Ecol Res 24: 127-136

Maki K, Kim C, Yoshimizu C, Tayasu I, Miyajima T, Nagata T (2010) Autochthonous origin of semi-labile dissolved organic carbon in a large monomictic lake (Lake Biwa): carbon stable isotopic evidence. Limnology 11: 143-153

Michener RM, Lajtha K (eds) (2007) Stable isotopes in ecology and environmental science. Blackwell Scientific, Oxford

Ogawa NO, Koitabashi T, Oda H, Nakamura T, Ohkouchi N, Wada E (2001) Fluctuations of nitrogen isotope ratio of gobiid fish (Isaza) specimens and sediments in Lake Biwa, Japan, during the 20th century. Limnol Oceanogr 46: 1228-1236

Ogawa NO, Nagata T, Kitazato H, Ohkouchi N (2010) Ultra-sensitive elemental analyzer/isotope ratio mass spectrometer for stable nitrogen and carbon isotope analyses. In: Ohkouchi N, Tayasu I, Koba K (eds) Earth, Life, and Isotopes. Kyoto University Press, Kyoto, pp 339-353

Ohkouchi N, Tayasu I, Koba K (eds) Earth, Life, and Isotopes. Kyoto University Press, Kyoto

Paul D, Skrzypek G, Fórizs I (2007) Normalization of measured stable isotopic compositions to isotope reference scales – a review. Rapid Commun Mass Spectrom 21: 3006–3014

Post DM (2002) Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology 83: 703-718

Post DM, Pace ML, Hairston NG (2000) Ecosystem size determines food-chain length in lakes. Nature 405: 1047-1049

Qi H, Coplen TB, Geilmann H, Brand WA, Böhlke JK (2003) Two new organic reference materials for δ^{13}C and δ^{15}N measurements and a new value for the δ^{13}C of NBS 22 oil. Rapid Commun Mass Spectrom 17: 2483–2487

Skrzypek G, Sadler R, Paul D (2010) Error propagation in normalization of stable isotope data: a Monte Carlo analysis. Rapid Commun Mass Spectrom 24: 2697-2705

Vander Zanden MJ, Casselman JM, Rasmussen JB (1999) Stable isotope evidence for the food web consequences of species invasions in lakes. Nature 401: 464-467

Wada E (2009) Stable δ^{15}N and δ^{13}C isotope ratios in aquatic ecosystems. Proc Jpn Acad Ser B 85: 98-107

Yamada Y, Ueda T, Koitabashi T, Wada E (1998) Horizontal and vertical isotopic model of Lake Biwa ecosystem. Jap J Limnol 59: 409-427

Yamada Y, Ueda T, Wada E (1996) Distribution of carbon and nitrogen isotope ratios in the Yodo River watershed. Jpn J Limnol 57: 467-477

Table 1 $\delta^{13}C_{VPDB}$ values corrected by two-point calibration indicated by asterisk

ID[a]	Substrate	Literature value[b]	#1 Corrected value	SD	n	#2 Corrected value	SD	n	#3 Corrected value	SD	n	#4 Corrected value	SD	n	#5 Corrected value	SD	n	#6 Corrected value	SD	n	#7 Corrected value[c]	SD	n	Average	SD	n
USGS41	L-Glutamic Acid	37.63	37.37	0.08	5	37.62	0.10	12				37.41	0.08	7	37.43	0.12	12	37.43	0.15	5	37.35	0.13	3	37.44	0.09	6
NBS19	Calcium carbonate	1.95	1.95 *	0.05	5	1.95 *	0.08	9	1.95 *	0.04	16	1.95 *	0.04	7	1.95 *	0.08	11	1.95 *	0.07	5				1.95	0.05	6
CERKU-05	L-Threonine		-9.40	0.04	5	-9.42	0.05	12				-9.44	0.07	8	-9.42	0.08	12	-9.53	0.09	5	-9.46	0.01	3	-9.45	0.04	6
BG-P	L-Proline											-10.30	0.04	4	-10.28	0.06	7	-10.23	0.07	5				-10.27	0.04	3
IAEA-CH-6	Sucrose	-10.45	-10.42	0.03	5	-10.41	0.08	12				-10.43	0.05	8	-10.46	0.10	12	-10.44	0.13	6	-10.45	0.00	3	-10.43	0.02	6
CERKU-07	Starch-C4					-10.79	0.05	12				-10.78	0.06	8	-10.71	0.11	12							-10.76	0.04	3
CERKU-02	L-Alanine								-19.00	0.07	16	-19.09	0.05	8	-19.01	0.12	12				-19.05	0.01	3	-19.04	0.04	4
BG-T	L-Tyrosine														-20.87	0.06	8	-20.72	0.08	6	-20.91	0.03	3	-20.83	0.10	3
CERKU-10	L-Alanine					-21.92	0.06	4				-21.92	0.07	8	-21.87	0.10	12	-21.72	0.10	6	-21.91	0.01	4	-21.87	0.09	5
CERKU-08	DL-Alanine		-23.80	0.06	5	-23.79	0.04	12	-23.79	0.08	16	-23.81	0.07	8	-23.81	0.07	12	-23.72	0.09	6	-23.82	0.01	4	-23.79	0.03	7
CERKU-06	Starch-C3					-24.01	0.05	12				-24.05	0.07	8	-23.94	0.06	12							-24.00	0.06	3
IAEA-CH-3	Cellulose	-24.72	-24.75	0.04	5	-24.75	0.05	12				-24.79	0.07	8	-24.76	0.10	12							-24.76	0.02	4
CERKU-01	DL-Alanine					-25.33	0.03	12	-25.30	0.05	16	-25.35	0.09	8	-25.34	0.13	12	-25.51	0.06	5	-25.36	0.02	3	-25.36	0.08	6
USGS40	L-Glutamic Acid	-26.39	-26.37	0.04	5	-26.37	0.06	12				-26.39	0.05	8	-26.36	0.12	12	-26.33	0.07	6	-26.38	0.01	3	-26.36	0.02	6
CERKU-09	L-Tyrosine		-26.60	0.05	5	-26.56	0.05	12				-26.63	0.05	8	-26.54	0.07	12							-26.58	0.04	4
BG-A	L-Alanine											-26.89	0.02	4	-26.80	0.09	8	-26.88	0.06	5	-26.85	0.01	5	-26.86	0.04	4
CERKU-04	L-Leucine		-29.07	0.09	5	-29.04	0.07	12				-29.10	0.08	8	-29.05	0.13	12							-29.06	0.03	4
CERKU-03	Glycine		-34.93	0.04	5	-34.96	0.03	11				-34.98	0.06	8	-34.82	0.11	12							-34.92	0.07	4
L-SVEC	Lithium carbonate	-46.6	-46.60 *	0.04	5	-46.60 *	0.14	10	-46.60 *	0.06	16	-46.60 *	0.08	5	-46.60 *	0.07	11	-46.60 *	0.16	5				-46.60		6

#1-#5 were measured in the Center for Ecological Research (CER), Kyoto University; #6 was measured in the BioGeos, Japan Agency for Marine-Earth Science and Technology (JAMSTEC); #7 was measured in the Department of Environmental Chemistry and Engineering, Tokyo Institute of Technology

ID identification, SD standard deviation

[a]Reference materials were arranged in descending order of isotope ratios.

[b]Coplen et al (2006)

[c]Two-point linear calibration of IAEA-CH-6 and IAEA-CH-7

Table 2 $\delta^{15}N_{air}$ values corrected by two-point calibration indicated by asterisk

ID[a]	Substrate	Literature value	#1 Corrected value	SD	n	#2 Corrected value	SD	n	#3 Corrected value	SD	n	#4 Corrected value	SD	n	#5 Corrected value	SD	n	#6 Corrected value	SD	n	#7 Corrected value[d]	SD	n	Average	SD	n
USGS32	Potassium nitrate	180[b]										180.00 *	0.13	12	180.00 *	0.10	12							180.00		2
USGS41	L-Glutamic Acid	47.57[c]	47.68	0.02	5	47.64	0.06	16				47.51	0.06	12	47.50	0.05	12	47.52	0.12	6				47.57	0.08	5
CERKU-02	L-Alanine								22.80	0.10	16	22.72	0.11	12	22.65	0.10	12	22.67	0.08	6				22.71	0.06	4
IAEA-N-2	Ammonium sulfate	20.41[b]	20.41 *	0.05	5	20.41 *	0.09	15	20.41 *	0.09	16	20.40	0.10	12	20.37	0.08	12	20.41 *	0.16	6				20.40		6
BG-P	L-Proline											13.50	0.07	8	13.49	0.08	8	13.53	0.05	6				13.51	0.02	3
CERKU-04	L-Leucine		8.86	0.04	5	8.72	0.08	16				8.75	0.09	12	8.72	0.12	12							8.76	0.07	4
BG-T	L-Tyrosine											8.75	0.18	8	8.83	0.21	8	8.65	0.33	6				8.74	0.09	3
CERKU-09	L-Tyrosine		5.63	0.08	5	5.59	0.05	16				5.60	0.07	12	5.56	0.08	12							5.60	0.03	4
CERKU-03	Glycine		2.23	0.05	5	2.19	0.06	16				2.18	0.07	12	2.12	0.11	12							2.18	0.04	4
IAEA-N-1	Ammonium sulfate	0.43[b]	0.43 *	0.05	5	0.43 *	0.09	16	0.43 *	0.08	15	0.43 *	0.08	12	0.43 *	0.09	12	0.43 *	0.10	4				0.43		6
CERKU-08	DL-Alanine		-1.63	0.11	5	-1.62	0.06	16	-1.59	0.07	16	-1.58	0.10	11	-1.69	0.06	8	-1.53	0.10	5	-1.74	0.07	4	-1.61	0.07	6
BG-A	L-Alanine											-1.67	0.07	8				-1.58	0.06	6				-1.65	0.06	3
CERKU-05	L-Threonine		-2.88	0.13	5	-2.89	0.05	16				-2.87	0.06	12	-2.88	0.06	12							-2.88	0.01	4
CERKU-01	DL-Alanine					-2.88	0.06	16	-2.83	0.05	16	-2.86	0.07	12	-2.91	0.07	12	-2.94	0.06	6				-2.89	0.04	5
USGS40	L-Glutamic Acid	-4.52[c]	-4.57	0.04	5	-4.62	0.06	16				-4.53	0.06	12	-4.60	0.07	12	-4.44	0.09	5				-4.55	0.07	5
CERKU-10	L-Alanine					-5.19	0.06	8				-5.19	0.05	12	-5.21	0.10	12	-5.22	0.06	4	-5.31	0.08	4	-5.22	0.05	5

#1–#5 were measured in the Center for Ecological Research (CER), Kyoto University; #6 was measured in the BioGeos, Japan Agency for Marine-Earth Science and Technology (JAMSTEC); #7 was measured in the Department of Environmental Chemistry and Engineering, Tokyo Institute of Technology

ID identification, SD standard deviation

[a]Reference materials were arranged in descending order of isotope ratios.

[b]Böhlke and Coplen (1995)

[c]Qi et al. (2003)

[d]See text for the correction method