Res. Org. Geochem. **28,** 13−17 (2012)

Article

Stable nitrogen isotopic fractionation associated with transamination of glutamic acid to aspartic acid: implications for understanding ^{15}N trophic enrichment in ecological food webs

Kasumi Miura* and Akiko S. Goto*,**,a

(Received October 14, 2012; Accepted December 14, 2012)

Abstract

The stable nitrogen isotope ratio of glutamic acid is increased by ～8.0‰ during trophic level changes in ecological food webs. It has been hypothesized that significant isotopic fractionation occurs associated with glutamic acid metabolism during transamination or deamination in heterotrophic animals. However, few studies have investigated the isotopic fractionation factor (α) in this process, even though knowledge of this factor is critical in quantitatively evaluating the flux of amino acid metabolism in animals. In this study we determined the value of α associated with an enzymatic transamination of glutamic acid to aspartic acid *in vitro*. We found 0.1‰ and 2.2‰ enrichment of ^{15}N following the enzymatic transamination of glutamic acid for 10 and 60 min, respectively. Using a Rayleigh model, the α value in this reaction was estimated to be 0.9958. Although this value is associated with much uncertainty (0.9949−0.9968) when applying it to test for an 8.0‰ enrichment, 8‰ of ^{15}N-enrichment for a α value of 0.9958 corresponds to 86% of the glutamic acid being reacted during transamination.

Key words: glutamic acid, transamination, isotopic fractionation, food web

1. Introduction

The stable nitrogen isotope ratio of amino acids has attracted recent attention as a useful tool for accurately determining the trophic position of heterotrophic organisms in ecological food webs. Amino acids are the basic subunits of protein and form a major pool of nitrogen in animals. Consequently, the ^{15}N-enrichment of amino acids in food webs can provide important information including the baseline isotope ratio and the trophic position of individual organisms in the studied food webs (e.g. McCarthy et al., 2007; Popp et al., 2007; Chikaraishi et al., 2009a). This is because the isotope ratio ($\delta^{15}N$) of glutamic acid increases by ～8.0‰ with a shift to a higher trophic level, while that of phenylalanine increases by only ～0.4‰ (Chikaraishi et al., 2009a).

Isotopic fractionation is generally defined using the isotopic fractionation factor (α) and the kinetic flux in an enzymatic reaction, whereby the flux can be calculated when α is known. Thus, it is possible to estimate the flux during amino acid metabolism if the α values in the corresponding enzymatic reactions are known.

Chikaraishi et al. (2007) recently suggested that the ^{15}N-enrichment in some amino acids (particularly the approximately 8‰ enrichment in glutamic acid) results

*Department of Chemistry, Tokyo Metropolitan University, 1-1 Minami-Osawa, Hachioji, Tokyo 192-0397 Japan
**Present address: Institute of Science and Engineering, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan
[a]Corresponding author. e-mail: akigoto@staff.kanazawa-u.ac.jp Tel: +81-76-264-6513; Fax: +81-76-264-6545

from isotopic fractionation during transamination (or deamination) because the C−N bond in the amino acid is cleaved. More than two decade ago, in a study of ^{15}N-enrichment (～3.4‰) of bulk animals in food webs, Macko et al. (1986) reported an α value of 0.9923 for the enzymatic transamination of glutamic acid to aspartic acid. Based on this α value, the 8‰ ^{15}N-enrichment that is commonly observed during glutamic acid transfer from diets to animals (Chikaraishi et al., 2009a) corresponds to approximately 64% of the glutamic acid being metabolized for the production of energy and the other ～36% being used for the construction of animal tissues (e.g. muscle), assuming that transamination only involves glutamic acid during animal metabolism. However, except for Macko et al. (1986), no information is available on the α values for enzymatic reactions related to amino acid metabolism (e.g. transamination and deamination), and this prevents a fundamental understanding of isotopic fractionation and associated fluxes during animal metabolism, and in each step of the trophic levels in food webs. Knowledge of α is thus a critical issue in physiological and ecological studies.

In this study we determined the α value for an enzymatic transamination of glutamic acid to aspartic acid *in vitro*, enabling comparison with the value reported by Macko et al. (1986). However, Macko et al. (1986) used an off-line method (dual-inlet stable isotope analysis for the glutamic acid collected using cation exchange column chromatography) to obtain the α value, whereas in this study we used an on-line method (compound-specific stable isotope analysis with on-line chromatographic separation of glutamic acid by gas chromatography). Comparison of the two methods is important because the former method potentially has a risk for much uncertainty resulting from: (1) non-quantitative purification of the amino acids of interest from the sample; and (2) isotopic modification because of the potential presence of impurities such as co-eluted chemicals and column chromatography resins. These problems, associated with the off-line method, are thought to be negligible in the on-line method used in this study.

2. Methods

Glutamic acid (Tokyo Chemical Industry Co. Ltd.; 99%), oxaloacetic acid (Wako Pure Chemical Industries Ltd.; 95%), and glutamic-oxaloacetic transaminase (GOT: Sigma-Aldrich Co. EC 2.6.1.1; from porcine heart; 434 units/mg) were used in the study. The stable nitrogen isotope ratio (δ^{15}N; relative to AIR: atmospheric nitrogen) of glutamic acid was determined by conventional elemental analyzer/isotope ratio mass spectrometry (EA/IRMS) using a Thermo Fisher Scientific Flash EA (1112HT) coupled to a Delta V Advantage IRMS by a ConFlo Ⅲ interface. The standard deviation (1σ) of isotope measurements was better than 0.3‰. Glutamic acid (30 mg) and oxaloacetic acid (180 mg) were dissolved in 120 ml of phosphate buffer at pH 7.6 together with the GOT enzyme (2 μl). In this reaction the amino group in glutamic acid is removed (glutamic acid is converted to α-ketoglutaric acid) and transferred to oxaloacetic acid to form aspartic acid. Glutamic acid was reacted with the transaminase for 10 or 60 min at a constant temperature at 37℃, at which time the enzyme in solution was denatured by the addition of a few ml of concentrated (12N) hydrochloric acid.

The remaining glutamic acid and the aspartic acid formed were derivatized to pivaloyl/isopropyl esters for isotope ratio measurement according to the method described by Chikaraishi et al. (2009b). The esterification process was repeated twice to achieve complete esterification of the amino acids. The stable nitrogen isotope ratios of glutamic acid and aspartic acid were determined by gas chromatography/isotope ratio mass spectrometry (GC/IRMS) using a Delta XP IRMS interfaced with a gas chromatograph through a GC-C Ⅲ interface (Thermo Fisher Scientific). The standard deviation (1σ) of the isotope measurement was better than 0.5‰ in this study. We also determined the transamination ratio from the conversion of glutamic acid to aspartic acid by comparison of the *m/z* 28 peak areas for glutamic acid in the GC/IRMS chromatograms prior to and following transamination. The quantification accuracy was approximately 10% in replicate analyses.

3. Result and Discussions

3.1. Isotopic fractionation factor

The $\delta^{15}N$ value for glutamic acid prior to the commencement of transamination was −5.1‰; this increased by 0.1‰ after transamination for 10 min, and by 2.2‰ after 60 min (Table 1). Although the magnitude of the ^{15}N-enrichment was somewhat different from that reported by Macko et al. (1986) (see below), the trend of change in the $\delta^{15}N$ value (i.e. ^{15}N-enrichment) during transamination was consistent between the two studies.

To calculate the α value we used a Rayleigh model defined by Equation (1):

$$\delta^{15}N_{Glu\_t} = (1000 + \delta^{15}N_{Glu\_o}) \times F^{(\alpha-1)} - 1000 \quad (1)$$

where $\delta^{15}N_{Glu\_t}$ is the isotope ratio of the glutamic acid remaining following enzymatic transamination for 10 min (−5.0‰) or 60 min (−2.9‰), $\delta^{15}N_{Glu\_o}$ is the initial isotope ratio for glutamic acid (−5.1‰), and F is the transamination ratio of the glutamic acid remaining according to Equation 2:

$$F = (C_t / C_o\ (\%)) / 100 \quad (2)$$

where C_o and C_t are the abundances of glutamic acid prior to and following transamination, respectively. Thus Equation (3) can be derived from Equation (1) as follows:

$$\ln[(1000 + \delta^{15}N_{Glu\_t})/(1000 + \delta^{15}N_{Glu\_o})] = (\alpha-1) \times \ln F \quad (3)$$

A plot of $\ln[(1000 + \delta^{15}N_{Glu\_t})/(1000 + \delta^{15}N_{Glu\_o})]$ *vs.* $\ln F$ should give a straight line of slope $(\alpha-1)$, meaning that the α value can be determined from the slope.

As shown in Fig. 1, a liner relationship between $\ln[(1000 + \delta^{15}N_{Glu\_t})/(1000 + \delta^{15}N_{Glu\_o})]$ and $\ln F$ was found in this study. The resulting value of $(\alpha-1)$ was −0.0042, giving a value for $\alpha = 0.9958$. As this α value is <1.0, it indicates that the lighter isotope (^{14}N) was preferentially removed from glutamic acid during the transamination to form of aspartic acid, whereas the heavier isotope (^{15}N) was concentrated in the remaining glutamic acid. This result is consistent with an ～8.0‰ change in the isotope ratio ($\delta^{15}N$) of glutamic acid during passage to one trophic level higher. Based on the use of $\alpha = 0.9958$ and the assumption of 8‰ ^{15}N enrichment in glutamic acid (i.e. $\delta^{15}N_{Glu\_t} = +2.9$‰ and $\delta^{15}N_{Glu\_o} = -5.1$‰) in equation (1), the calculated F value is = 0.14: this indicates that 86% of the glutamic acid was reacted during transamination, while 14% remained unreacted.

However, if the α value (0.9923) calculated by Macko et al. (1986) is used in the above calculation, an F value of 0.36 is obtained, indicating that 64% of the glutamic acid is reacted during transamination and 36% is unreacted. The method used for isotope analysis in this study avoided the potential problems associated with the off-line method used by Macko et al. (1986), including isotopic modification and contamination. The

Table 1. The proportion and $\delta^{15}N$ change of remaining glutamic acid in transamination.

	0 min	10 min	60 min
C_t / C_o (%)	1	99.6	58.8
$\delta^{15}N_{Glu}$ (‰)	−5.1	−5.0	−2.9
$\delta^{15}N_{Asp}$ (‰)	n.d.	n.d.	−9.3
$\delta^{15}N_{Glu\_t} - \delta^{15}N_{Glu\_o}$ (‰)	0	0.1	2.2

C_o and C_t are abundance of glutamic acid before and after transamination. $\delta^{15}N_{Glu\_t} - \delta^{15}N_{Glu\_o}$ is the $\delta^{15}N$ difference of glutamic acid between before and after transamination.

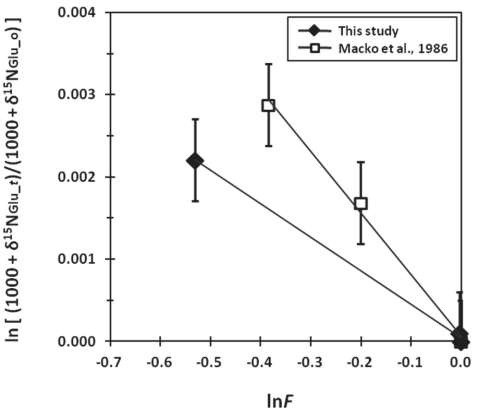

Fig. 1. A correlation line of $\ln[(1000+\delta^{15}N_{Glu\_t})/(1000+\delta^{15}N_{Glu\_o})]$ *vs.* $\ln F$ for the determination of isotope fractionation factor (α) using a Rayleigh model. Each slope $(\alpha-1)$ is −0.0042 (this study) and −0.0077 (Macko et al., 1986), respectively.

difference in the α values between this study and that of Macko et al. (1986) may have been a consequence of the different methods used in the two studies. However, we were unable to evaluate whether the difference was significant because of the large uncertainties associated with the in α values in each study. In particular, the levels of enrichment found by Macko et al. (1986) and in this study (2.9‰ and 2.2‰, respectively) were much less than is commonly reported (e.g. 8‰) for food webs. In addition, when the analytical error in the isotope analysis (0.5‰) is taken into account, the α value in this study includes much uncertainty (0.9949−0.9968), which corresponds to a large potential variation in F (from 7.6% to 20%) relative to an 8‰ enrichment level. Thus, future studies should investigate changes in the δ^{15}N value at higher transamination ratios than used to date, which will reduce uncertainties in calculation of the isotopic fractionation factor associated with this transamination process.

3.2. Implication

Lindeman (1942) proposed the "ten percent law" for the transfer of energy from one trophic level to the next. According to this law only ～10% of the energy in one trophic level is stored as organic matter in the next level, and the remaining ～90% is lost during transfer or is broken down in respiration. Thus, the biomass size is decreased by a factor of 10 with each transfer to a higher trophic level in the food web. The ratio of the biomass size (or net production) between one level and the next highest level is termed the conversion efficiency. A number of studies have evaluated the conversion efficiency using biomass information (e.g. size and population of individuals) and models (e.g. Lindeman, 1942; Pauly & Christensen, 1995; Lin et al., 2004; Das & Chakrabarty, 2006). However, accurate estimation of this parameter requires long-term measurements of biomass, which may limit the representative food webs available for such studies.

An understanding of isotopic fractionation in amino acids in food webs may help overcome this limitation. An 8.0‰ level of enrichment of ^{15}N during glutamic acid metabolism between diet and consumer has been reported for various trophic relationships (e.g. phytoplankton and zooplankton, zooplankton and fish, plant leaves and caterpillars; Chikaraishi et al., 2009a, 2011), implying that the isotopic fractionation factor and flux during glutamic acid metabolism is common to a range of organisms including herbivores, omnivores, and carnivores.

In this study we estimated the isotopic fractionation factor associated with enzymatic transamination of glutamic acid to aspartic acid *in vitro*, and obtained a value of $\alpha = 0.9958$. When these results are related to the 8‰ ^{15}N enrichment during glutamic acid metabolism, it suggests that 86% of the glutamic acid was reacted during transamination, while 14‰ remained unreacted. This implies that 8‰ of the ^{15}N-enrichment corresponds to 86% of the glutamic acid being metabolized for energy production and 14% used for the construction of animal tissues, assuming that transamination of only glutamic acid occurs in the animals. If the amount of glutamic acid controls the biomass of individual members in food webs, the implication is that the biomass is decreased by 86% at each higher trophic level in the food webs. To enable accurate conversion efficiencies in food webs to be established it will be necessary to investigate and integrate all α values for individual metabolic reactions that potentially involve nitrogen isotopic fractionation of glutamic acid. Determining the isotopic fractionation factor associated with amino acid metabolism is thus central to evaluating conversion efficiency in ecological food webs.

4. Summary

In this study, the isotopic fractionation factor (α) for the transamination of glutamic acid to aspartic acid *in vitro* was estimated to be 0.9958. As this α value is <1.0, it indicates enrichment of the ^{15}N in glutamic acid remaining after deamination by a transaminase reaction, thereby suggesting that such an enzymatic step could be a potential cause of the 8‰ enrichment of the δ^{15}N of glutamic acid during transfer to the next highest trophic level. This 8‰ ^{15}N-enrichment corresponds to 86% of the glutamic acid having been reacted and 14%

not reacted during the transamination reaction, if the α value was 0.9958. However, the estimated α value includes much uncertainty (0.9949−0.9968). To obtain a more accurate estimation of α, transamination experiments longer (i.e. high transamination ratio) than the 10 and 60 min experiments used in this study will be required.

Acknowledgments

We acknowledge Prof. T. Korenaga (Tokyo Metropolitan Univ./Chiba Institute of Science) for a big support in the study, Ms. A. Yamanaka (Tokyo Metropolitan Univ.) and Mr. K. Aoyagi (Tokyo Metropolitan Univ.) for assistance in the experiments, and Dr. S. Mihara (Tokyo Metropolitan Univ.) and all members of the laboratory of environmental and analytical chemistry (Tokyo Metropolitan Univ.) for discussion on the results. We also thank Drs Y. Kashiyama (JST PRESTO Researcher) and Y. Chikaraishi (JAMSTEC) for their valuable suggestions and thoughtful comments that are very helpful for improving the manuscript.

References

Chikaraishi Y., Kashiyama Y., Ogawa N.O., Kitazato H. and Ohkouchi N. (2007) Metabolic control of nitrogen isotope composition of amino acids in macroalgae and gastropods: implications for aquatic food web studies. *Mar. Ecol. Progr. Ser.* **342**, 85-90.

Chikaraishi Y., Ogawa N.O., Doi H. and Ohkouchi N. (2011) $^{15}N/^{14}N$ ratios of amino acids as a tool for studying terrestrial food webs: a case study of the terrestrial insects (bees, wasps, and hornets). *Ecol. Res.* **26**, 835-844.

Chikaraishi Y., Ogawa N.O., Kashiyama Y., Takano Y., Suga H., Tomitani A., Miyashita H. and Ohkouchi N. (2009a) Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. *Limnol. Oceanogr.: Methods*. **7**, 740-750.

Chikaraishi Y., Takano Y. and Ohkouchi N. (2009b) GC/MS analysis of amino acids as pivaloyl/isopropylesters. *Res. Org. Geochem.* **25**, 61-70. (in Japanese)

Das S.K. and Chakrabarty D. (2006) Utilization and flow of energy in tropical ecosystem: A test case for two Ox-bow lakes. *Int. J. Environ. Sci. Tech.* **3**, 141-146.

Lin H.J., Shao K.T., Hwang J.S., Lo W.T., Cheng I.J. and Lee L.H. (2004) A trophic model for Kuosheng bay in Northern Tiwan. *J. Mar. Sci. Tech.* **12**, 424-432.

Lindeman R.L. (1942) The trophic-dynamic aspect of ecology. *Ecology* **23**, 399-418.

Macko S.A., Estep M.L.F., Engel M.H. and Hare P.E. (1986) Kinetic fractionation of stable nitrogen isotopes during amino acid transamination. *Geochim. Cosmochim. Acta* **50**, 2143-2146.

McCarthy M.D., Benner R., Lee C. and Fogel M.L. (2007) Amino acid nitrogen isotopic fractionation patterns as indicators of heterotrophy in plankton, particulate, and dissolved organic matter. *Geochim. Cosmochim. Acta* **71**, 4727-4744.

Pauly D. and Christensen V. (1995) Primary production required to sustain global fisheries. *Nature* **374**, 255-257.

Popp B.N., Graham B.S., Olson R.J., Hannides C.C.S., Lott M., López-Ibarra G. and Galván-Magaña F. (2007) Insight into the trophic ecology of yellowfin tuna, Thunnus albacares, from compoundspecific nitrogen isotope analysis of proteinaceous amino acids. In: Dawson T.E. and Siegwolf R.T.W. (eds) *Stable isotopes as indicators of ecological change.* pp. 173-190. Academic Press, Dublin.