Proceedings of the 12th International Symposium on Applied Isotope Geochemistry (AIG-12)

Amino acid δ^2H as indicator of metabolic, ecological, and biogeochemical processes

Patrick Griffin[a], Arndt Schimmelmann[a], Peter E. Sauer[a], Katarina Topalov[a], Yoshito Chikaraishi[b]

[a] Department of Earth and Atmospheric Sciences, Indiana University, 1001 East 10th Street, Bloomington IN 47405, USA
[b] Department of Biogeochemistry, Japan Agency for Marine-Earth Science and Technology, Hokkaido University, Kita-19, Nishi-8, Kita-ku, Sapporo 060-0819, Japan

Abstract

Hydrogen isotope ratios reflect phenomena that span every spatial scale of interest in the Earth sciences, from global and regional hydrology to the invisible chemical products of microbial metabolism. Proteins, and the amino acids that compose them, are abundant, hydrogen-bearing biomolecules, often comprising the majority of cellular dry mass, that also preserve over geological time. Laboratory experiments with the model microorganism *Escherichia coli* give clues to the biochemical underpinnings that dictate how hydrogen from the environment – both as water and organic nutrients – is fractionated during metabolism. A comparison of the δ^2H distribution of amino acids from bacteria and animals also provides a basis for interpreting the ecological origin of amino acids obtained from unknown sources, as may occur in the sedimentary record. Finally, potential application to paleo-environmental reconstruction – both hydrology and geochemistry – are discussed.

Introduction

Hydrogen is the most abundant element in biogeochemical systems and its isotope systematics are perhaps the most complicated to understand, and therefore employ as an analytical tool. Due to the mass difference between the isotopes, H is more strongly fractionated than any other element, leading to a wider range of natural values. Also unlike the other elements, some H in biomolecules readily exchanges with ambient moisture, erasing the isotopic signal imparted during biosynthesis. Transient H-bonding is responsible for an array of molecular interactions, including the base pairing of nucleic acids and the transport of many compounds during membrane transport. Finally, a family of enzymes is dedicated to the transfer of H from activated carriers, both to provide energy for ATP production (from NADH) as well as reducing power during biosynthesis (NADPH). Most importantly, however, hydrogen is central to the fundamental interaction of living organisms with their geochemical milieu: the acquisition of food and nutrients to be turned into biological molecules and tissues. Understanding these interactions is central to understanding biogeochemical processes.

Despite the tremendous potential utility of H as a proxy for these processes, its application has so far yielded limited success. Largely, this owes to the difficulty inherent in the interpretation of δ^2H from bulk materials. Simply stated, bulk materials are complex chemical mixtures of many distinct chemical species, each with individual isotope ratios that vary by hundreds of mUr (or ‰), have undergone varying degrees of exchange, and bear the isotopic signal of unknown biological processes.

Compound specific isotope analysis of amino acids addresses these problems. Amino acid derivatives are readily separated by GC, allowing for the “unmixing” of complex tissues. The metabolic pathways that produce them are well-studied, allowing researchers to develop an understanding of the physicochemical underpinnings that give rise to observed biochemical fractionations. Amino acids are also abundant, persist over geological time, and branch from central metabolism at multiple points. All of these features make them ideal candidates for the development of a new analytical framework for understanding biogeochemical fractionation of H.

Methods

Culturing of E. coli – Cultures of *E. coli* were grown from monoclonal colonies and incubated in the appropriate medium at 37 °C until stationary phase. Cell mass was harvested via centrifugation and lyophilized.

Preparation of keratin and collagen specimens – Collagen and keratin were rinsed first to remove particulate matter and then to remove fatty acids and solvent-soluble contaminants. Bird feathers were rinsed under a stream of deionized water then with a mixture of dichloromethane (DCM) and methanol (MeOH) (9:1, v/v). Collagen soaked overnight in warm DCM/MeOH.

Hydrolysis and derivatization –1 mg of material was placed in a hydrolysis vial to which 1 mL of 6 N HCl was added. Headspace was evacuated with streaming N_2 prior to closure with a PFTE-lined cap. After 20 hours at 110 °C, hydrolysates were dried under streaming N_2 at 110 °C. Amino acids were rendered volatile through a process of *N*-trifluoroacetic anhydride isopropyl ester derivatization (Fogel et al., 2016).

Isotope analysis – δ^2H values were determined via gas chromatography-thermal conversion-isotope ratio mass spectrometry. A Trace GC Ultra with High Temperature unit was coupled with a Delta Plus^{XP} for analysis.

Derivatives were dissolved in ~200 µL DCM, and 1 µL aliquots were injected through a S/SL port to a DB-1 column.

Results

When cultured in liquid media with glucose as the only source of organic carbon, δ^2H of amino acids synthesized by *E. coli* reflected a systematic enrichment in response to δ^2H of media water (Fig. 1). Culture media that included amino acids reflected compound-specific patterns of direct incorporation (not shown).

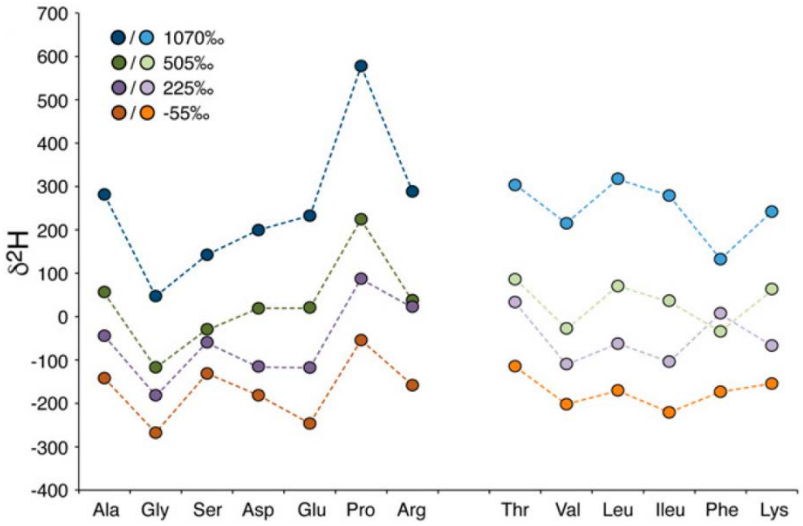

Fig. 1. δ^2H of amino acids from *E. coli* cultured in ^{2}H-labeled water with glucose as sole carbon source.

Data from *E.coli* experiments as well as analyses of collagen and keratin from natural animals were combined and subjected to principle component analysis to determine if groupings could be determined. (Fig. 2).

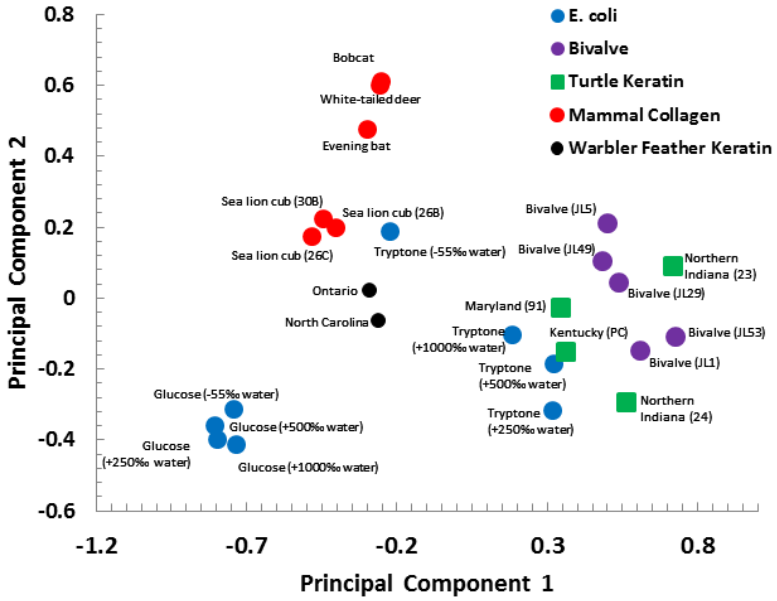

Fig. 2. PCA of amino acid distributions from several mammals, turtles, bivalves, and *E. coli* grown on different nutrient substrates.

Discussion

These results indicate the feasibility of compound specific isotope analysis of hydrogen in amino acids. Experiments with *E. coli* demonstrate several key facts. First, the isotopic composition of environmental waters is recorded in the stable hydrogen of amino acids (Fig. 1). Further, each amino acid draws a different amount of its H from water (Table 1). Second, the *E. coli* cultured in media with amino acids show a distinct pattern of incorporation that differs from the *E. coli* cultured in media containing only glucose as an organic carbon source. Indeed, as shown in Fig. 2, amino acids from *E. coli* grown in a tryptone-based medium more closely resemble the keratin from turtle shells than they do amino acids from *E. coli* grown on glucose.

AA	Glucose, $\%H_{H2O}$	Tryptone, $\%H_{H2O}$
Ala	49.1 ± 7.2	46.1 ± 6.8
Gly	36.1 ± 5.3	19.8 ± 2.9
Ser	50.8 ± 7.4	17.3 ± 2.5
Pro	45.4 ± 6.6	11.6 ± 1.7
Asp	55.4 ± 8.1	18.6 ± 2.7
Glu	73.3 ± 10.7	22.2 ± 3.3
Thr	71.6 ± 10.5	12.4 ± 1.8
Val	57.0 ± 8.3	9.0 ± 1.3
Leu	48.3 ± 7.1	8.3 ± 1.2
Ileu	58.2 ± 8.5	3.8 ± 0.6

Table 1. Percentage of amino acid H estimated to have been derived from media water.

Another important consequence of the analysis depicted in Fig. 2 is the applicability of microbial model systems to preliminary investigations of H isotope distribution in animals. Microbial experiments are far more rapid and cost-effective than similar experiments with animals, and are subject to less regulatory intervention. Further we can conclude that *E. coli*, in particular, is a suitable model organism for understanding how H from water and organic nutrients are recorded in amino acids. There are compelling reasons to anticipate this finding, namely that *E. coli* is a common gut inhabitant that passes amino acids to its host organism, as well as sharing the same central metabolic pathways as animals when cultured aerobically. These results are proof of that concept. They also open the door to using *E. coli* as a model for understanding which aspects of cellular physiology impart the observed fractionations. These experiments, too, are far more tractable in microbial models.

Finally, the data shown in Table 1 offer researchers a starting point for the development of the amino acid system as proxy for paleo-environmental reconstruction, particularly as it pertains to hydrological cycling. Each amino acid obtains a different amount of its H from environmental water. As a result, some amino acids are more sensitive to changes in aridity than others. This information, combined with analyses of fossil assemblages, may provide researchers with a means to predict the extent of ecological disruption that accompanies changes in aridity, such as those driven by large scale warming.

Reference

Fogel M. L., Griffin P. L. and Newsome S. D. (2016) Hydrogen isotopes in individual amino acids reflect differentiated pools of hydrogen from food and water in *Escherichia coli*. Proceedings of the National Academy of Sciences, 113, E4648–E4653