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Fractionation of stable nitrogen isotopes ($^{15}N/^{14}N$) during enzymatic deamination of glutamic acid: Implications for mass and energy transfers in the biosphere

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Isotopic fractionation of nitrogen associated with the trophic transfer of amino acids in food webs has recently been used as a powerful tool to estimate the accurate trophic levels of heterotrophic organisms. During the grazing process (i.e., trophic transfer), the amino acid, glutamic acid, is significantly enriched in ^{15}N (by ~6–9‰) from diets to consumer heterotrophs; this is most likely caused by isotopic fractionation with the preferential deamination of ^{14}N-amino group in glutamic acid during its metabolism. However, few studies determined the isotopic fractionation factor (α) of this process, which limits our understanding of the mechanism responsible for the isotopic fractionation and thus restricts its applicability in assessing the mass and energy transfers with respect to the amino acid assimilation/dissimilation cycle in the biosphere. In this study, we evaluate the α value associated with the enzymatic deamination of glutamic acid *in vitro*. Glutamic acid is gradually enriched in ^{15}N by up to 4.0‰, when the deamination flux is increased up to 45.4%. The α value calculated is 0.9938 ± 0.0005 if the Rayleigh fractionation model is applied to the enrichment in ^{15}N. Thus, we demonstrate the relationship between isotopic fractionation and deamination flux: for example, 8.0‰ fractionation corresponds to that 72 ± 3% of the diet-derived glutamic acid is deaminated in the consumer species at each shift of trophic levels in a food web.

Keywords: glutamic acid, deamination, isotopic fractionation factor (α), nitrogen isotopic fractionation, energy transfer

Introduction

In the biosphere, amino acids not only are major pools as the basic subunit of biomass protein but also have function as a major metabolic energy source for almost all organisms, including large animals (i.e., megafauna) and plants, fungi, and bacteria (e.g., Steffan *et al*., 2013, 2015; Takizawa and Chikaraishi, 2014, 2017; Takizawa *et al*., 2017). During the last decade, analysis of stable nitrogen isotope ratios ($\delta^{15}N$, ‰ vs. AIR) in amino acids has been used as a powerful tool accurately to determine the positions of heterotrophic organisms in the trophic level hierarchy of ecological food webs (e.g., McClelland and Montoya, 2002; McCarthy *et al*., 2007; Popp *et al*., 2007; Chikaraishi *et al*., 2009, 2011; Steffan *et al*., 2013; Bradley *et al*., 2015; Nielsen *et al*., 2015; McMahon *et al*., 2015a, b; Blank *et al*., 2017; Ohkouchi *et al*., 2017).

Amino acids show a reproducible trend of the isotopic fractionation in ^{15}N associated with enzymatic processes (e.g., deamination) in heterotrophic metabolism. For example, the nitrogen isotopic ratio ($\delta^{15}N$) of the major amino acid, glutamic acid, predictably increases by ~6–9‰ during each step in the trophic level of food webs, which is generally attributed to the fact that deamination (the initial step in transamination) is a dominant reaction in the metabolism of glutamic acid (Fig. 1, Chikaraishi *et al*., 2007, 2009; Ohkouchi *et al*., 2015). Deamination can operate a preferential elimination of the ^{14}N-amino group in amino acids to form ammonia, leaving behind the ^{15}N-amino group to form biomass protein. In contrast, the change in the $\delta^{15}N$ value of phenylalanine is always limited to ~0–1‰ at each trophic step of the food web, which is consistent with the fact that there is no deamination in the first step of phenylalanine metabolism (Fig. 1, Chikaraishi *et al*., 2007, 2009; Ohkouchi *et al*., 2015).

Theoretically, the isotopic fractionation of nitrogen within amino acids correlates primarily with the proportions of the total amino acid flux during multiple steps of

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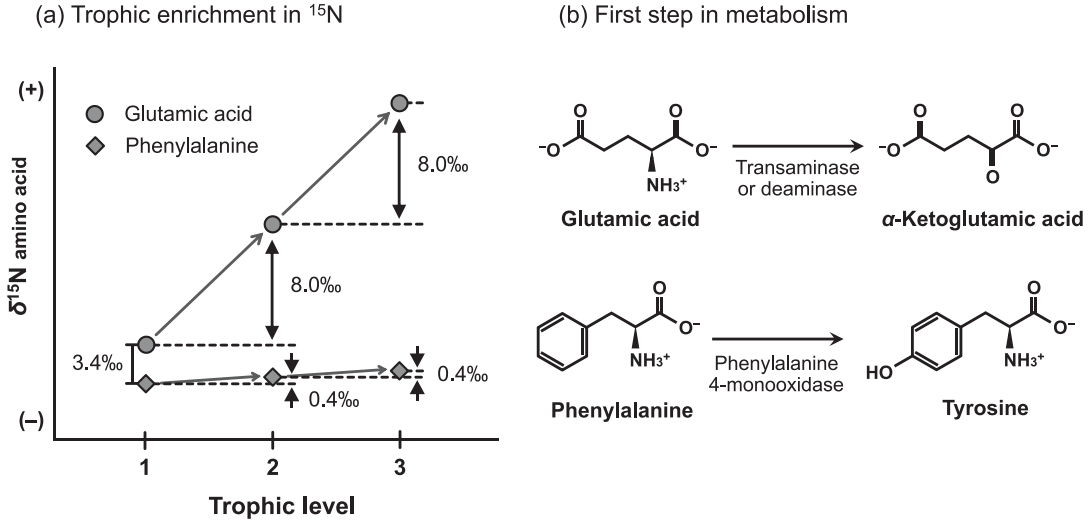

Fig. 1. Trophic enrichment in ^{15}N of amino acids along a food chain. (a) Relationship between the trophic level and the $\delta^{15}N$ values of amino acids (i.e., significant and little enrichments in ^{15}N are empirically observed in glutamic acid and phenylalanine, respectively) in grazing processes (after Chikaraishi et al., 2007). (b) First step in the metabolism of glutamic acid and phenylalanine in consumer species (after Chikaraishi et al., 2007).

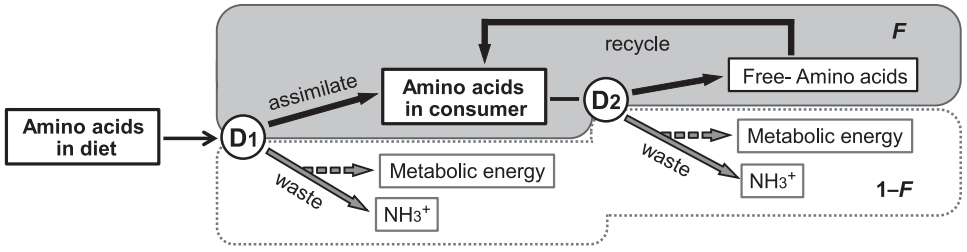

Fig. 2. Flow of the amino acid metabolic branches ($D_1 + D_2$) in consumer species. Diet-derived amino acids are deaminated at D_1, whereas the residual pool of amino acids is assimilated to construct biomass protein. Similar to D_1, aged amino acids are deaminated at D_2. Sum of amino acid flux into biomass protein is defined by F while that into deamination is defined by 1 – F.

deamination in consumer metabolism (e.g., D1 and D2 in Fig. 2): ^{15}N is highly concentrated in biomass protein if a large proportion of the amino acids is deaminated (Fig. 3a), whereas ^{15}N is slightly or negligibly concentrated in biomass if only a small proportion of amino acids is deaminated (Fig. 3b). The Rayleigh isotopic fractionation model has frequently been used so far, to quantify the relationship between the magnitude of isotopic fractionation and the flux of reactions, for example, in studies on the enzymatic deamination of amino acids (Macko *et al.*, 1986; Miura and Goto, 2012) and on the biological and chemical degradation of other organic compounds (e.g., Morasch *et al.*, 2001; Pond *et al.*, 2002). According to the Rayleigh model, such a relationship for the deamination of amino acids is defined as follows:

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

where $\delta^{15}N_{AA\_o}$ and $\delta^{15}N_{AA\_t}$ represent the isotopic ratios of an amino acid presenting before and remaining against deamination, respectively; F ($0 < F < 1$) represents the proportion of the remaining pool of amino acid against deamination (i.e., $[1 - F]$ represents the proportion of amino acid that is deaminated); and α represents the isotopic fractionation factor of the deamination. If the α value is <1.0, the ^{15}N-enrichment of the remaining amino acid ($\Delta\delta^{15}N_{AA\_t}$) should increase inversely against the value of F. For example, a $\Delta\delta^{15}N_{AA\_t}$ value of +8.0‰ corresponds to an 80% deamination flux ($F = 0.2$) for a given amino acid, when the α value is 0.995. Conversely, if the proportion of deamination flux is fixed at 40% ($F = 0.6$) for a given amino acid, the α value should be lower for generating a large $\Delta\delta^{15}N_{AA\_t}$ value, which is a pair like 0.992 and 4‰ or 0.984 and 8‰ (as α and $\Delta\delta^{15}N_{AA\_t}$, respectively).

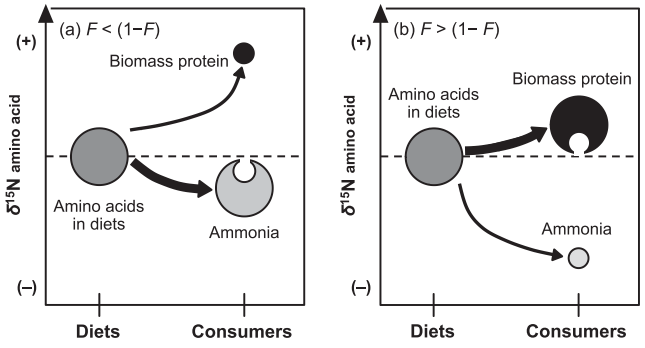

Fig. 3. Schematic view of the isotopic fractionation between ammonia and biomass protein during deamination. Flux to biomass protein construction against deamination is denoted by F: (a) Biomass protein is produced by using only a small portion (F) of the diet-derived amino acids while the large portion (1 – F) is deaminated. (b) Biomass protein is produced using a large portion (F) of the diet-derived amino acids while the small portion (1 – F) is only deaminated.

Estimating the α value is, thus, essential for quantifying the flux in the amino acid deamination and also for illustrating the amino acid assimilation/dissimilation balance (or cycle) associated with the deamination in heterotrophic metabolism. It is also required to understand the mass and energy transfers in turn (or integrated) trophic transfers along food chains in the biosphere. However, to our knowledge, only few studies have reported the isotopic fractionation factor (α) for the enzymatic deamination. Macko *et al*. (1986) first reported that ^{15}N-enrichment is ~2.9‰, when 31.9% of glutamic acid is deaminated during the *in vitro* enzymatic transamination from glutamic acid to aspartic acid. More recently, Miura and Goto (2012) reported similar results that the ^{15}N-enrichment observed is 2.2‰, when 41.2% of glutamic acid is deaminated in the same enzymatic reaction. The Rayleigh model estimate 0.9923 (Macko *et al*., 1986) and 0.9958 (Miura and Goto, 2012) for the α value, which are both consistent with the hypothesis that the ^{15}N-enrichment of amino acids is caused by the preferential deamination of the ^{14}N-amino group in the amino acid metabolism of heterotrophic animals. However, these α values still involve a large degree of uncertainty because the limited experimental data (i.e., two points in each study) were obtained in the relatively early stage of the reaction (~30–40% deamination). This is probably caused by the difficulties inherent in controlling enzymatic deamination *in vitro* and in purifying the remaining amino acids from the reagents and enzyme in the reaction mixture. The α value estimated by Macko *et al*. (1986) is far different from that estimated by Miura and Goto (2012), which shows 64% and 86% deamination, respectively, to interpret 8‰ trophic isotopic fractionation (a standard ^{15}N-enrichment of glutamic acid per trophic level in food webs). In this study, we further evaluate the α value based on the data found in this study and those available from the previous studies (Macko *et al*., 1986; Miura and Goto, 2012). Moreover, applying the α value observed, we illustrate an ecosystem model to assess the mass and energy transfers with respect to the amino acid assimilation/dissimilation cycle in the biosphere.

Experimental and analytical Methods

Glutamic acid (Tokyo Chemical Industry Co. Ltd.; 99%), oxaloacetic acid (Wako Pure Chemical Industries Ltd.; 95%), glutamic-oxaloacetic transaminase (GOT: Sigma-Aldrich Co. EC 2.6.1.1; from porcine heart; 434 units/mg), and the same reagents and enzyme used by Miura and Goto (2012) were used in this study. The δ^{15}N value of glutamic acid (–5.1‰) was determined in the previous study (Miura and Goto, 2012).

The deamination of the amino group of glutamic acid and its subsequent transfer to oxaloacetic acid to form aspartic acid were performed according to a modified procedure outlined by Miura and Goto (2012). In brief, deamination was initiated by the addition of GOT (2 μL) to glutamic acid (30 mg) and oxaloacetic acid (180 mg) in 120 mL of phosphate buffer (pH 7.6) at 37°C. The sixfold excess of oxaloacetic acid (the amino-group acceptor) relative to glutamic acid increased the deamination flux by approximately 5–15% compared with that reported in previous studies. This minimized the reverse transamination from aspartic acid to glutamic acid. The reaction, after occurring for 180 or 240 mines, was stopped by the addition of a few drops of concentrated HCl (aq.). The residual glutamic acid and the aspartic acid produced were purified with a cation-exchange chromatograph (Takano *et al*., 2010) and then derivatized to pivaloyl/isopropyl (Pv/iPr) esters for quantitative and isotopic ratio analyses (Chikaraishi *et al*., 2009).

The δ^{15}N values for glutamic acid and aspartic acid were determined with a gas chromatograph-isotope ratio mass spectrometer (GC-IRMS: a Thermo Fisher Scientific Delta XP IRMS interfaced with an Agilent 6980GC through a GC-C III interface). The Pv/iPr derivatives of amino acids were injected into an Agilent HP Ultra-2 capillary column (50 m length, 0.32 mm i.d., 0.52 μm film thickness) and then converted to N_2 gas in combustion (950°C) and reduction (550°C) furnaces. The N_2 gas was purified through a countercurrent dryer (Permeable membrane, Nafion^{TM}) and a liquid nitrogen CO_2 trap. The carrier gas (He) flow rate was controlled at a constant rate of 1.4 mL min^{-1}. The isotopic composition of the amino acids was expressed relative to that of atmospheric nitrogen (AIR) on the scale normalized to the known δ^{15}N values of the reference amino acids (ranging from –26.1

Table 1. Molar ratio (C_t/C_o) and the $\delta^{15}N$ value of glutamic acid before and after deamination

Deamination rate of glutamic acid (%)	45.2	45.4	0*	0**	0.4*	18.2**	31.9**	41.2*
C_tC_o (%)	54.8	54.6	100*	100**	99.6*	81.8**	68.1**	58.8*
$\delta^{15}N_{Glu\_t}$ (‰)	–1.2	–1.1	–5.1*	7.2**	–5.0*	8.9**	10.1**	–2.9*
$\delta^{15}N_{Asp\_t}$ (‰)	–8.8	–8.3	n.d.*	n.d.**	n.d.*	–0.5**	–0.6**	–9.3*
$\delta^{15}N_{Glu\_t} - \delta^{15}N_{Glu\_o}$ (‰)	3.9	4.0	0*	0**	0.1*	1.7**	2.9**	2.2*

n.d. = not determined.
**Data from Miura and Goto (2012).*
***Data from Macko et al. (1986).*
C_t/C_o is the molar ratio of glutamic acid remaining against deamination with time t. $\delta^{15}N_{Glu\_t}$ is the $\delta^{15}N$ value of glutamic acid after deamination with time t and $\delta^{15}N_{Asp\_t}$ is the $\delta^{15}N$ value of aspartic acid produced by transamination (caused by deamination) with time t. $\delta^{15}N_{Glu\_t} - \delta^{15}N_{Glu\_o}$ is the difference in the $\delta^{15}N$ value of glutamic acid remaining against and presenting before deamination.

to +45.7‰, Indiana University, Shoko Science Co., Sato *et al*., 2014). The standard deviations of the isotope measurements were better than 0.5‰ (Chikaraishi *et al*., 2010). The deamination flux (F) was determined by comparison of the m/z 28 peak areas for glutamic and aspartic acids on the GC-IRMS chromatogram with an error of <10%.

Results and Discussion

The $\delta^{15}N$ value for glutamic acid is clearly increased by 3.9‰ (from –5.1‰ to –1.2‰) and 4.0‰ (from –5.1‰ to –1.1‰), when the deamination flux is 45.2% and 45.4%, respectively, in the enzymatic reaction in this study (Table 1). Although the degree of ^{15}N-enrichment and the deamination flux varies somewhat among previous studies (Macko *et al*., 1986; Miura and Goto, 2012) and this study, the largest ^{15}N-enrichment is found in this study. The ^{15}N-enrichment in this study is thus greater (i.e., 4.0‰) with high deamination flux (i.e., 45.4%) than that in the previous two studies, which further supports the theoretical assumption that a significant ^{15}N-enrichment in the residual pool of amino acids is caused by a large proportion of the amino acids being deaminated (Fig. 3).

To calculate the isotopic fractionation factor (α), we applied these data to the Rayleigh isotopic fractionation model (Eq. (1)). Rearrangement of Eq. (1) yields Eqs. (2) and (3), which are as follows:

$$(1000 + \delta^{15}N_{AA\_t})/(1000 + \delta^{15}N_{AA\_o}) = F^{(\alpha - 1)} \quad (2)$$

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

Plotting $\ln[(1000 + \delta^{15}N_{AA\_t})/(1000 + \delta^{15}N_{AA\_o})]$ vs. $\ln F$ should show a linear correlation with a slope of $(\alpha - 1)$, if the deamination of glutamic acid follows the Rayleigh model. Indeed, when we plot the data collected in this study together with those from the two earlier studies (Macko *et al*., 1986; Miura and Goto, 2012), $\ln[(1000 + \delta^{15}N_{AA\_t})/(1000 + \delta^{15}N_{AA\_o})]$ shows the strong negative linear correlation with $\ln F$ with the correlation factor (R^2) for the regression being 0.9652 (Fig. 4). The Rayleigh model is thus simply applicable in the observed isotopic fractionation that occurs during the deamination of glutamic acid. Since the slope of the linear regression line, –0.0062 ± 0.0005, is equivalent to $(\alpha - 1)$, the α value is 0.9938 ± 0.0005. This α value (<1.0) clearly indicates that the ^{15}N-enrichment of glutamic acid ($\Delta\delta^{15}N_{AA\_t}$) increases inversely against the F value, implying the preferential deamination of the ^{14}N-amino group of glutamic acid during the transamination.

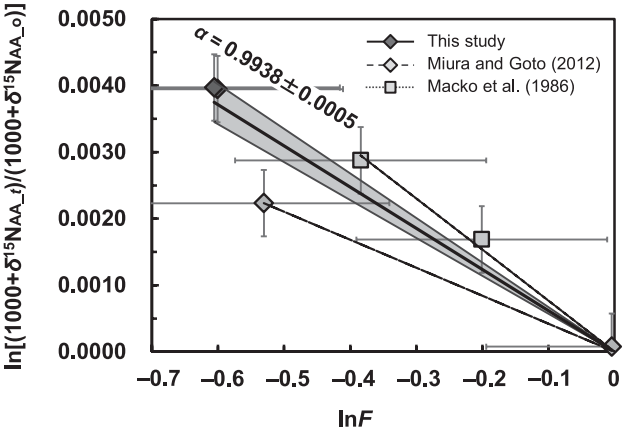

Fig. 4. The isotopic fractionation factor (α) for the deamination of glutamic acid. The α value was estimated to be 0.9938 ± 0.0005 based on the slope of a linear correlation among all data found in this study (solid line) and reported by Miura and Goto (2012) (dashed line) and Macko et al. (1986) (short dashed line). The Rayleigh fractionation model "$\ln[(1000 + \delta^{15}N_{AA\_t})/(1000 + \delta^{15}N_{AA\_o})] = (\alpha - 1) \times \ln F$" indicates that $\ln[(1000 + \delta^{15}N_{AA\_t})/(1000 + \delta^{15}N_{AA\_o})]$ correlates with $\ln F$ on a straight line with slope of $(\alpha - 1)$. The residual portion of glutamic acid against deamination is denoted by F.

The nitrogen isotopic fractionation of glutamic acid

during grazing processes has frequently been observed in various laboratory-cultured species and diet-characterized wild species (e.g., McClelland and Montoya, 2002; Chikaraishi *et al*., 2009; Bradley *et al*., 2014; Downs *et al*., 2014; Steffan *et al*., 2013, 2015; Blank *et al*., 2017). For example, Chikaraishi *et al*. (2009) reported that the ^{15}N-enrichment of glutamic acid is 8.0 ± 1.2‰ in cultured marine zooplankton and fish and in wild marine gastropods: this value is certainly confirmed in trophic levels 1–4 in cultured insects and in trophic levels 1–5 in wild insects (Steffan *et al*., 2013) as well as in long-term cultured tuna (Bradley *et al*., 2014), shrimp (Downs *et al*., 2014), and freshwater fish (Blank *et al*., 2017), as well as fungi and bacteria (Steffan *et al*., 2015). In this study, the fractionation factor (α) determined is 0.9938 ± 0.0005 for the deamination of glutamic acid. If the ^{15}N-enrichment of glutamic acid is caused only by this deamination, the ^{15}N-enrichment of 8.0‰ is simply explained as 72% (i.e., $1 - F = 0.72$) of diet-derived glutamic acid being deaminated for energy production, and as the remaining 28% (i.e., $F = 0.28$) being used for the construction of biomass protein in consumer species. In addition, the error observed in this study (±0.0005) corresponds approximately to ±0.6‰ of the error in the ^{15}N-enrichment of glutamic acid, which falls within the error observed during grazing process in the previous study (i.e., ±1.2‰: Chikaraishi *et al*., 2009). On the other hand, so far, several studies reported a large (i.e., expanded) or small (i.e., compressed) ^{15}N-enrichment of glutamic acid in wild and cultured species (e.g., Dale *et al*., 2011; Germain *et al*., 2013; Hoen *et al*., 2014; Chikaraishi *et al*., 2015; McMahon *et al*., 2015a, b; McMahon and McCarthy, 2016). For example, Germain *et al*. (2013) reported a highly-compressed (2.9 ± 0.6‰) isotopic fractionation in the harbor seal. These results suggest that magnitude of isotopic fractionation is intrinsically somewhat diverse in consumer species. According to the present results (i.e., $\alpha = 0.9938$), this variation in isotopic fractionation can possibly be interpreted as diversity in the metabolic flux of amino acid deamination. A small degree in the fractionation can be caused by a large F value (e.g., 4.0‰ enrichment in ^{15}N corresponds to $F = 0.53$); in contrast, a large degree in the fractionation can be caused by a small F value (e.g., 16.0‰ enrichment in ^{15}N corresponds to $F = 0.08$). The former case (e.g., 4.0‰ enrichment) implies that the consumer species requires relatively small amounts of metabolic energy from diet-derived amino acids; e.g., when the species undergoes diapause or when the species can derive sufficient metabolic energy from other resources, such as fat or carbohydrates, instead of protein (Chikaraishi *et al*., 2015; McMahon *et al*., 2015a; Blank *et al*., 2017). In contrast, the latter case (e.g., 16.0‰ enrichment) may correspond to a species that requires relatively large amounts of metabolic energy from diet-derived amino acids; e.g., when a species can take a limited amount of diet, but they need much energy, particularly for high activities, such as swimming, flying, or hunting, even during periods of strong starvation. Thus, knowing the isotopic fractionation factor (α) for the deamination of amino acids is essential to better understand the proportion of metabolic flux (i.e., assimilation/dissimilation balance) of diet-derived amino acids in consumer species.

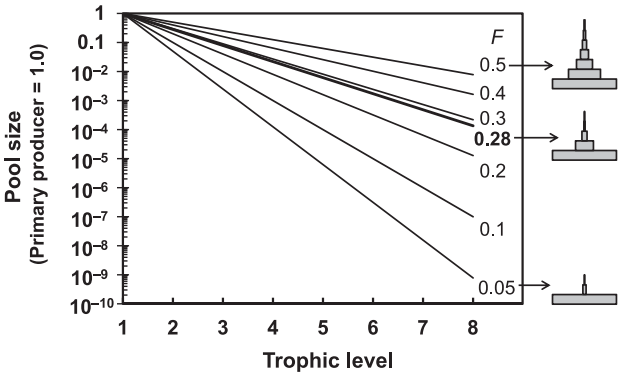

Fig. 5. Relationship between pool sizes of biomass glutamic acid and trophic level of organisms. Pool size of glutamic acid in primary producers is normalized to 1.0, which is reduced for the higher trophic level consumer species, particularly in cases, where the F value is small.

IMPLICATIONS

It is found that the degree of enrichment in ^{15}N of glutamic acid is frequently similar or almost the same among fish, insects, and mammals as well as bacteria and archaea (Steffan *et al*., 2015; Yamaguchi *et al*., 2017), implying that the isotopic fractionation factor (α) and metabolic flux (F) for the deamination of glutamic acid remain constant and universal for these consumers at any trophic level in food webs in the biosphere. In this case, the size of the glutamic acid pool at each trophic level in a steady state food web is given as $F^{(n-1)}$ for the species at trophic level n, where the pool size of primary producers (i.e., $n = 1$) is defined as 1.0 (Fig. 5). In this case, if the metabolic flux is 0.5 ($F = 0.5$), half of the glutamic acid derived from primary producers (trophic level [TL] 1) is degraded to produce metabolic energy and the other half residue to make biomass in the primary consumers (TL 2), and ultimately, 6.25% of the basal resource-derived glutamic acid is inherited in the consumer species at TL 5. However, if the metabolic flux is 0.05 ($F = 0.05$), only 5% and ultimately 0.000625% of the glutamic acid is inherited in the consumers at TL 2 and TL 5, respectively. Thus, the degree of metabolic flux is an essential

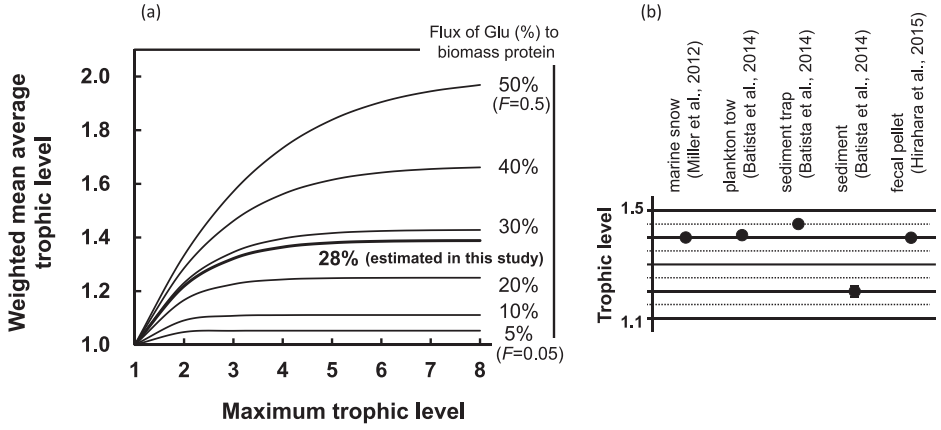

Fig. 6. The relationship between the maximum and weighted mean average trophic levels of food webs. (a) The weighted mean average trophic level ($TL_{weighted\ mean}$) of an entire food web shows asymptotic curves with the maximum trophic level of the food webs (e.g., $TL_{weighted\ mean}$ converges toward approximately 1.4 if 72% of diet-derived glutamic acid is deaminated while the remaining 28% is used to construct biomass protein, i.e., F = 0.28). (b) Apparent trophic levels of environmental samples (e.g., marine snow, plankton tow, sediment trap, sediment, and fecal pellet) reported in the previous studies, which are likely consistent with the $TL_{weighted\ mean}$ value at F = 0.28.

factor for evaluating food web sharpness (i.e., narrow vs. broad umbrella) and energy transfers in the ecosystem. If the metabolic flux is 0.28 (F = 0.28, corresponding to 8‰ enrichment in ^{15}N at each step moving toward a higher trophic level), the primary producer-derived glutamic acid is inherited by 28% in primary consumers, and ultimately by 0.61% in the consumers at TL 5. Assuming that the annual primary production of glutamic acid is 300 kg/km^2/year, we can calculate that 84, 23.52, 6.59, and 1.84 kg/km^2/year of glutamic acid are responsible for the annual growth of biomass at each trophic level (TL 2, 3, 4, and 5), respectively. This dramatic decrease of the pool size is disadvantageous to maintaining a high population for large-biomass carnivorous animals, such as high TL megafauna, in the biosphere.

To evaluate whether the F values observed are realistic in ecosystems, we calculated the weighted mean of trophic level for an entire food web (i.e., $TL_{weighted\ mean}$). The $TL_{weighted\ mean}$ value has asymptotic curves regarding the food chain length, into a single value that is decided by the F value (Fig. 6). Primary producers and primary consumers at low trophic levels contribute considerably to the $TL_{weighted\ mean}$ value because of their large biomass, especially if the F value is small (Fig. 5). In contrast, heterotrophic animals at higher trophic levels contribute only slightly to the $TL_{weighted\ mean}$ value because of their small biomass, even though the ^{15}N-enrichment in glutamic acid increases considerably in turn deamination along food chains. For example, the $TL_{weighted\ mean}$ value estimated is 1.05, when only 5% of diet-derived glutamic acid remains as biomass protein in heterotrophs (i.e., F = 0.05) from one trophic level to the next. However, the $TL_{weighted\ mean}$ value gradually increases as the F value increases (e.g., 1.97 at F = 0.5). In this study, we illustrate that the ^{15}N-enrichment of glutamic acid of 8.0‰ at each step in the trophic level corresponds to F = 0.28. If this is the case, it (i.e., F = 0.28) leads to a $TL_{weighted\ mean}$ value of 1.38–1.39. Moreover, the $TL_{weighted\ mean}$ value is 2.08 and 1.09 if the F values are 0.53 (corresponding to 4.0‰ ^{15}N-enrichment) and 0.08 (corresponding to 16.0‰ ^{15}N-enrichment), respectively. The actual $TL_{weighted\ mean}$ value of the entire food web is not available at the moment and may vary across ecosystems and environments. However, Miller *et al.* (2012) calculated the apparent trophic level of the potential diet of eel larvae (e.g., marine snow) in the western Pacific to be ~1.4 based on the estimated trophic level of the eel larvae (TL ~2.4) in the food web. Batista *et al.* (2014) reported that apparent trophic levels of mixed plankton tow and sediment trap samples in the Santa Barbara Basin are 1.41 and 1.45, respectively. Hirahara *et al.* (2015) reported that apparent trophic level of fecal pellets (TL 1.5) of a calanoid copepod (TL 2.1) is similar to these values, and is 0.4 units higher than that of the food algae (TL 1.1). Interestingly, the $TL_{weighted\ mean}$ value (1.38–1.39) based on 8.0‰ ^{15}N-enrichment (F = 0.28 and α = 0.9938) estimated in this study is well-consistent with these apparent trophic levels in the marine snow, the mixed plankton tow sample, the sediment trap sample, and the zooplankton fecal pellet. If these samples represent the entire food web from the primary producers to the heterotrophs within a small ecosystem, this consistency may be an evidence that the F value observed here is likely realistic in ecosystems.

On the other hand, the ^{15}N-enrichment of glutamic acid

in consumers may not be explained solely by the isotopic fractionation during deamination associated with GOT, because it is potentially caused by multiple reactions (e.g., deamination by glutamate dehydrogenase, Kelly and Stanley, 2001) occurring parallel to the transamination of glutamic acid. Moreover, various amino acids may react in complex metabolic networks, in which more than 50 substrate-specific enzymes have been identified in animals (Bender, 2012). Similar to glutamic acid, strong ^{15}N-enrichment is commonly found in alanine, valine, leucine, isoleucine, and proline during the grazing process between consumer and resource species (e.g., Chikaraishi *et al*., 2007, 2009; McCarthy *et al*., 2007; Popp *et al*., 2007; McMahon *et al*., 2015a, b), whereas even ^{15}N-depletion occurs in threonine (Bradley *et al*., 2014; Downs *et al*., 2014; McMahon *et al*., 2015a, b). Macko *et al*. (1986) calculated the α value to be 0.9987 for the transamination of aspartic acid to form glutamic acid, which is larger than the value for the deamination of glutamic acid determined in this study. Moreover, the α value might be affected by the velocity of the enzymatic reaction, the molar balance between substrate and enzyme, and the taxonomic diversity of the enzyme. Therefore, the factors that affect isotope fractionation during the various reactions (at least in major processes) involving the 20 protein-forming amino acids in heterotrophs should be determined in future studies, to better understand the mass and energy transfers with respect to the amino acid assimilation/dissimilation balance (or cycle) in the grazing process within a single heterotroph species as well as in the integrated food webs in the biosphere.

Summary

We determined the isotopic fractionation factor (α) associated with the enzymatic deamination of glutamic acid to better understand and quantify the mass and energy transfers responsible for the fractionation of nitrogen isotopes in amino acids occurring along ecological food webs in the biosphere. The data obtained in this study and those reported in previous studies can illustrate 0.9938 ± 0.0005 for the α value of the deamination, which indicates that the glutamic acid in biomass is enriched in ^{15}N after deamination. This supports the hypothesis that increase in the δ^{15}N value for glutamic acid along the trophic shifts in food webs is caused by the deamination of glutamic acid in heterotrophs. Our results indicate that 72 ± 3% of the diet-derived glutamic acid is deaminated to produce metabolic energy in heterotrophic animals at each step of the trophic level in grazing food webs if the trophic isotopic fractionation of glutamic acid is 8.0‰. In this case, 28 ± 3% of the diet-derived glutamic acid resists deamination and is retained by heterotrophs in biomass protein.

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