N isotopic composition of Phenylalanine and Glutamic Acid from a number of organisms, demonstrating new HPLC protocol for precise isotopic measurements

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Project
» The Use of Nitrogen Isotopes of Amino Acids To Understand Marine Sedimentary 15N Records (Amino Acid Sediment 15N)

Contributors	Affiliation	Role
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Abstract
N isotopic composition of Phenylalanine and Glutamic Acid from a number of organisms, demonstrating new HPLC protocol for precise isotopic measurements.

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Dataset Description

N isotopic composition of Phenylalanine and Glutamic Acid from a number of organisms.

These data were published in:
Broek, T, and McCarthy, M.D. (2014) A New Approach to ä^{15}N compound-specific amino acid trophic position measurements: preparative high pressure liquid chromatography technique for purifying underivatized amino acids for stable isotope analysis. Limnology and Oceanography Methods, 12, 2014, 840-852. doi:10.4319/lom.2014.12.840

Methods & Sampling

Methodology described in Broek & McCarthy (2014):

AA standards
Standard L-AA powders were purchased from Alfa Aesar and Acros Organics and used to prepare individual liquid standards (0.05 M), which were then combined as an equimolar mixture of 16 individual AAs ("16 AA Standard") for developing separations. The 16 AA Standard contained the proteinaceous AAs: glycine (Gly), L-alanine (Ala), L-arginine (Arg), L-aspartic acid (Asp), L-glutamic acid (Glu), L-histidine His), L-isoleucine (Ile), L-leucine (Leu), L-lysine (Lys), D/L-methionine (Met), L-phenylalanine (Phe), L-proline (Pro), L-serine (Ser), L-threonine (Thr), L-valine (Val); and nonprotein AA nor-leucine(Nle), which ;is commonly used as an internal standard (Popp et al. 2007; McCarthy et al. 2013). The δ15N and δ13C values for dry standards were determined by standard EA-IRMS at the University of California, Santa Cruz Stable Isotope Laboratory (UCSC-

SIL) following standard protocols (http://es.ucsc.edu/ ~ silab). Average precision of EA-IRMS δ15N standard values was 0.11 ± 0.07 ‰. Additionally, a commercially available equimolar AA standard mixture "Pierce Amino Acid Standard H" (Pierce H)(Thermo Scientific) containing the same AAs as the "16 AA Standard" with the exception of the nonprotein AA Nle and addition of the proteinaceous AAscysteine (Cys) and tyrosine (Tyr) was used to construct individual calibration curves, so as to verify relative molar abundance of individual AAs in natural samples.

Sample preparation:
The cyanobacteria sample (*Spirulina Sp.)* was obtained as a bulk commercial dry powder (Spirulina Pacifica, Nutrex Hawaii, Kailua-Kona, HI). This same sample has been used previously as a McCarthy laboratory internal quality control standard, and its CSI-AA values have been measured repeatedly by GC-C-IRMS, allowing an investigation of the long-term accuracy and precision of the GC-C-IRMS instrument.

Coastal mussel (*Myilitus Califorianus)* sample was collected in 2012 from Santa Cruz, CA. The mussel was previously dissected, and the adductor muscle tissue removed and lyophilized prior to storage. We used a subsample of adductor muscle collected for a prior study (Vokhshoori and McCarthy 2014) hydrolyzing the bulk lyophilized adductor muscle tissue directly without lipid extraction.

The deep-sea bamboo coral (genus *isidella*) sample was previously collected in 2007 from Monterey Bay, CA, USA (36 44.6538N, 122 2.2329W, 870.2 m) (Hill, *pers. comm.* 2011). A proteinaceous node was separated from the calcium carbonate skeleton and oven dried (60 degrees C, 24 h).

White sea bass muscle tissue was subsampled from an incidental recreational catch in 2007, landed from Santa Cruz Island, Channel Islands, CA (J. Patterson, *pers. comm.* 2007). Fish muscle tissue was also lyophilized prior to hydrolysis.

Harbor seal blood was collected in May-June 2007 from a wild animal in Tomales Bay, CA (38 13.9N, 122 58.1W) under NMFS Research Permit no. 555-1565. Blood serum was purified, lipid extracted, and lyophilized prior to hydrolysis, as described previously (Germain et al. 2011).

For all sample types, proteinaceous material was hydrolyzed by adding 40-50 mg of bulk dry sample to an 8 mL glass vial, followed by 5 mL of 6 N hydrochloric acid (HCl) at room temperature. The vials were flushed with nitrogen gas, sealed, and allowed to hydrolyze under standard conditions (110 degrees C, 20 h). Hydrolysis under acidic conditions quantitatively deaminates asparagine (Asn) to aspartic acid, and glutamine (Gln) to glutamic acid (Barrett 1985). Therefore, in this protocol (and all others based on acid hydrolysis), measured Glu in fact represents Gln+Glu, and measured Asp represents Asp+Asn. We note that while the abbreviations Glx and Asx are sometimes used to denote these combined Gln+Glu and Asp+Asn fractions, we have elected to simply use Asp and Glu as abbreviations, as defined above, in order to correspond better with prior TPCSIA literature. Additionally, acid hydrolysis is known to destroy cysteine (Cys), precluding it from analysis (Barrett 1985). Resulting hydrolysates were dried to completion under nitrogen gas and brought up in 0.1 N HCl to a final concentration of 1 mg tissue/ 100 L HCl. Approximately 75% of each of the resulting mixtures was reserved for HPLC/EA-IRMS analysis, and the remaining material was dried to completion for derivatization and subsequent GC-C-IRMS analysis.

GC-C-IRMS Analysis:
Trifluoroacetyl isopropyl ester (TFA-IP) AA derivatives were prepared using standardized lab protocols, as described previously (McCarthy et al. 2013). Briefly, hydrolyzed samples were esterified in 300 uL 1:5 mixture of acetyl chloride:2-propanol (110 degrees C, 60 minutes). The resulting amino acid isopropyl esters were then acylated in 350 uL 1:3 mixture of dichloromethane (DCM):trifluoroacetic acid anhydride (100 degrees C, 15 minutes). Derivatized AAs were dissolved in DCM to a final ratio of 1 mg of original proteinaceous material to 50 uL DCM. Isotopic analysis was conducted on a Thermo Trace GC Ultra (Thermo Fisher Scientific, West Palm Beach, FL, USA) coupled via a Thermo GC IsoLink to a ThermoFinnigan DeltaPlus XP isotope ratio monitoring mass spectrometer (Thermo Fisher Scientific). Derivatives (1 L) were injected (injector temp. 250 degrees C constant) onto an Agilent DB-5 column (50 m x 0.32 mm ID x 0.52 um film thickness, Agilent Technologies, Inc., Santa Clara, CA, USA), with a He carrier flow rate of 2 mL/min (constant-flow). Separations were achieved with a four-ramp oven program: 52 deg C, 2 min hold; ramp 1 = 15 deg C /min to 75 deg C, hold for 2 min; ramp 2 = 4 deg C /min to 185 deg C, hold for 2 min; ramp 3 = 4 deg C /min to 200 deg C; ramp 4 = 30 deg C /min to 240 deg C, hold for 5 min. This method allows for the determination of 11-15 AAs depending on derivatization efficiency and instrument sensitivity. Values are typically obtained for Gly, Ala, Glu, Ile, Leu, Phe, Pro, Ser, Thr, Val, Nle, and Lys. Values for Met, His and Arg are obtained only in some samples, depending on concentration and derivatization efficiency. For δ15N AA values, samples were analyzed in quadruplicate (n=4) with bracketed lab AA isotopic standard mix for subsequent standard offset and drift corrections. Corrections based on authentic external standards were applied using previously published protocols (McCarthy et al. 2013).

HPLC/EA-IRMS:
Liquid chromatographic separations were conducted using a Shimadzu HPLC system (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA) equipped with: system controller (SCL-10A vp), degasser (DGU-20A5), 2 pumps (LC-20AD), autosampler (SIL-20A) with an adjustable injection volume of 0.1-100 uL, and coupled to a Shimadzu automated fraction collector (FRC-20A). An adjustable flow splitter (Analytical Sales and Services, Inc., Pompton Plains, NJ, USA) was used inline following the chromatography column to direct ~15% of the flow to a SEDERE (Alfortville, France) evaporative light scattering detector (ELSD-LT II, Sedex 85LT) for peak detection and quantitation. A semi-preparative scale SiELC Primesep A column (10 x 250 mm, 100 angstrom pore size, 5 um particle size; SiELC Technologies Ltd., Prospect Heights, IL, USA) was used for amino acid purification. The Primesep A column used here is a reverse-phase semi-preparative scale column embedded with strong acidic ion-pairing groups. Such mixed phase columns have been developed specifically for the separation of charged organic compounds as the acidic sites in the stationary phase interact with the charged functional groups and provide additional retention mechanisms to increase chromatographic separation potential. For a more detailed description of the retention mechanisms of the Primesep A column see (McCullagh et al. 2006; 2010).

Typically, 75-100 uL of sample solution was loaded onto the HPLC instrument. A binary solvent ramp program was used consisting of 0.1% trifluoroacetic acid (TFA) in HPLC grade water (aqueous phase) and 0.1% TFA in acetonitrile (organic phase). The final solvent ramp program used for optimal separation was as follows: starting with 100% aqueous / 0% organic; increased from 0 to 0.5% organic from 0-30 minutes; increased to 15% organic from 30-35 minutes; increased to 22.5% from 35-70 minutes; increased to 30% from 70-95 minutes; held at 30% until 140 minutes. The column was then cleaned and equilibrated by increasing to 100% and holding for 20 minutes; then decreasing to 50% and holding for an additional 15 minutes; then decreasing to 0% and holding until the method ends at 180 minutes. A flow rate ramp is also employed in which the total flow rate is held at 2.5 mL/minute for 0-30 minutes; increased to 4.5 mL/minute from 30-35 minutes; held at 4.5 mL/min from 35-170 minutes; then decreased back to 2.5 mL/minute from 170-175 minutes and held until the completion of the analysis.

Purified AAs were collected into 3.5 mL tubes via the automated fraction collector using time-based collections, and then transferred to 20 mL glass vials. The solvent was removed under vacuum using a Jouan centrifugal evaporator (Societe Jouan, Saint-Herblain, France) at a chamber temperature of 60 degrees C. Dry AA residues were then re-dissolved into a small volume (~30 L) of 0.1 N HCl, transferred into pre-ashed tin (Sn) EA capsules, and dried to completion in a 60 degrees C oven for 12 hours. Capsules were then pressed into cubes and analyzed for δ15N and δ13C values by EA-IRMS. EA-IRMS analysis was conducted in the UCSC shared Stable Isotope Laboratory facility (UCSC-SIL), using an EA-IRMS analyzer dedicated to smaller samples. This system uses a Carlo Erba CHNS-O EA1108-Elemental Analyzer, interfaced via a Thermo Finnigan Gasbench II device to a Thermo Finnigan Delta Plus XP isotope ratio mass spectrometer (Thermo Fisher Scientific), configured after Polissar et al. (2009). For AAs in this study, we found that ≤ 100 nmol quantities of purified AA material could be routinely measured using this instrument, although as discussed below a standard EA configuration could also equally be used. Raw EA-IRMS δ15N and δ13C values were corrected for instrument drift and size effects using AA isotopic standards and standard correction protocols used by the UCSC-SIL (http://es.ucsc.edu/~silab).

Data Processing Description

BCO-DMO Processing:
- modified parameter names to conform with BCO-DMO naming conventions;
- removed the extraneous values that were in original spreadsheet, as advised by data submitter;
- replaced missing data/blanks with "nd" ("no data")

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Data Files

File
d15N.csv (Comma Separated Values (.csv), 909 bytes) MD5:61c3f7a15de4a05d7ff335504af8536f Primary data file for dataset ID 711958

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Related Publications

Broek, T. A. B., & McCarthy, M. D. (2014). A new approach to δ15N compound-specific amino acid trophic position measurements: preparative high pressure liquid chromatography technique for purifying underivatized amino acids for stable isotope analysis. Limnology and Oceanography: Methods, 12(12), 840–852. doi:10.4319/lom.2014.12.840
Results

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Parameters

Parameter	Description	Units
sample	Description of the sample type.	unitless
Glu_d15N_GCC	Glutamic Acid d15N value; determined by GC-C-IRMS. Average precision: +/- 0.5 per mil	per mil
Glu_d15N_GCC_stdev	Standard deviation of the Glutamic Acid d15N value determined by GC-C-IRMS.	per mil
Phe_d15N_GCC	Phenylalanine d15N value; determined by GC-C-IRMS. Average precision: +/- 1 per mil	per mil
Phe_d15N_GCC_stdev	Standard deviation of the Phenylalanine d15N value determined by GC-C-IRMS.	per mil
TP_GCC	Trophic Position; determined by GC-C-IRMS.	unitless
Glu_d15N_HPLC	Glutamic Acid d15N value; determined by HPLC/EA-IRMS. Average precision: +/- 0.5 per mil	per mil
Glu_d15N_HPLC_stdev	Standard deviation of the Glutamic Acid d15N value determined by HPLC/EA-IRMS.	per mil
Phe_d15N_HPLC	Phenylalanine d15N value; determined by HPLC/EA-IRMS. Average precision: +/- 1 per mil	per mil
Phe_d15N_HPLC_stdev	Standard deviation of the Phenylalanine d15N value determined by HPLC/EA-IRMS.	per mil
TP_HPLC	Trophic Position; determined by HPLC/EA-IRMS.	unitless
d15N_EA	15N/14N isotopic ratio; determined by EA-IRMS. Average precision: +/- 0.1	permil (‰)
d15N_EA_stdev	Standard deviation of 15N/14N isotopic ratio determined by EA-IRMS.	permil (‰)

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Instruments

Dataset-specific Instrument Name	Carlo Erba CHNS-O EA1108-elemental analyzer
Generic Instrument Name	Elemental Analyzer
Dataset-specific Description	EA-IRMS analysis was conducted in the UCSC shared Stable Isotope Laboratory facility (UCSC-SIL), using an EA-IRMS analyzer dedicated to smaller samples. This system uses a Carlo Erba CHNS-O EA1108-Elemental Analyzer, interfaced via a Thermo Finnigan Gasbench II device to a Thermo Finnigan Delta Plus XP isotope ratio mass spectrometer (Thermo Fisher Scientific).
Generic Instrument Description	Instruments that quantify carbon, nitrogen and sometimes other elements by combusting the sample at very high temperature and assaying the resulting gaseous oxides. Usually used for samples including organic material.

Dataset-specific Instrument Name	Thermo Trace GC Ultra
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	Isotopic analysis was conducted on a Thermo Trace GC Ultra (Thermo Fisher Scientific, West Palm Beach, FL, USA) coupled via a Thermo GC IsoLink to a ThermoFinnigan DeltaPlus XP isotope ratio monitoring mass spectrometer (Thermo Fisher Scientific).
Generic Instrument Description	Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)

Dataset-specific Instrument Name	Agilent DB-5 column
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	Derivatives (1 L) were injected (injector temp. 250 degrees C constant) onto an Agilent DB-5 column.
Generic Instrument Description	Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)

Dataset-specific Instrument Name	Shimadzu HPLC
Generic Instrument Name	High-Performance Liquid Chromatograph
Dataset-specific Description	Liquid chromatographic separations were conducted using a Shimadzu HPLC system (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA) equipped with: system controller (SCL-10A vp), degasser (DGU-20A5), 2 pumps (LC-20AD), autosampler (SIL-20A) with an adjustable injection volume of 0.1-100 µL, and coupled to a Shimadzu automated fraction collector (FRC-20A).
Generic Instrument Description	A High-performance liquid chromatograph (HPLC) is a type of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by high pressure pumping of the sample mixture onto a column packed with microspheres coated with the stationary phase. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Dataset-specific Instrument Name	ThermoFinnigan DeltaPlus XP isotope ratio monitoring mass spectrometer
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	Isotopic analysis was conducted on a Thermo Trace GC Ultra (Thermo Fisher Scientific, West Palm Beach, FL, USA) coupled via a Thermo GC IsoLink to a ThermoFinnigan DeltaPlus XP isotope ratio monitoring mass spectrometer (Thermo Fisher Scientific).
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

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Project Information

The Use of Nitrogen Isotopes of Amino Acids To Understand Marine Sedimentary 15N Records (Amino Acid Sediment 15N)

Coverage: California Margin , Santa Barbara Basin , CA current system, Eastern Tropical Pacific

The bioavailability of nutrients plays a crucial role in oceanic biological productivity, the carbon cycle, and climate change. The global ocean inventory of nitrogen (N) is determined by the balance of N-fixation (sources) and denitrification (sinks). In this three-year project, a researcher from the University of California, Santa Cruz, will focus on developing compound-specific N isotope (d15N) analysis of amino acids as a new tool for understanding N source and transformation of organic matter in paleo-reservoirs. The offsets in the isotopic ratios of individual amino acid groups may yield information about trophic transfer, heterotrophic microbial reworking, and autotrophic versus heterotrophic sources. By measuring and comparing the bulk and amino acid d15N in size-fractioned samples from plankton tows, sediments traps, and multi-cores in oxic and suboxic depositional environments, the researcher will: (1) Provide a proxy of the d15N of average exported photoautotrophic organic matter; and (2) Provide a new level of detail into sedimentary organic N degradation and preservation.

Broader impacts:
This project will improve understanding of the fundamental underpinnings and behaviors of d15N amino acid

patterns and how they behave in contrasting sedimentary environments, while also developing a potential paleoceanographic proxy. Funding will support a graduate student and undergraduate research at the institution. The researcher will also conduct community outreach in the form of a workshop/tutorial on the proxy development.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1131816

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