

## Position-specific N isotope analysis of Glutamine extracted from cultured phytoplankton

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Glutamine (Gln) and glutamic acid (Glu) provide the first step of incorporating inorganic nitrogen (N) into cellular organic N in photoautotrophic primary producers. Both are present at higher concentrations than other amino acids (AAs). The N atom in cellular ammonia forms the amide group of Gln, which is subsequently used in producing Glu. Although Glu supplies the N for most other amino acids via transamination reaction, Gln and Glu interconvert with each other via  $\alpha$ -ketoglutarate. We hypothesize that the  $\delta^{15}\text{N}$  of Gln's amino-N ( $\delta^{15}\text{N}_{\text{Gln-amino}}$ ), amide N ( $\delta^{15}\text{N}_{\text{Gln-amide}}$ ), and intracellular ammonium have the same values under equilibrium conditions due to fast turnover. In addition, the amide-N of Gln provides N for the nucleobases and one of the sidechain N atoms in histidine. Thus, position-specific N isotope analysis of Gln will provide key information on AA and nucleotide biosynthesis in organisms.

Such analysis is made possible with our newly developed method. Intracellular Gln was extracted from lysed phytoplankton cells, followed by the separation and collection by Ion-exchange Chromatography. The collected Gln fraction was divided in 2. One sub-fraction was oxidized by hypochlorite, converting the amino-N to nitrite; and the other one was hydrolyzed with hydrochloric acid to convert amide-N into ammonium, which was further oxidized to nitrite by hypobromite. The nitrite produced in the two sub-fractions was then converted to nitrous oxide and analyzed using Purge-and-Trap Isotope Ratio Mass Spectrometry, yielding both  $\delta^{15}\text{N}_{\text{Gln-amino}}$  and  $\delta^{15}\text{N}_{\text{Gln-amide}}$ .

We will compare the  $\delta^{15}\text{N}_{\text{Gln-amino}}$  and  $\delta^{15}\text{N}_{\text{Gln-amide}}$  with the  $\delta^{15}\text{N}$  of intracellular ammonium in cultured phytoplankton to verify our hypothesis. This study will shed light on the  $\delta^{15}\text{N}$  patterns of Gln and other amino acids in different phytoplankton phylogenetic groups under various metabolic conditions, which will further advance the use of  $\delta^{15}\text{N}$ -AA patterns in trophic ecology and paleo-N cycle reconstructions.

