Stable Nitrogen Isotope Analysis of Amino Acids by GC/C/IRMS

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Measurements of the $\delta^{15}N$ values of individual amino acids have provided very specific information about the biogeochemical, environmental, and ecological processes. The combination of gas chromatography with IRMS has become a hopeful tool for nitrogen isotopic analyses of individual amino acids in mixtures.

Amino acids before isotopic determination need to be derivatized using MTBSTFA and separated by gas chromatography, and gas chromatographic effluents were combusted and sent to the mass spectrometer continuously in a helium carrier stream. The GC column temperature was programmed for the tBDMSi derivatives separation as follows: isothermal 90 ºC for 1 min, then heating up to 140 ºC at rate of 8 ºC/min and keeping it for 5 min, to 220 ºC at rate of 3 ºC/min and then to 285 ºC at rate of 12 ºC/min, and hold at final temperature 285 ºC for 12.5 min until the elution of the last component. In this study, 0.8 ~ 4.5 nmol of each of the 20 amino acids (Ala, Gly, Val, Leu, Ile, Pro, Asn, Met, Ser, Thr, Phe, Asp, Glu, Lys, Gln, Arg, His, Tyr, Trp and GABA) was injected for isotopic determination.

All the 20 derivatived amino acids could be completely resolved in 60 min by GC programm. We found a high isotopic correlation (R^2=0.9987, $p<0.0001$) between the determined values and the real values for most of the amino acids except three amino acids (Asn, Gln and Arg) which signals were much depleted. But for the three amino acids, there also existed a high isotopic correlation (R^2=0.9999, $p<0.0001$). Reproducible $\delta^{15}N$ values were obtained within different injected amounts. The reproducibility of all the 20 derivatives was between 0.3‰ and 0.8‰. The mean precision of reproducibility was 0.5‰. After calibration with the 2 correlation equations, the isotopic difference between the calibrated values and the real values was in the range of 0.1‰ to 0.5‰ for the 20 amino acids.

Using the method developed, we successfully analysed the $\delta^{15}N$ values of 20 free amino acids in tree leaves and barks.

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Cell Alive System (CAS): A new method of core sample freezing for shore-based biological analyses and sample storage

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We report a novel freezing technology for the long-term preservation of subseafloor core samples. Subseafloor core samples recovered by scientific ocean drilling provide unprecedented opportunities to study deep subseafloor life and biogeocheminal cycles. For the future analyses of cores using newly devleoped life science technologies, archiving precious core materials under the appropriate condition is fundamentary significant. Given such scientific requirements, the Kochi Core Center (KCC), one of the offical core repositories of the Integrated Ocean Drilling Program (IODP), has started storing some biological core samples in -80˚C deep freezers and/or in liquid N_2 tanks, so called “DeepBIOS” (Deep Biosphere Samples).

To keep quality assuarance and control (QA/QC) of the DeepBIOS, the intital freezing process is a key: however, using the conventional way (e.g., quick transfer to deep freezer), it has been confirmed that formation of ice crystals decompose biological signetures. During the JAMSTEC *Chikyu* Expedition 905, we tested a new technology called “Cell Alive System (CAS)”, which utilizes magnetic field to vibrate water molecur in the sample, following snap and hence uniform freezing of core samples at the supercooling temperature. The core samples from various depths were sub-sampled, and immediately frozen in the CAS system along with the standard freezing method under the temperature of -20˚C, -80˚C, and -196˚C. Analysis of cell abundance showed that conventional freezing methods decreased the number of microbial cells, whereas the CAS freezing resulted in almost no loss of the cells. We also tested the paleomagnetic characteristics after the CAS freezing, indicating no or very little change in remnant magnetism. No visible changes in volume of sediment was observed after the CAS freezing. Consequently, our results indicate that the CAS freezing technique is highly useful for QA/QC of scientific frozen core samples to preserve intact biological signetures, as well as other non-biological characteristics, for the long-term storage.

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