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High-precision compound-specific carbon isotopic analysis of underivatized amino acids using a multi-dimensional-HPLC and nano-EA/IRMS

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We have developed an analytical method for the precise $\delta^{13}C$ measurement of individual amino acid using a multi-dimensional high-performance liquid chromatography (HPLC) and a nano-scale elemental analyzer/isotope ratio mass spectrometry (EA/IRMS). Although this method is time-consuming, it can offer higher precision and accuracy than does the conventional analytical method such as GC/C/IRMS, because the derivatization of amino acids is not required. A reversed-phase column (CAPCELL PAK C18, Shiseido, Japan) and a mixed-mode column (Primesep A, SIELC Technologies, U.S.A.) were applied for the HPLC (Agilent Technologies, U.S.A.) with a charged aerosol detector (Thermo Fisher Scientific, U.S.A.) (Ishikawa et al., 2018). We conducted the isolation of underivatized amino acids in a standard mixture containing 15 proteinogenic amino acids (Gly, Ala, Glu, Arg, Val, Pro, Met, Tyr, Ile, Leu, Phe, Thr, His, Asp, Ser), and confirmed that all these amino acids were successfully isolated. Each collected amino acid was filtered through a 0.45 μm membrane filter (Pall, U.S.A.) and washed with diethyl ether to remove hydrophobic impurities. The $\delta^{13}C$ values of these amino acids before and after the separation and purification were consistent, which proved that the whole experimental procedure did not change the $\delta^{13}C$ values of amino acids. We applied this method to several aquatic organisms. The results show that the $\delta^{13}C$ values of amino acids vary as large as 30‰ with Gly being most enriched in ^{13}C.

Reference

Ishikawa et al., (2018) *Anal. Chem.*, 90, 20, 12035-12041.