Modeling stable isotope fractionation in microbial methane production

J. GROPP[1], Q. JIN[2], M.A. IRON[3], I. HALEVY[1]

[1] Dept. of Earth and Planetary Sciences, Weizmann Institute of Science, Israel (jonathan.gropp@weizmann.ac.il)
[2] Dept. of Earth Sciences, U. of Oregon, Eugene, OR, USA
[3] Dept. of Chemical Research Support, Weizmann Institute of Science, Israel

Methanogenic archaea produce methane (CH_4) from CO_2 and H_2 or from organic matter in anoxic environments. This process is associated with characterstic isotopic fractionations of C and H isotopes between the produced methane and its substrates (denoted by $^{13}\alpha$ and $^{2}\alpha$ for singly substituted isotopes, and Δ_{18} for clumped isotopologues of CH_4). Accordingly, the isotopic composition of CH_4 is used to trace its sources in the environment. The isotopic fractionation during methanogenesis is generally correlated with the net thermodynamic drive of the methanogenic reaction (ΔG_r) [*1–3*]. However, there is no mechanistic explanation for larger-than-equilibrium fractionations of C isotopes at relatively small ΔG_r, and for the weaker dependence of H isotope fractionation on ΔG_r, that are observed in experiments.

We developed a bio-isotopic model, that relates the concentrations of intracellular metabolites in methanogens and ΔG_r to $^{13}\alpha$, $^{2}\alpha$ and Δ_{18} at a steady state. The model is based on a framework proposed for sulfate-reducing bacteria [*4*], and considers the full metabolic pathway, including electron carrier cycling, sodium pumping, and incorporation of H atoms from both H_2O and H_2. We calculated the theroretical equilibrium fractionations associated with each step of the metablolic pathway, and used the model to predict the reversibility degree of these steps at different conditions, to ultimately get the isotopic composition of CH_4 as a function of ΔG_r.

Our model elucidates the intracelullar controls over stable isotope fractionation in methanogenesis. We find that as ΔG_r becomes increasingly negative, the enzymatically catalyzed reactions depart from equilibrium differentially. Departure from equilibrium of a pathway step inhibits expression of isotope fractionation of downstream steps, and shapes the observed net fractionation during methanogenesis. The model successfully predicts $^{13}\alpha$- and $^{2}\alpha$-ΔG_r relationships observed in lab cultures. In addition, it predicts Δ_{18}-$^{2}\alpha$-ΔG_r relationships, and identifies the key metabolic steps generating Δ_{18} disequilibria.

[1] Valentine *et al.*, 2004, *GCA*. **68**, 1571–1590. [2] Okumura *et al.*, 2016, *PEPS.* **3**, 14. [3] Wang *et al.*, 2015, *Science*. **348**, 428–431. [4] Wing & Halevy, 2014, *PNAS.* **111**, 18116–25.