Active site constraints on nitrogen stable isotope fractionation by Mo-dependent nitrogenase

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Biological nitrogen (N) fixation by the metalloenzyme nitrogenase is the main source of biologically available, fixed N to terrestrial and marine ecosystems. The N stable isotope composition of biomass ($^{15}N/^{14}N$ typically expressed as $\delta^{15}N$) provides a powerful tool to reconstruct past and present N cycling, but its interpretation depends on a full understanding of the isotopic signature of N fixation. N fixation by Mo-, V-, and Fe-only isoforms of nitrogenase results in systematic differences in the organism-level expressed isotope effect of nitrogen fixation ($^{15}\varepsilon_{fix} = \delta^{15}N_{dissolved\ N2} - \delta^{15}N_{biomass}$; $^{15}\varepsilon_{fix\text{-}Mo}$= ~ 2‰, $^{15}\varepsilon_{fix\text{-}V}$ and $^{15}\varepsilon_{fix\text{-}Fe}$ = ~ 6-8‰), suggesting enzyme active site structure is a key constraint on fractionation. Here, we investigate how the nitrogenase active site environment influences $^{15}\varepsilon_{fix}$. We measured $\delta^{15}N_{biomass}$ for strains of the model N fixer, *Azotobacter vinelandii,* which have mutations in the α-70 valine residue, an amino acid that modulates substrate access to the Mo-nitrogenase active site. Cellular level ^{15}N fractionations for a mutant with α-70 valine substituted by the smaller amino acid alanine ($^{15}\varepsilon_{fix\text{-}Mo\text{-}small}$ = 3.4±0.1‰, n=11) and for a mutant with α-70 valine substituted by the larger amino acid isoleucine ($^{15}\varepsilon_{fix\text{-}Mo\text{-}large}$=5.5±0.0‰, n=4) were significantly different from each other and higher than published wild-type values for *Azotobacter* ($^{15}\varepsilon_{fix\text{-}Mo}$ = 0-2‰). The results imply that the active site environment plays a key role in controlling expressed isotope effects. Further investigations on the biochemistry and physiology of nitrogen fixation are ongoing and will help unravel how nitrogenase sequence, the physiological context of nitrogenase, and environmental parameters affect the $\delta^{15}N$ of nitrogen fixer biomass.