Hydrogen Isotope Fractionation in Amino Acids as a Tracer of Microbial Metabolism

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Hydrogen is the most abundant element in biogeochemical systems and is central to both energetic and biosynthetic metabolic processes. The isotopic fractionation of hydrogen in biological systems has garnered great interest in recent decades as a proxy for diet and environmental conditions. Most of this research has focused on fatty acids due to their chemical recalcitrance in sediments as well as their well-characterized biosynthetic pathways. However, the fundamental mechanisms that control H isotope fractionation remain incompletely understood. The amino acids, which are both more abundant and biochemically varied than the fatty acids, are a promising avenue to advance this like of inquiry.

Here we present data on an the hydrogen isotope ratios of amino acids harvested from from laboratory cultures of the model microbe *Escherichia coli* as measured by combined gas chromatography-pyrolysis-isotope ratio mass spectrometry (GC-P-IRMS).

Cultures incubated in aqueous, glucose-based media produce a systematic distribution of isotope ratios in the amino acids. The fractionation and incorporation of media water, however, differs for each of the amino acids measured. When grown in the presence of typtone, a protein digest, the amino acids reflect direct incorporation from the medium for some residues but differing level of *de novo* biosynthesis in others.

In an effort to understand the enzymatic processes controlling the fractionation of hydrogen as it enters microbial metabolism, we focus on alanine biosynthesis. Alanine is the most abundant amino acid in bulk protein and is synthesized from the crucial metabolic intermediary pyruvate. By tracing the incorporation of hydrogen from both organic food and cytosolic water, we develop a model for alanine as an indicator of environmental waters. These results provide a significant first step in understanding the hydrogen isotope fractionation in microbial metabolism.