Reduction and Condensation of Aldehydes by the Isolated Cofactor of Nitrogenase

Chi Chung Lee,† Yilin Hu,*†‡ and Markus W. Ribbe*†‡

†Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92697-3900, United States
‡Department of Chemistry, University of California, Irvine, California 92697-2025, United States

ABSTRACT: Isolated nitrogenase cofactors can reduce CO, CN−, and CO2 to short-chain hydrocarbons in reactions driven by a strong reductant. Here, we use activity analyses and isotope labeling experiments to show that formaldehyde and acetaldehydes can be reduced as-is or reductively condensed into alkanes and alkenes by the isolated cofactor of Mo-nitrogenase in the presence of EuII-dithylenetriamine pentaacetate (DTPA). Further, we demonstrate that aldehydes can be condensed with CO by the isolated cofactor under the same reaction conditions, pointing to aldehyde-derived species as possible intermediates of nitrogenase-catalyzed CO reduction. Our deuterium labeling experiments suggest the formation of a cofactor-bound hydroxymethyl intermediate upon activation of the formaldehyde, as well as the release of C2H4 as a product upon β-hydride elimination of an acetaldehyde-derived hydroxyethyl intermediate. These findings establish the reductive condensation of aldehydes as a previously unobserved reactivity of a biogenic catalyst while at the same time shed light on the mechanism of enzymatic CO reduction and C–C bond formation, thereby providing a useful framework for further exploration of the unique reactivity and potential applications of nitrogenase-based reactions.

INTRODUCTION

Nitrogenase is known for its key role in the global nitrogen cycle, catalyzing the ambient reduction of atmospheric N2 to bioavailable NH4+.4−7 Recently, nitrogenase was shown to reduce C1 substrates, such as CO and CN−,5−7 to short-chain alkanes and alkenes under ambient conditions, thereby gaining recognition as a versatile metalloenzyme capable of both Haber-Bosch9 and Fischer–Tropsch10,11 like reactions. The “conventional” Mo-nitrogenase utilizes a two-component system for catalysis, with a reductase component (Fe protein) transferring electrons—concomitant with ATP hydrolysis—to the cofactor site (M-cluster) of the catalytic component (MoFe protein) for substrate reduction (Figure S1A). Interestingly, the protein-bound M-cluster only shows a marginal activity of CO reduction that is 700–800 times lower than that of its protein-bound counterpart in the “alternative” V-nitrogenase; upon extraction into an organic solvent, however, the isolated M-cluster is comparable to its V-counterpart in total carbon turnover yields when catalyzing the reduction of CO and other C1 substrates (i.e., CN− and CO2), in the presence of a strong reductant (e.g., EuIII-DTPA or SmIII) (Figure S1B).12,13 The ability of the extracted M-cluster to catalyze the reduction of C1 substrates is important, as it presents a unique opportunity for us to investigate the reactivity of isolated cofactors toward certain oxygenated carbon species, such as aldehydes, alcohols, and acetone, which cannot be directly applied to the protein-bound cofactors because of their destabilizing effects on protein structures. Knowledge in this regard will not only enable a further expansion of the catalytic repertoire of nitrogenase cofactors, but also lead to a better understanding of the mechanism of enzymatic CO reduction and C–C bond formation, as these oxygenated carbon species are potential intermediates of this nitrogenase-catalyzed reaction.14,15

RESULTS AND DISCUSSION

We started out by testing if oxygenated C1 and C2 species, including aldehydes, alcohols, and acetone, could be converted by the isolated M-cluster into hydrocarbon products. (Note: no unexpected safety hazards were encountered.) Driven by EuIII-DTPA (E0 = −1.14 V at pH 8)16 in a H2O-based reaction, the isolated M-cluster was capable of converting aldehydes (CH3O, CH2OH), but not alcohols (CH3OH, CH3CH2OH) or acetone ((CH3)2CO), into hydrocarbons (Figure 1A). GC-MS analysis confirmed that the hydrocarbon products were derived from the aldehyde substrates, showing the expected mass shifts and fragmentation patterns upon substitution of 13C-aldehyde for the corresponding 12C-aldehyde (Figure 1B,C; also see Figure S5). Notably, formaldehyde (CH2O) gave rise to C1–C4 alkanes (C2H6, C3H8, C4H10) and acetaldehyde (CH3CHO) gave rise to C2 and C4 alkanes (C2H4, C2H6, C3H6, C4H8), suggesting that aldehyde was either reduced as-is or coupled with each other into the respective products (see Figure 1A). Moreover, compared to CO, both formaldehyde...
and acetaldehyde were converted by the isolated M-cluster to hydrocarbons at considerably higher yields (see Figure 1A). Together, these observations firmly established aldehyde as a substrate of the isolated nitrogenase cofactor, which can undergo direct reduction or reductive condensation into hydrocarbon products.

The newly discovered reactivity of the isolated cofactor toward aldehydes provided a platform for us to use isotope labeling experiments to probe the mechanism of cofactor-based aldehyde reduction. The first question we asked was whether activation of formaldehyde (CH₂O) would result in a cluster-bound hydroxymethyl intermediate via several plausible
routes. Should this be the case, the hydroxymethyl species would need to undergo a series of proton/electron transfer steps, coupled with removal of oxygen as water, to generate CH4 that contains two substrate-derived hydrogens and two solution-derived hydrogens (Figure 2A). To test our hypothesis, we monitored the amount of H/D labels in product CH4 when CH2O and CD2O were supplied as the respective substrates to D2O- and H2O-based reactions (Figure 2A, ①, ②). GC-MS analysis revealed formation of CH2D2 as the predominant species upon reduction of CH2O in a D2O-based reaction (Figure 2B, ①), or upon reduction of CD2O in an H2O-based reaction (Figure 2B, ②; also see Figure S6A). The appearance of two solution-derived hydrogens in CH4 would be consistent with the appearance of a cluster-bound hydroxymethyl intermediate as an activated C1 species in the cofactor-catalyzed reaction of aldehyde reduction (see Discussion in the Supporting Information for consideration of alternative mechanisms).

Having tackled the question related to the identity of the activated C1 species, we then asked the question of whether the product formed upon C−C bond formation could be released via β-hydride elimination. In this scenario, a partially reduced, cofactor-bound C2 species—such as a cofactor-bound hydroxyethyl group derived from acetaldehyde (C2H4O)—could undergo multiple proton/electron transfer events that are coupled with the removal of its oxygen atom as water, as well as β-hydride elimination that occurs concurrently with the formation of a C=C bond, which results in the release of C2H4 as a product (Figure 3A). To test our hypothesis, we monitored the amount of D labels in product C2H4 when CD3CDO (i.e., CD3CHO or CD3CDO) was used as a substrate in an H2O-based buffer system (Figure 3A, ①, ②). GC-MS analysis demonstrated a predominant formation of C2H3D3 (i.e., CD2CDH) when CD3CDO was used as a substrate (Figure 3B, ①), and the predominant formation of C2H2D2 (i.e., CD2CH2) when CD3CHO was used as a substrate (Figure 3B, ②; also see Figure S6B). The observation that only one β-hydrogen of C2H4O was replaced in both cases strongly pointed to a mechanism of product release via β-hydride elimination (see Discussion in the Supporting Information for consideration of alternative mechanisms).

The successful identification of formaldehyde- and acetaldehyde-derived species as catalytically competent intermediates for cofactor-based hydrocarbon formation led to the question of whether these intermediates could be shared by other carbon substrates, such as CO and CN−, in the same type of reactions catalyzed by the cofactor of nitrogenase. To address
In this question, we examined whether the C1 or C2 aldehyde could be coupled with CO into C2 or C3 products. Excitingly, when CO was supplied in addition to formaldehyde (CH2O), there was an increase in the yield of C2 products (C2H4, C2H6), whereas when CO was supplied in addition to acetaldehyde (C2H4O), C3 products (C3H6, C3H8) were absent when CH3CHO was supplied as the sole substrate were detected in the reaction (Figure 4A, highlights). Similar effects were observed when CN−, another known C1 substrate of the nitrogenase cofactor, was supplied in addition to CH2O or C2H4O to the reaction (Figure 4A, highlights). GC-MS analysis further confirmed a cross condensation between CH2O (left) or CH3CHO (right) and CO or CN− are highlighted. TON, turnover number, calculated based on the number of reduced carbons in hydrocarbon products. (B, C) GC-MS fragmentation patterns of C2 (B) and C3 (C) hydrocarbons generated from the cross condensation between 13CH2O (B) or 13CH3CHO (C) and 12CO or 12CN−. The masses of the base peaks of products are indicated (B, C). Note that, as expected, C1 products were not detected in the activity assay (A) and GC-MS analysis (C) when CH3CHO was supplied alone as a substrate.

Interestingly, Fe-bound C1 (hydroxymethyl) and C2 (hydroxyethyl) species have been suggested by density functional theory (DFT) calculations as potential intermediates of CO2 reduction by synthetic [Fe4S4] clusters in a recent study that excluded condensation between two neighboring, cofactor-bound CO moieties and pointed to aldehydes as possible intermediates along the reaction pathway of CO reduction. Interestingly, Fe-bound C1 (hydroxymethyl) and C2 (hydroxyethyl) species have been suggested by density functional theory (DFT) calculations as potential intermediates of CO2 reduction by synthetic [Fe4S4] clusters in a recent study that excluded condensation between two neighboring, cofactor-bound CO moieties and pointed to aldehydes as possible intermediates along the reaction pathway of CO reduction.
reaction driven by SmI₂ (E° ≈ −1.5 V in DMF). It is appealing, therefore, to consider the plausible scenario that the cofactor of nitrogenase—a complex FeS cluster variant—also reduces CO via certain aldehyde-derived intermediates. The significant increase of hydrocarbon yields when aldehydes are supplied as substrates instead of CO seems to support this argument, as aldehydes are further reduced and, therefore, “easier” substrates to enter the CO reduction pathway.

The involvement of β-hydride elimination in the termination step of alkene formation does not come as a complete surprise, not only because of its prevalence in organometallic chemistry, but also because of its implications in nitrogenase-catalyzed reactions. Previously, it was observed that ethene was predominately produced in the enzymatic CO condensation reaction catalyzed by nitrogenase, which points to a possible product release mechanism via β-hydride elimination in this case. Recently, it was proposed that metal hydride could play a central role in the reduction of N₂ by nitrogenase. In this proposal, the protein-bound M-cluster would first accumulate hydrides as a form of reducing equivalents, which can then be reductively eliminated to generate a “super reduced” state of the metal center for N₂ activation. While the involvement of a β-hydride elimination step in CO reduction seems to serve a different purpose in terminating the reaction and facilitating product release, it could act in a manner analogous to that proposed for N₂ reduction by “shortcutting” the reduction cycle via a “recharge” of the metal center with one hydride for the subsequent reduction events along the CO reduction pathway.

The reductive condensation of aldehydes that directly leads to the formation of hydrocarbons is, to our knowledge, unprecedented in biochemistry. The aldehyde dehydrogenating oxygenase (ADO), a diiron oxygenase that converts aldehyde to hydrocarbon and formate via oxidative cleavage of the aldehydic carbon, is one loosely related biological example along this line of reactivity. In the context of organometallic chemistry, reactions catalyzed by transition metals seem to utilize a mechanism that is distinct from the reaction catalyzed by the M-cluster in that they typically generate alcohols by coupling aldehydes and ketones with olefins or other reactive species. The formation of hydrocarbons in these reactions would require much harsher conditions.

It is important to note that a large variety of homogeneous systems that mimic the Fischer–Tropsch synthesis (FTS) have been documented in the literature. Early development of these systems focused on direct reduction of CO with hydrides, such as the reduction of CpFe(CO)₃⁺ with NaBH₄ or conversion of CO to reduced C₂ species, by metal hydride complexes. In addition, substantial efforts were dedicated to development of metal clusters as FTS models based on the premise that metal clusters, particularly heterobimetallic systems, could exhibit metal surface reactivity patterns in CO activation and C–C bond coupling. One relatively well-known example of this genre is the lanthanide-hydride tetraramer, which react with CO under ambient conditions to generate ethylene. This is one of the few systems capable of performing the facile conversion of CO to hydrocarbons, encompassing some of the difficult steps, such as C–O bond cleavage and product release. However, upon removal of the oxygen atom, the starting cluster is converted into lanthane-dioxide cubanes, rendering the reaction not catalytic. Another outstanding example that deals with a similar oxygen removal scenario is the terphenyl-diphosphone coordinated molybdenum complex, which is capable of scission of the C–O bond, C–C coupling, and spontaneous dissociation of the resulting C fragment. In this case, Me₃SiCl is utilized to efficiently attack and remove oxygen in the presence of K⁺ to form reactive metal carbene species. It is worth noting that, as observed in the transition metal-catalyzed reactions of ketone/aldehyde reduction, our M-cluster-based reaction is efficient in removing oxygen and hydroxyl because of the protic/aqueous reaction system.

Interestingly, it has been demonstrated for the industrial FTS that aldehyde can be incorporated into hydrocarbons when it is supplied along with syngas in the feedstock, which allows for more selective synthesis of desired hydrocarbon products, depending on the aldehyde additive supplied to the reaction. Given the observation of aldehyde condensation with CO, a similar functionality may also be achieved by catalysts based on nitrogenase cofactors. Continued exploration of this reactivity, in combination with further mechanistic investigations into the enzymatic CO reduction and C–C bond formation, could facilitate future developments of nitrogenase-based catalysts for synthesis of valuable chemical commodities.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscentsci.8b00553.

Materials and methods, Discussions, and eight supporting figures (PDF)

**AUTHOR INFORMATION**

**Corresponding Authors**

*Y.H.) E-mail: yilinh@uci.edu.

*(M.W.R.) E-mail: mrribbe@uci.edu.

**ORCID**

Yilin Hu: 0000-0002-9088-2865

Markus W. Ribbe: 0000-0002-7366-1526

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This work was supported by NSF Grant CHE-1608926 (to M.W.R. and Y.H.).

**REFERENCES**

(1) Burgess, B. K.; Lowe, D. J. Mechanism of molybdenum nitrogenase. Chem. Rev. 1996, 96, 2983–3012.

(2) Howard, J. B.; Rees, D. C. Structural basis of biological nitrogen fixation. Chem. Rev. 1996, 96, 2965–2982.

(3) Eady, R. R. Structure-function relationships of alternative nitrogenases. Chem. Rev. 1996, 96, 3013–3030.

(4) Hoffman, B. M.; Lukoyanov, D.; Yang, Z. Y.; Dean, D. R.; Seefelder, L. C. Mechanism of nitrogen fixation by nitrogenase: the next stage. Chem. Rev. 2014, 114, 4041–4062.

(5) Lee, C. C.; Hu, Y.; Ribbe, M. W. Vanadium nitrogenase reduces CO. Science 2010, 329, 642.

(6) Hu, Y.; Lee, C. C.; Ribbe, M. W. Extending the carbon chain: hydrocarbon formation catalyzed by vanadium/molybdenum nitrogenases. Science 2011, 333, 753–755.

(7) Hu, Y.; Ribbe, M. W. Nitrogenases—a tale of carbon atom(s). Angew. Chem., Int. Ed. 2016, 55, 8216–8226.
Schögl, R. Catalytic synthesis of ammonia—a "never-ending story"? Angew. Chem., Int. Ed. 2003, 42, 2004–2008.

Cherkasov, N.; Ihabdon, A. O.; Fitzpatrick, P. A review of the existing and alternative methods for greener nitrogen fixation. Chem. Eng. Process. 2015, 90, 24–33.

Roer-DePoofter, C. K. A comprehensive mechanism for the Fischer–Tropsch synthesis. Chem. Rev. 1981, 81, 447–474.

Miglio, R.; Zennaro, R.; de Klerk, A. Environmental sustainability. In Greener Fischer–Tropsch Processes for Fuels and Feedstocks; Maitlis, P.; de Klerk, A., Eds.; Wiley-VCH: Weinheim, 2013; pp 311–336.

Lee, C. C.; Hu, Y.; Ribbe, M. W. Insights into hydrocarbon formation by nitrogenase cofactor homologs. mBio 2015, 6, e00307–e00315.

Lee, C. C.; Hu, Y.; Ribbe, M. W. Catalytic reduction of CN\(^{-}\), CO, and CO\(_2\) by nitrogenase cofactors in lanthanide-driven reactions. Angew. Chem., Int. Ed. 2015, 54, 1219–1222.

Dance, I. How does vanadium nitrogenase reduce CO to hydrocarbons? Dalton Trans 2011, 40, 5516–5527.

Dance, I. Calculated vibrational frequencies for FeMo-co, the active site of nitrogenase, bearing hydrogen atoms and carbon monoxide. Dalton Trans 2011, 40, 6480–6489.

Vincent, K. A.; Tilley, G. J.; Quamme, N. C.; Streeter, I.; Burgess, B. K.; Cheesman, M. R.; Armstrong, F. A. Instantaneous, stoichiometric generation of powerfully reducing states of protein active sites using Eu(II) and polyaminocarboxylate ligands. Chem. Commun. 2003, 20, 2590–2591.

Vargas, R. M.; Theys, R. D.; Hossain, M. M. A new reaction for the synthesis of carbene precursors from aldehydes and Cp-(CO)\(_2\)Fe\(^{M+}\) (M = Na, K). J. Am. Chem. Soc. 1992, 114, 777–778.

Wang, Q.; Försterling, F. H.; Hossain, M. M. Enantiomeric cis-cyclopropane synthesis using the chiral iron carbene complexes S-\(\eta^5\)-C\(_5\)H\(_5\))(CO)\(_2\)Fe = CH\(((\eta^5-C\(_5\)H\(_5\))(CO))\(_2\)Fe\). Organometallics 2002, 21, 2596–2598.

Theys, R. D.; Vargas, R. M.; Wang, Q.; Hossain, M. M. A method for the synthesis of carbene precursors from aldehydes and the metallas(\(\eta^5\)-C\(_5\)H\(_5\))(CO)\(_2\)Fe\(^-\) and (\(\eta^5\)-C\(_5\)H\(_5\))(CO)\(_2\)Fe\(^+\)). Organometallics 1998, 17, 1333–1339.

Crabtree, R. H. Insertion and elimination. In The Organometallic Chemistry of the Transition Metals, 6th ed.; John Wiley & Sons: Hoboken, NJ, 2014; pp 185–203.

Lee, C. C.; Wilcoxen, J.; Hiller, C. J.; Britt, R. D.; Hu, Y. Evaluation of the catalytic relevance of the CO-bound states of V-nitrogenase. Angew. Chem., Int. Ed. 2018, 57, 3411–3414.

Stiebritz, M. T.; Hiller, C. J.; Sickerman, N. S.; Lee, C. C.; Tanifuji, K.; Ohki, Y.; Hu, Y. Ambient conversion of CO\(_2\) to hydrocarbons by biogenic and synthetic [Fe\(_{5}\)S\(_{5}\)] clusters. Nat. Catal. 2018, 1, 444–451.

Harris, D. F.; Lukoyanov, D. A.; Shaw, S.; Compton, P.; Tokmina-Lukaszewska, M.; Bothner, B.; Kelleher, N.; Dean, D. R.; Hoffman, B. M.; Seefeldt, L. C. Mechanism of N\(_2\) reduction catalyzed by Fe-nitrogenase involves reductive elimination of H\(_2\). Biochemistry 2018, 57, 701–710.

Gu, N. X.; Oyala, P. H.; Peters, J. C. An S = 1/2 iron complex featuring N\(_2\), thiolate, and hydride ligands: reductive elimination of H\(_2\) and relevant thermochemical Fe-H parameters. J. Am. Chem. Soc. 2018, 140, 6374–6382.

Li, N.; Chang, W. C.; Warui, D. M.; Booker, S. J.; Krebs, C.; Bollinger, J. M. Evidence for only oxygenative cleavage of aldehydes to alk(a/e)nones and formate by cyanobacterial aldehyde decarboxylases. Biochemistry 2012, 51, 7908–7916.

Holmes, M.; Schwartz, L. A.; Krische, M. J. Intermolecular metal-catalyzed reductive coupling of dienes, alenes, and enynes with carbonyl compounds and imines. Chem. Rev. 2018, 118, 6026–6052.

West, N. E.; Miller, A. J. M.; Labinger, J. A.; Bercaw, J. E. Homogeneous syngas conversion. Coord. Chem. Rev. 2011, 255, 881–898.

Cutler, A. R.; Hanna, P. K.; Vites, J. C. Carbon monoxide and carbon dioxide fixation: relevant C1 and C2 ligand reactions emphasizing (\(\eta^5\)-C\(_5\)H\(_5\))Fe-containing complexes. Chem. Rev. 1988, 88, 1363–1403.

Wolczanski, P. T.; Bercaw, J. E. Mechanisms of carbon monoxide reduction with zirconium hydrides. Acc. Chem. Res. 1980, 13, 121–127.

Rittleng, V.; Chetcuti, M. J. Hydrocarbyl ligand transformations on heterobimetallic complexes. Chem. Rev. 2007, 107, 797–858.

Wheatley, N.; Kalck, P. Structure and reactivity of early–late heterobimetallic complexes. Chem. Rev. 1999, 99, 3379–3420.

Shima, T.; Hou, Z. Hydrogenation of carbon monoxide by tetranuclear rare earth metal polyhydrido complexes. Selective formation of ethylene and isolation of well-defined polyoxo rare earth metal clusters. J. Am. Chem. Soc. 2006, 128, 8124–8125.

Buss, J. A.; Agapie, T. Four-electron deoxygenative reductive coupling of carbon monoxide at a single metal site. Nature 2016, 529, 72–75.

Buss, J. A.; Agapie, T. Mechanism of molybdenum-mediated carbon monoxide deoxygenation and coupling: mono- and i-carbene complexes precede C–O bond cleavage and C–C bond formation. J. Am. Chem. Soc. 2016, 138, 16466–16477.

Ordosmany, V. V.; Khodakov, A. Y. Syngas to chemicals: the incorporation of aldehydes into Fischer–Tropsch synthesis. ChemCatChem 2017, 9, 1040–1046.