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Vanadium Nitrogenase Reduces CO*

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Abstract

Vanadium nitrogenase not only reduces dinitrogen to ammonia but also reduces carbon monoxide to ethylene, ethane, and propane. The parallelism between the two reactions suggests a potential link in mechanism and evolution between the carbon and nitrogen cycles on Earth.

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 (N₂) and hydrogen (H₂) gases into ammonia (NH₃), whereas the latter converts a mixture of carbon monoxide (CO) and H₂ 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 utilize a catalytic mechanism that involves 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 N₂ at the cofactor site (*i.e.*, FeMoco or Fe-Vco) of the latter. Unlike the HB-process, the nitrogenase-based NH₃ synthesis involves addition of separated protons and electrons (rather than intact H₂) across the N₂ triple bond, and H₂ is liberated as a side product (4, 5). In the absence of N₂, H₂ is the sole electron-accepting product of nitrogenase catalysis. Such H₂-evolution by Mo-nitrogenase is unaffected by CO; yet, the activity of H₂-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 H₂ evolution by V-nitrogenase originate from the diversion of electrons toward CO reduction?

Indeed, we detected ethylene (C₂H₄), ethane (C₂H₆), and propane (C₃H₈) 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/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 C₂H₄, C₂H₆ and C₃H₈ upon substitution of ¹²CO with ¹³CO (Fig. 1).

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Like the concomitant evolution of H₂, 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 H₂, a well-established inhibitor for N₂-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 N₂ reduction.

The ability of V-nitrogenase to catalyze both CO and N₂ reductions suggests a potential link between the evolution of the 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?

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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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).
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Fig. 1. CO-reducing activity of V-nitrogenase. Time-courses of C_2H_4 (A, upper), C_2H_6 (B, upper), and C_3H_8 (C, upper) formation by V- (●) and Mo- (●) nitrogenases in the presence of 100% CO [Data are presented as the means $\pm$ SD ($N = 5$).]; and GC-MS analysis of C_2H_4 (A, lower), C_2H_6 (B, lower), and C_3H_8 (C, lower) formed by V- (red lines) and Mo- (black lines) 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 m/z (lower panels): A,1 and A,2: 28.031; A,3 and A,4: 30.047; B,1 and B,2: 30.047; B,3 and B,4: 32.054; C,1 and C,2: 44.062; C,3 and C,4: 47.073.