

Supporting Information

Materials and Methods

Unless otherwise specified, all chemicals were purchased from Fisher Scientific (Pittsburgh, PA) or Sigma-Aldrich (St. Louis, MO). ^{12}CO (99.5% purity) was purchased from Praxair Inc. (Danbury, CT). All isotope-labeled compounds ($\geq 98\%$ isotopic purity) were purchased from Cambridge Isotope Labs Inc. (Tewksbury, MA).

Protein Purification. *Azotobacter vinelandii* strain expressing a His-tagged form of vanadium-iron (VFe) protein was grown as described elsewhere.^[1,2] Published methods were adapted for the purification of this protein.^[1,2]

Enzymatic Assays. Each H_2O -based reaction contained, in a total volume of 15 mL (in a 27 mL reaction vial), 100 mM Tris (pH 8.0), 20 mM Eu(II) DTPA and 1 M NaHCO_3 as a CO_2 source. Reactions were equilibrated for 1 h (when pH stabilized at 8.3) before they were initiated by addition of 100 mg VFe protein. Subsequently, reaction vials were shaken in a water bath at 30°C and samples were taken at 10, 30, 60, 120, 180, 240, 300 and 360 min. The D_2O -based reaction had the same composition as the H_2O -based assay, except that all components were dissolved in 100 mM (D11)-Tris [*i.e.*, (DOCD2)3CND2] buffer and that all protein samples were exchanged into the same deuterated buffer. The pD of this buffer was adjusted to 8.0 with DCl and NaOD, as determined by the previously established equation [pD = measured pH + 0.40].^[3] In addition, pH indicator strips were used for further verification. For GC-MS analysis, $\text{NaH}^{12}\text{CO}_3$ was replaced by $\text{NaH}^{13}\text{CO}_3$. For experiments using CO as a substrate, NaHCO_3 was omitted and CO was added to the headspace of the reaction vial in a concentration mimicking that achieved by VFe protein from CO_2 reduction at 180 min: 136 ppm for H_2O -based assays, or 178 ppm for D_2O -based assays.

Activity Analysis. The products CH_4 , C_2H_4 , C_2H_6 , C_3H_6 , C_3H_8 , C_4H_8 and C_4H_{10} were quantified by GC-FID using a previously published method.^[1,4,5] For each assay, 250 μL of headspace was

applied on an activated alumina column (Grace, Columbia, MD), which was held at 55°C for 1 min, then heated to 180°C at 12.5°C/min and held at 180°C for another 2.6 min. The quantities of all products were standardized using a Scott gas mixture containing 15 ppm of each hydrocarbon compound (Houston, TX). The product CO was determined by GC coupled to a reduction gas detector (RGD) from SRI (Torrance, CA). For each assay, 250 μ l of headspace was applied on a molecular sieve column (Grace, Columbia, MD) which was run at a constant temperature of 50°C. The amount of CO was determined over a linear standard curve ($R^2 \geq 98$), which was derived by the injection of varying amounts of ^{12}CO (99.5% purity). The detection limit for the products in nmol product/ μ mol protein was as follows: 0.27 (CH_4), 0.11 (C_2H_4), 0.091 (C_2H_6), 0.087 (C_3H_6), 0.066 (C_3H_8), 0.086 (C_4H_8), 0.053 (C_4H_{10}), and 0.1 (CO).

GC-MS Analysis. The hydrocarbon products were identified by GC-MS using an Agilent 6890 GC system coupled to a Waters GCT-Premier time-of-flight mass spectrometer. The identities of CH_4 , C_2H_4 , C_2H_6 , C_3H_6 , C_3H_8 , C_4H_8 and C_4H_{10} were confirmed by comparing their masses and retention times with those of the Scott standard alkane and alkene gas mixture. A total of 50 μ L gas was injected into a split/splitless injector operated at 125°C in split mode, with a split ratio of 5. A 1-mm ID liner was used to optimize sensitivity. Gas separation was achieved with a HP-PLOT-Q capillary column (0.320 mm ID x 30 m length, Agilent Technologies, Santa Clara, CA), which was held at 40°C for 1 min, heated to 45°C at a rate of 5°C/min, heated to 200°C at 20°C/min, and held at 200°C for another 4 min. The carrier gas, helium, was passed through the column at a rate of 1.1 mL/min. The mass spectrometer was operated in electron impact (EI) ionization mode.

References

- [1] C. C. Lee, Y. Hu, M. W. Ribbe, *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 9209-9214.
- [2] Y. Hu, A. W. Fay, M. W. Ribbe, *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 3236-3241.
- [3] P. K. Glasoe, F. A. Long, *J. Phys. Chem.* **1960**, *64*, 188-190.
- [4] Y. Hu, C. C. Lee, M. W. Ribbe, *Science* **2011**, *333*, 753-755.
- [5] C. C. Lee, Y. Hu, M. W. Ribbe, *Science* **2010**, *329*, 642.