Supporting Information

Reduction of CO_2 by Nitrogenases**

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SUPPLEMENTARY FIGURES

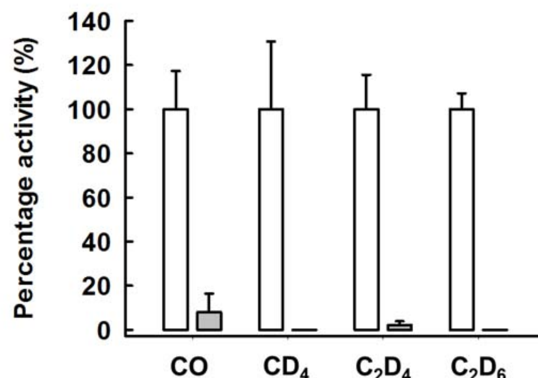

Fig. S1. ATP-dependent product formation by V-nitrogenase from CO_2 reduction. Percentage activities of product formation by V-nitrogenase from CO_2 in the presence (white bars) or absence (grey bars) of ATP in D_2O-based reactions. The percentage activities in the absence of ATP were calculated relative to those in the presence of ATP, with the latter set at 100%. The actual specific activities of product formation in the presence of ATP are, in nmol/μmol protein/h, 194 $\pm$ 17 (CO), 12.4 $\pm$ 3.8 (CD_4), 21.8 $\pm$ 1.7 (C_2D_4) and 1.31 $\pm$ 0.05 (C_2D_6).

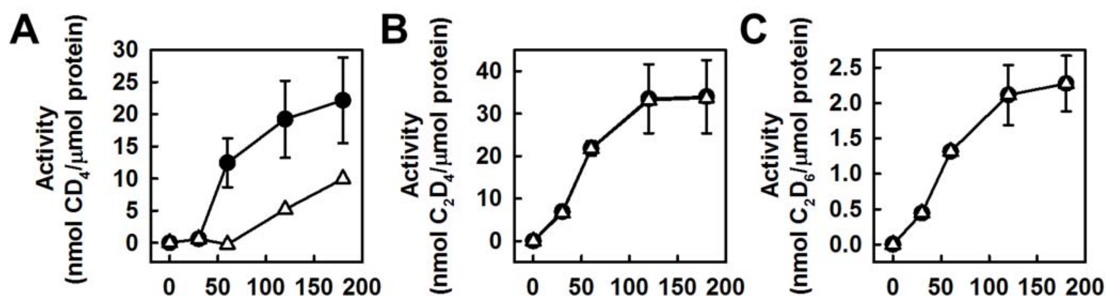

Fig. S2. Contributions of CO_2 or CO_2-derived intermediate(s) other than CO to product formation by V-nitrogenase. Data are taken from Fig. 4. Shown are the CO_2-based time courses (●) and the difference curves between the CO_2- and CO-based time courses (△) of CD_4 (*A*), C_2D_4 (*B*) and C_2D_6 (*C*), which suggests partial (*A*) or full (*B* and *C*) contribution of CO_2 or CO_2-derived intermediate(s) other than CO to product formation by V-nitrogenase. The difference curve in *A* (△) was generated by 'shifting' the time course of CD_4 formation from CO (Fig. 4*A*, ⊙) 30 min to the right and subsequently subtracting it from the time course of CD_4 formation from CO_2 (Fig. 4A, ●).

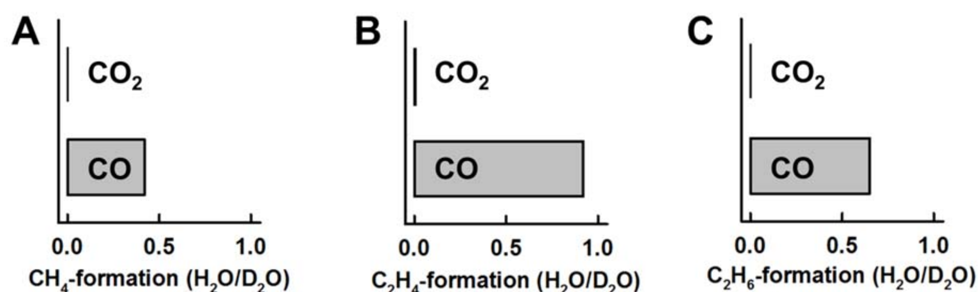

Fig. S3. Formation of hydrocarbon products by V-nitrogenase. Comparison between the CO_2- and CO-based reactions with regard to the percentage activity of CH_4 (*A*), C_2H_4 (*B*) and C_2H_6 (*C*) formation in H_2O relative to that in D_2O. The bar labeled 'CO' was calculated by dividing the activity of product formation from CO in H_2O by that in D_2O; whereas the bar labeled 'CO_2' was calculated by dividing the activity of product formation from CO_2 in H_2O by that in D_2O.

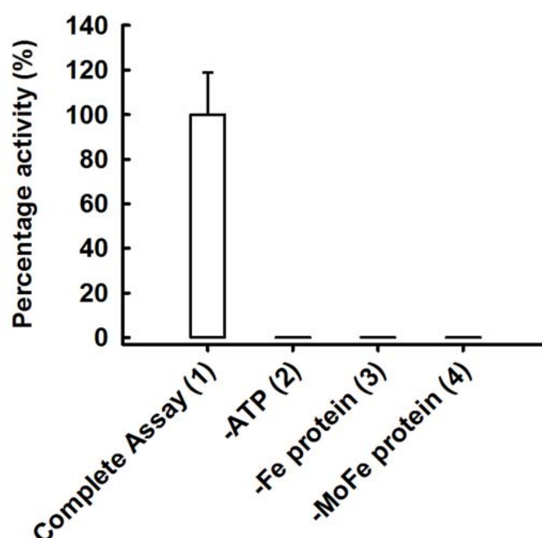

Fig. S4. ATP- and component protein-dependent CH_4 formation by Mo-nitrogenase in the presence of CO_2. Percentage activities of CH_4 formation by Mo-nitrogenase in a complete assay containing ATP and both component proteins (*1*), in the absence of ATP (*2*), in the absence of Fe protein (*3*), and in the absence of MoFe protein (*4*) in H_2O-based reactions. The percentage activity in the absence of ATP or either component protein of Mo-nitrogenase was calculated relative to that in the presence of ATP and dithionite, with the latter set at 100%. The actual specific activity of CH_4 formation in the presence of ATP and dithionite is 3.7 $\pm$ 0.7 nmol/μmol protein/h.

EXPERIMENTAL SECTION

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

Protein Purification. *Azotobacter vinelandii* strains expressing His-tagged MoFe and VFe proteins and non-tagged *vnfH*- and *nifH*-encoded Fe proteins (designated VnfH and NifH, respectively) were grown as described elsewhere.[1-4] Published methods were used for the purification of these nitrogenase protein.[1-4]

Enzymatic Assays. Each H_2O-based reaction of CO_2 reduction had, in a 37.5 mL reaction vial, a total volume of 25 mL, which contained 100 mM Tris (pH 8.0), 20.4 mM MgATP, 43.2 mM $MgCl_2$, 245 mM creatine phosphate, 420 U/mL creatine phosphokinase, 20 mM sodium dithionite and 1 M $NaHCO_3$ as a CO_2 source. This reaction mixture was allowed to equilibrate for 1 h, followed by the adjustment of pH to 8.5. The reaction was started by the addition of 150 mg VnfH plus 100 mg MoFe protein, or 150 mg NifH plus 100 mg VFe protein. Subsequently, the reaction was shaken in a water bath at 30°C and samples were taken at 0, 30, 60, 120 and 180 min, respectively. $NaHCO_3$ was omitted from the assays addressing whether the hydrocarbons generated by CO_2 reduction came indirectly from CO reduction. In these assays, CO were added in the headspaces of the vials and equilibrated at 110 ppm, which represented the maximum amount of CO produced from CO_2 reduction. The D_2O-based assay 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. In addition, all protein samples used in these assays were exchanged into the same deuterated buffer. The pD of this buffer was adjusted to 8.0 with DCl and NaOD by using the pH indicator strips and subsequently determining the pD by a previously established equation:[5] pD = measured pH + 0.40. For GC-MS analysis (*see below*), the H_2O- or D_2O-based assays of CO_2 reduction were prepared as described above except for the substitution of $NaH^{13}CO_3$ for $NaH^{12}CO_3$.

Activity Determination. The products CH_4, C_2H_4 and C_2H_6 were quantified by GC-FID using a previously published method.[6-8] For each assay, a total of 250 μL headspace was injected onto a Grace activated alumina column (Columbia, MD), which was held at 55°C for 1 min, heated to 180°C at 12.5°C/min, and held at 180°C for another 2.6 min. The quantities of these products were determined based on a Scott standard gas mixture (Houston, TX) containing 15 ppm of each hydrocarbon compound. The product CO was quantified by GC coupled to an SRI reduction gas detector (Torrance, CA). For each assay, a total of 250 μL headspace was applied onto a Grace molecular sieve column, which was held at a constant temperature of 50°C. The quantity of CO was determined based on a linear standard curve (R^2 ≥ 98) derived from the injection of varying amounts of ^{12}CO (99.5% purity). The detection limits for the products were, in nmol product/μmol protein, 0.38 (CH_4), 0.23 (C_2H_4), 0.36 (C_2H_6) and 0.1 (CO).

GC-MS Analysis. The hydrocarbon products were identified by GC-MS using an Agilent 6890 GC system (Santa Clara, CA) coupled to a Waters GCT-Premier time-of-flight mass spectrometer (Milford, MA). The identities of CH_4, CD_4, C_2D_4 and C_2D_6 were confirmed by comparing their masses and retention times with those of the Scott standard alkane and alkene gas mixture. For each assay, a total of 50 µL headspace 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. Separation of gas was achieved with an Agilent 0.320 mm (ID) x 30 m (length) HP-PLOT-Q capillary column, which was held at 40°C for 1 min, heated to 45°C at a rate of 5°C/min, heated further to 200°C at a rate of 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.

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