REMARKABLE FRACTIONATION OF NITROGEN ISOTOPES IN ANAEROBIC AMINO ACID METABOLISMS

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Introduction

The stable isotopic compositions (e.g., $^{15}N/^{14}N$) of organic compounds (δ) have long been used in studies for biogeochemistry to understand who, how to, or how much organic compounds were produced or consumed in natural environments. During the last decade, the nitrogen isotopic composition of amino acids ($\delta^{15}N_{AAs}$) in organisms has been employed as a potential powerful tool to estimate the diet-resource utilization and trophic energy transfer among organisms in food webs (e.g., Chikaraishi et al., 2007; McCarthy et al., 2007; Popp et al., 2007; Takizawa et al., 2017). This method is proposed by empirical observations that a single 'universal' isotopic enrichment in ^{15}N is found in glutamic acid for diverse species, because of a certain metabolism (i.e., deamination) of glutamic acid during grazing processes. Glutamic acid is indeed directly incorporated into the tricarboxylic acid cycle (i.e., TCA cycle) and extracted its energy under aerobic metabolism. However, little knowledge is available for change in the $\delta^{15}N_{AAs}$ value associated with 'anaerobic metabolism' in organisms who are major life in the earth. For instance, the glycolysis and fermentation is representative processes for the anaerobic metabolism.

Therefore, in this study, we investigated laboratory-cultured samples to see the change in the $\delta^{15}N_{AAs}$ value with respect to the anaerobic metabolism such as glycolysis and fermentation in the samples. The eukaryote *Saccharomyces cerevisiae* and the bacteria *Lactococcus lactis* were incubated with respective organic substrates, and were compared the $\delta^{15}N_{AAs}$ values between before (i.e., t=0, non-fermented) and after incubation (i.e., t=f, fermented). The $\delta^{15}N_{AAs}$ values were measured with a gas chromatograph-isotope ratio mass spectrometer and reported for eight amino acids (i.e., glycine, alanine, valine, leucine, isoleucine, proline, glutamic acid, and phenylalanine). We use Δ values (i.e., $\Delta\delta^{15}N = \delta^{15}N_{t=f} - \delta^{15}N_{t=0}$) to see change in the $\delta^{15}N_{AAs}$ value for the anaerobic metabolisms.

Results and Discussion

To our knowledge for aerobic metabolism (that composes of glycolysis and TCA cycle), the magnitude of the $\delta^{15}N$ values highly depends on the metabolic flux how many percentage of amino acids is deaminated in samples. In general, the $\delta^{15}N$ values of several amino acids (e.g., phenylalanine, called 'source' amino acids) do not change during the metabolism because they have very little flux of deamination in organisms. In contrast, the $\delta^{15}N$ values of the other amino acids (e.g., glycine, alanine, valine, leucine, isoleucine, proline, and glutamic acid, called 'trophic' amino acids) are elevated because they have a large flux of deamination (Fig. 1a).

However, for the anaerobic metabolism (that composes of glycolysis but not TCA cycle), we found negligible change in the $\Delta\delta^{15}N$ value for phenylalanine, as well as for isoleucine, proline, and glutamic acid, but variable change for glycine, alanine, valine, and leucine in this experiment (Fig 1b). Similar to aerobic metabolism, phenylalanine does not change the $\delta^{15}N$ values under neither aerobic nor anaerobic metabolism. On the other hand, trophic amino acids indicate two distinct trends in the $\Delta\delta^{15}N$ value:

(1) Substantially zero in the $\Delta\delta^{15}$N value is found for isoleucine, proline, and glutamic acid. This is consistent with the anaerobic metabolism that these amino acids cannot be employed as energy fuels because of 'no-activity' of the TCA cycle, resulting in microbes cannot deaminate these amino acids.
(2) Significantly positive in the $\Delta\delta^{15}$N value is found for glycine, alanine, valine, and leucine. This is also consistent with the anaerobic metabolism that these amino acids can be employed as energy fuels because of 'high-activity' in the glycolysis, resulting in microbes have deaminated these amino acids.

Our results further suggest that activity of anaerobic metabolism can be recorded in glycine, alanine, valine, and leucine but not isoleucine, proline, glutamic acid, and phenylalanine, resulting in that comparison of the δ^{15}N values between these two metabolically-different amino acids (e.g., alanine and glutamic acid) will be useful to evaluate the contribution of anaerobic metabolism in biological and environmental samples. Moreover, we predict that enhancing knowledge of the isotopic fractionation of amino acids in diverse anaerobic metabolisms (e.g., reversed TCA cycle) will be required in further studies.

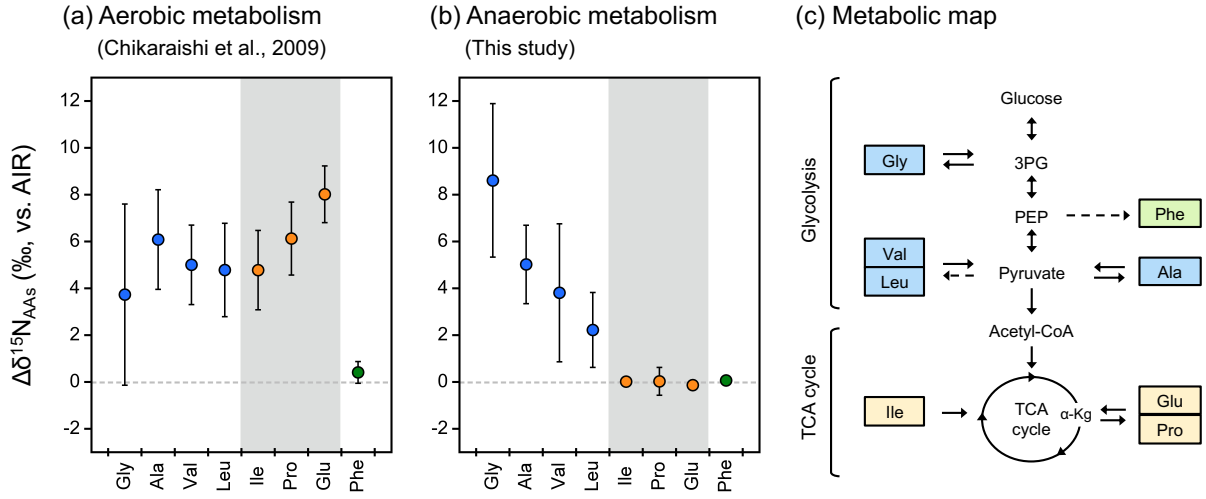

Figure 1 *The comparison of the $\Delta\delta^{15}N_{AAs}$ values between (a) aerobic metabolism reported in Chikaraishi et al. (2009), and (b) anaerobic metabolism found in this study, and of the (c) metabolic map. In anaerobic metabolisms, substantially zero in the $\Delta\delta^{15}N$ value is found for isoleucine, proline, and glutamic acid, because of no activity of the TCA cycle.*

References

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