Theoretical Estimation of the $^{13}C/^{12}C$ fractionation between Pyruvic Acid and Alanine at the α Carbon through the Alanine Transaminase Reaction

*BROOKE DALLAS[1], JOHN EILER[1]

[1] Division of Geological and Planetary Science, California Institute of Technology, Pasadena CA, USA
(*bdallas@caltech.edu, eiler@gps.caltech.edu)

The ALT enzyme catalyzes the transfer of an amino group from alanine to α-ketoglutarate to produce pyruvate and glutamate. An equilibrium isotopic fractionation is expected for the isotope exchange reaction:

$$^{13}C{=}O + {}^{12}CHNH_2 \leftrightarrows {}^{12}C{=}O + {}^{13}CHNH_2 \qquad (1)$$

with C=O preferentially enriched in ^{13}C because of the strength of the C=O bond relative to $C\text{-}NH_2$. We carried out electronic structure calculations to estimate the equilibrium constant K_1 for reaction (1). Calculations were done on the alanine-pyruvate couple as well as the analogous molecules methylamine-formaldehyde (MA-FA) and isopropylamine-acetone (IA-AC). We used density functional theory (DFT) to calculate the zero-point energies within the local density approximation (LDA) and also with the hybrid B3LYP functional. Solvent effects in the alanine-pyruvate system were approximated by protonating the pyruvate to pyruvic acid (to mimic hydrogen bond donation into the carboxylic acid group) and by protonating the amine group on alanine to create H-alanine$^+$. The MA-FA, IA-AC, and alanine-pyruvate systems all indicate fractionations of 7-12‰ ($1000\ln K_1$), while the solvent-corrected pyruvic acid-H-alanine$^+$ system predicts ~15-20‰. Benchmark calculations on the MA-FA system at the MP2-aug-cc-pvTZ level give a much lower fractionation, 4.4‰, relative to that predicted by DFT (8-9‰), indicating the necessity of additional high-level calculations on the larger systems. Anharmonic corrections, estimated by DFT-LDA calculation of the C=O and $C\text{-}NH_2$ potential surfaces, are estimated to be less than 0.5‰. Calculations on the hydrated from of pyruvate, measured by ^1H NMR to be present at 10-20%, predict, unexpectedly, a higher fractionation (2-5‰, depending on the DFT functional). It is also predicted that the presence of ^2H at the α-carbon site (rather than ^1H) should decrease the expected fraction by ~1/2 to 2/3. The fractionation predicted by DFT, even diluted over the three carbon sites, should be readily apparent even with non-site-specific isotope analysis.