

STABLE ISOTOPE ANALYSIS OF AMINO ACIDS: AS A NEW TOOL FOR ILLUSTRATING CENTRAL BIOSYNTHETIC AND METABOLIC FLUX

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Introduction

Organic compounds have a large variation in the stable isotopic compositions (e.g., D/H, ¹³C/¹²C, and ¹⁵N/¹⁴N) in biological, ecological, and geochemical samples, which are always closely related to isotopic fractionation due to the isotope effect and metabolic flux on ‘key’ biochemical processes in biosynthetic and metabolic pathways in organisms (e.g., Hayes, 2001; Chikaraishi, 2014; Ohkouchi et al., 2015). An identification of ‘key’ process in these pathways is indispensable for better understanding of ‘universality vs. specificity’ on the isotopic compositions or fractionations found in organisms, as well as of the accuracy and precision on the isotope evidence in application studies.

In case of amino acids, it has long been known that a large variation in the isotopic fractionation of nitrogen within amino acids is observed in the trophic transfer from resource to consumer species (e.g., Gaebler et al., 1966). More recently, the fractionation has been employed as a unified approach to provide unprecedented accuracy and precision in our understanding of trophic linkages in complex networks of diverse ecosystems and of the associated energy-flow cycles in biogeochemical processes of biosphere (e.g., Chikaraishi et al., 2014). For example, the following equation has been used to estimate the trophic position (TP) of organisms in food webs:

$$TP = [(\delta^{15}N_{Tr} - \delta^{15}N_{Src} + \beta) / TDF] + 1$$

where $\delta^{15}N_{Tr}$ and $\delta^{15}N_{Src}$ denote the isotopic composition of ‘trophic’ and ‘source’ amino acids (the most commonly represented by glutamic acid and phenylalanine, respectively), β denotes the isotopic difference between these amino acids in primary producers, and TDF ($= \Delta\delta^{15}N_{Tr} - \Delta\delta^{15}N_{Src}$) denotes the isotopic difference at each shift of trophic levels (Chikaraishi et al., 2009).

However, a little is known the biosynthetic and metabolic factors responsible for such trophic isotopic fractionation observed in consumer species. For example in case of amino acids, although the validity of the TP estimate is principally dependent on the consistency of TDF, compression and expansion on the TDF are frequently found in organisms under ‘specific’ and ‘unusual’ physiological conditions (e.g., they feed only on artificial diets with a high carbohydrate/protein ratio). Thus, the ‘key’ process affecting the isotopic fractionation in the biosynthetic and metabolic pathway should be identified.

Scientific objectives

To identify the isotopic fractionation for ‘key’ process in central metabolic and biosynthetic pathways, our studies currently have been focused on positive and negative changes in the carbon and nitrogen isotopic compositions of amino acids, as representative organic compounds close to the central pathways, between consumer and diet species in certainly-characterized conditions.

Results and Discussion

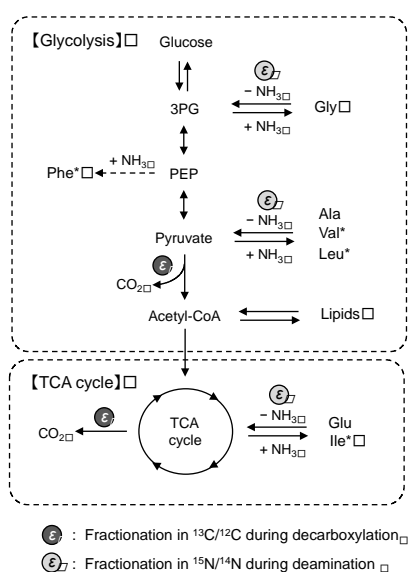
Our results indicate that the change in the nitrogen isotopic composition of amino acids for the consumer-resource combinations (e.g., Fig. 1) is well consistent with a general theory (Chikaraishi et al., 2009): the isotopic composition of amino acids mirrors metabolic activity of amino acid deamination, as a significantly-large change is found with increasing the deamination flux in amino

acid metabolism. On the other hand, the change in the carbon isotopic composition of amino acids for the consumer-resource combinations (e.g., Fig. 1) reveals another trends and allow us to predict the following processes:

- (1) preferential accumulation of ^{13}C on pyruvic acid (as a precursor of alanine and glycine) associated with its decarboxylation to acetyl-CoA;
- (2) preferential accumulation of ^{13}C on intermediates (e.g., α -ketoglutaric acid as a precursor of glutamic acid) associated with decarboxylation in the TCA cycle; and
- (3) propagation of these accumulated ^{13}C into the amino acids *via* re-biosynthesis (or metabolic routing) in consumer species.

We conclude that the proposed bridged perspective between the isotopic fractionation and its responsible process in the biosynthesis and metabolism of amino acids in consumers will be useful to refine our fundamental understanding in the isotopic compositions of individual compounds (including not only amino acids but also lipids) in biological as well as geochemical samples.

(a) Metabolic Pathway



(b) Isotopic fractionation

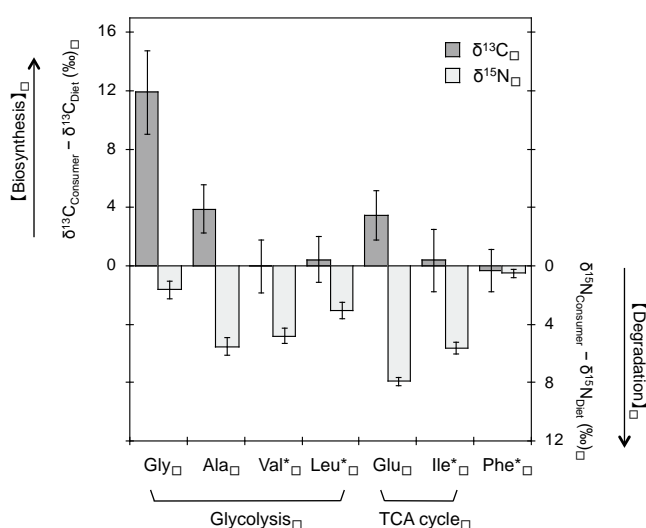


Figure 1 (a) Simplified view of the central pathway for biosynthesis and metabolism of amino acids and lipids, (b) stable isotopic fractionations in carbon and nitrogen of amino acids between a diet (the sponge *Halichondria okadai*) and its consumer species (the sea slug *Hypselodoris festiva*).

References

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