NITROGENASE CATALYZED DINITROGEN REDUCTION – A NEW MECHANISTIC APPROACH

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1. Introduction

The molecular mechanism of the enzymatic reduction of the inert gas N_2 remains an open question, despite the immense amount of work that has been done in this area (Burgess BK, Lowe DJ 1996, Rees DC, Howard JB 2000). We are conducting a mechanistic investigation of the dinitrogen (N_2) reduction by the enzyme. Our goal is to develop a unique methodology that will enable the examination of the N_2 reduction mechanism within the enzyme's complex kinetic cascade (Thorneley RNF, Lowe DF 1983, Thorneley RNF, Lowe DF 1996).

2. Procedure

A methodology is being established to measure competitive ^{15}N kinetic isotope effects (KIEs). Competitive KIEs are effects on the second order rate constant *V/K*. These KIEs are only sensitive to kinetic steps from the free N_2 binding to the first irreversible step (Cook PF 1991). Thus, these effects are not "masked" by the slow rate limiting steps. The enrichment of ^{15}N in the remaining dinitrogen substrate is measured at various fractional conversions (*f*) by isotope ratio mass spectrometry. The KIEs are calculated from: where R_t is the isotopic ratio at time t and R_0 the isotopic ratio at t=0 (Kohen A, Klinman JP 1999, Melander L, Saunders WH 1987). Triple labeling ^{15}N KIE experiments ($^{15}N_2$:$^{15}N^{14}N$:$^{14}N_2$) assist in the elucidation of the intrinsic mechanism (Cook PF 1991).

$$^{15}(V/K) = \frac{\ln(1-f)}{\ln\left(1-f\frac{R_t}{R_0}\right)}$$

3. Analysis and Discussion

KIEs are a manifestation of changes in bond order along the reduction path. The experimental findings will be used to reevaluate various theoretical models suggested and will lead to the identification of model (s) consistent with the results. We hope to provide one of the first experimental tools to shed light on this fascinating chemical process. In the future, D_2O and D_2 effects on the ^{15}N KIEs and studies with several mutants will enable examination of the reductive protonation of N_2.

4. References

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