Vanadium Nitrogenase Reduces CO

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The Haber-Bosch (HB) and Fischer-Tropsch (FT) syntheses are important industrial processes for fertilizer and fuel production: The former converts a mixture of dinitrogen (N_2) and hydrogen (H_2) gases into ammonia (NH_3), whereas the latter converts a mixture of carbon monoxide (CO) and H_2 gases into liquid hydrocarbons. Both reactions involve the hydrogenation of isoelectronic small molecules on late transition metal catalysts under high temperature and pressure (*1*, *2*). Yet despite these common traits, the two processes are rarely compared because of the clear distinction between their respective products. Here, we report an unanticipated link between these two formal reactions through a natural source, the vanadium nitrogenase of *Azotobacter vinelandii*.

Like the *nif*-encoded molybdenum nitrogenase, the *vnf*-encoded V nitrogenase is composed of a specific reductant and a catalytic component (*3*). Both nitrogenases use a catalytic mechanism that involves adenosine triphosphate (ATP)–dependent electron transfer from a reductant (i.e., *nifH*- or *vnfH*-encoded Fe protein) to the catalytic component (i.e., *nifDK*-encoded MoFe protein or *vnfDGK*-encoded VFe protein) and the reduction of N_2 at the cofactor site (i.e., FeMoco or FeVco) of the latter. Unlike the HB process, the nitrogenase-based NH_3 synthesis involves addition of separated protons and electrons (rather than intact H_2) across the N_2 triple bond, and H_2 is liberated as a side product (*4*, *5*). In the absence of N_2, H_2 is the sole electron-accepting product of nitrogenase catalysis. Such H_2 evolution by Mo nitrogenase is unaffected by CO, yet the activity of H_2 evolution by V nitrogenase is reduced by an average of 35% in the presence of 100% CO (fig. S1).

We observed that the rates of ATP hydrolysis by Mo and V nitrogenases were comparable under CO, which reflected a similar flux of electrons through the two nitrogenases (fig. S1). One question naturally follows: Could the diminished H_2 evolution by V nitrogenase originate from the diversion of electrons toward CO reduction?

Indeed, we detected ethylene (C_2H_4), ethane (C_2H_6), and propane (C_3H_8) by gas chromatography–mass spectrometry (GC-MS) analysis of the reaction catalyzed by V nitrogenase under 100% CO (Fig. 1, red) (*6*). In contrast, no alkane or alkene formation was observed in the reaction catalyzed by Mo nitrogenase (Fig. 1, black). Isotopic labeling confirmed CO as the carbon source in these products by showing mass shifts of 2, 2, and 3, respectively, of C_2H_4, C_2H_6, and C_3H_8 upon substitution of ^{12}CO with ^{13}CO (Fig. 1).

Like the concomitant evolution of H_2, the reduction of CO required the presence of both component proteins of V nitrogenase, the hydrolysis of ATP, and dithionite as an in vitro electron source (fig. S2). Furthermore, CO reduction by V nitrogenase was inhibited by the addition of increasing amounts of H_2, a well-established inhibitor for N_2 reduction by nitrogenase (fig. S3). The latter observation implies that the reaction mechanism likely involves proton (and electron) transfer to CO rather than direct hydrogenation of CO, in a similar manner to the native N_2 reduction.

The ability of V nitrogenase to catalyze both CO and N_2 reductions suggests a potential link between the evolution of carbon and nitrogen cycles. It has been shown that abiotic substances, such as minerals on submarine vents and nebular dust, are capable of catalyzing FT- and HB-type reactions under extreme conditions (*7*). Perhaps this dual catalytic capacity was assimilated by ancient microbes through a primitive form of nitrogenase (*8*), which evolved solely toward nitrogen fixation following the rise of photosynthesis for carbon fixation.

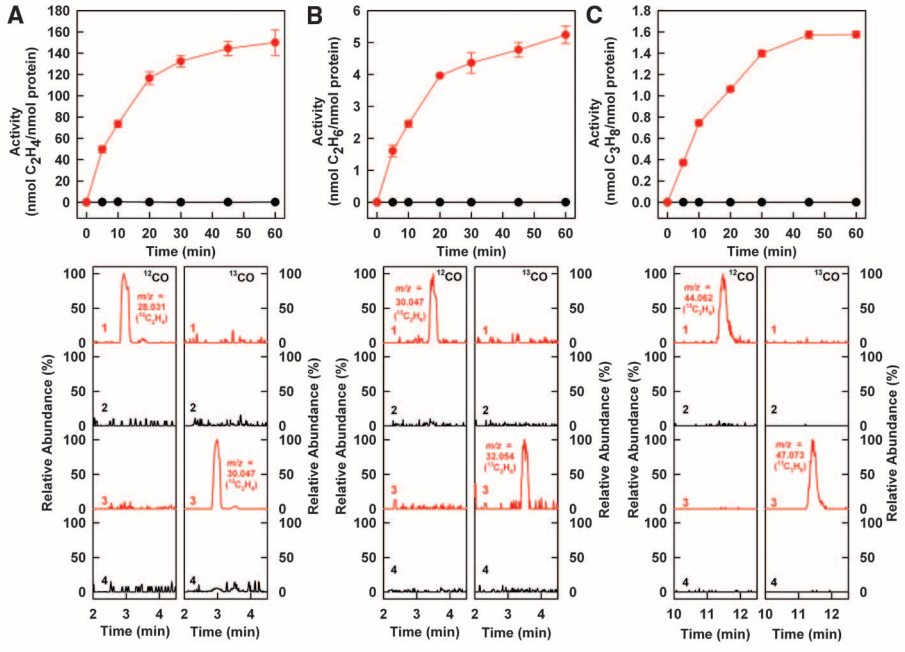

Fig. 1. CO-reducing activity of V nitrogenase. (Top) Time courses of C_2H_4 (**A**), C_2H_6 (**B**), and C_3H_8 (**C**) formation by V (red) and Mo (black) nitrogenases in the presence of 100% CO [data are presented as mean ± SD (N = 5)]. (Bottom) GC-MS analyses of C_2H_4 (A), C_2H_6 (B), and C_3H_8 (C) formed by V and Mo nitrogenases in the presence of 100% CO. The products were analyzed with ^{12}CO or ^{13}CO as the substrate and traced at the following mass-to-charge ratios (*m/z*): (A) 1 and 2, 28.031; 3 and 4, 30.047; (B) 1 and 2, 30.047; 3 and 4, 32.054; (C) 1 and 2, 44.062; 3 and 4, 47.073.

References and Notes

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5. Alternative substrates of Mo nitrogenase include alkynes, cyanides, nitriles, and nitrogen oxides, whereas alternative substrates of V nitrogenase have not been studied extensively (*4*).
6. Materials and methods are detailed in supporting material on *Science* Online.
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8. V nitrogenase is likely more ancient than Mo nitrogenase (*9*), which may explain why V nitrogenase retains the CO-reducing ability.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/329/5992/642/DC1
Materials and Methods
Figs. S1 to S3
References

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