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## Catalytic reduction of CN<sup>-</sup>, CO and CO<sub>2</sub> by nitrogenase cofactors in lanthanide-driven reactions\*\*

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### Abstract

Nitrogenase cofactors can be extracted into an organic solvent and added in an adenosine triphosphate (ATP)-free, organic solvent-based reaction medium to catalyze the reduction of cyanide (CN<sup>-</sup>), carbon monoxide (CO) and carbon dioxide (CO<sub>2</sub>) when samarium (II) iodide (SmI<sub>2</sub>) and 2,6-lutidinium triflate (Lut-H) are supplied as a reductant and a proton source, respectively. Driven by SmI<sub>2</sub>, the cofactors not only catalytically reduce CN<sup>-</sup> or CO to C1-C4 hydrocarbons, but also catalytically reduce CO<sub>2</sub> to CO and C1-C3 hydrocarbons. The observation of C-C coupling from CO<sub>2</sub> reveals a unique, Fischer-Tropsch-like reaction with an atypical carbonaceous substrate; whereas the achievement of catalytic turnover of CN<sup>-</sup>, CO and CO<sub>2</sub> by isolated cofactors suggests the possibility to develop nitrogenase-based electrocatalysts for hydrocarbon production from these carbon-containing compounds.

### Keywords

nitrogenase; carbon dioxide; carbon monoxide; C-C coupling; hydrocarbon

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Nitrogenase is a uniquely versatile metalloenzyme that catalyzes the reduction of various substrates, such as nitrogen (N<sub>2</sub>), carbon monoxide (CO) and cyanide (CN<sup>-</sup>), at its cofactor site.<sup>[1–4]</sup> The molybdenum (Mo)- and vanadium (V)-nitrogenases are two homologous members of this enzyme family, which contain homologous cofactors—the molybdenum-iron cofactor (designated the M-cluster) and the vanadium-iron cofactor (designated the V-cluster)—at their respective active sites.<sup>[1,5]</sup> The M-cluster (Fig. S1A) is a [MoFe<sub>7</sub>S<sub>9</sub>C] cluster that can be viewed as [Fe<sub>4</sub>S<sub>3</sub>] and [MoFe<sub>3</sub>S<sub>3</sub>] subclusters bridged by three equatorial

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Supporting information for this article including experimental procedures, Table S1 and Figures S1–S2 is given via a link at the end of the document.

$\mu_2$ -sulfides and one interstitial  $\mu_6$ -carbide. In addition, this cofactor has an endogenous compound, homocitrate, attached to its Mo end.<sup>[6–8]</sup> The V-cluster (Fig. S1B) is nearly identical to the M-cluster in structure except for the substitution of V for Mo and a slight elongation of the metal-sulfur core of this cluster.<sup>[9,10]</sup> Apart from the two cofactors, a third cluster species has been identified both as a biosynthetic intermediate and as a structural homolog of the M-cluster. Designated the L-cluster (Fig. S1C), this  $[\text{Fe}_8\text{S}_9\text{C}]$  cluster represents an all-iron version of the cofactor, as it closely resembles the core structure of the mature M-cluster except for the substitution of Fe for Mo and homocitrate at one end.<sup>[11–13]</sup> The structural homology between the L-cluster and the two cofactors is striking; more importantly, it suggests a close resemblance of these clusters to one another in their catalytic capacities.

Such a resemblance indeed exists between the M- and V-clusters, as both cofactors can be extracted from protein into an organic solvent, *N*-methylformamide (NMF),<sup>[10]</sup> and directly used as a catalyst to reduce  $\text{CN}^-$  or CO to hydrocarbons in the presence of a strong reductant, europium (II) diethylenetriamine-pentaacetate ( $\text{Eu}^{\text{II}}$ -DTPA).<sup>[14]</sup> Driven by  $\text{Eu}^{\text{II}}$ -DTPA ( $E^{0'} = -1.14$  V at pH 8), both cofactors generate alkanes and alkenes of varying lengths as products of  $\text{CN}^-$  or CO reduction at comparable efficiencies. Additionally, they both display a strong preference of  $\text{CN}^-$  over CO as a substrate, which may originate from a stabilizing effect of  $\text{CN}^-$  on certain oxidation states of the two cofactors.<sup>[14]</sup> However,  $\text{Eu}^{\text{II}}$ -DTPA is not a strong enough reductant to drive the catalytic turnover of CO by either cofactor, as the turnover numbers (TON) of CO by both cofactors are less than 1.<sup>[15]</sup> Moreover, this reductant does not support the reduction of  $\text{CO}_2$  by the cofactors, an event that requires more reducing power than the reduction of  $\text{CN}^-$  or CO.<sup>[16]</sup> This observation prompts the questions of (i) whether CO and  $\text{CO}_2$  can be catalytically turned over by these clusters in the presence of an appropriate reductant; and (ii) if the L-cluster resembles the M- and V-clusters in the conversion of carbon-containing compounds to hydrocarbons.

The answers to both questions are yes. When  $\text{Eu}^{\text{II}}$ -DTPA is replaced by a stronger reductant, samarium (II) iodide ( $\text{SmI}_2$ ),<sup>[17]</sup> the NMF-extracted M-, V- and L-clusters are all capable of turning over  $\text{CN}^-$ , CO and  $\text{CO}_2$  under ambient conditions in an organic solvent-based reaction medium. Driven by  $\text{SmI}_2$  [ $E^{0'} = -1.55$  V in tetrahydrofuran (THF)] and using protons supplied by 2,6-lutidinium triflate (Lut-H),<sup>[18]</sup> the three clusters not only can reduce  $\text{CN}^-$  (Fig. 1A, upper; Table S1) and CO (Fig. 1B, upper; Table S1) to  $\text{CH}_4$ ,  $\text{C}_2\text{H}_4$ ,  $\text{C}_2\text{H}_6$ ,  $\text{C}_3\text{H}_6$ ,  $\text{C}_3\text{H}_8$ , 1- $\text{C}_4\text{H}_8$  and *n*- $\text{C}_4\text{H}_{10}$ , but also can reduce  $\text{CO}_2$  to CO,  $\text{CH}_4$ ,  $\text{C}_2\text{H}_4$ ,  $\text{C}_2\text{H}_6$ ,  $\text{C}_3\text{H}_6$  and  $\text{C}_3\text{H}_8$  (Fig. 1C, upper; Table S1). Gas chromatograph-mass spectrometry (GC-MS) analysis confirms  $\text{CN}^-$ , CO and  $\text{CO}_2$  as the carbon sources for the hydrocarbons generated in these reactions, as all products display the expected mass shifts upon substitution of  $^{13}\text{CN}^-$ ,  $^{13}\text{CO}$  and  $^{13}\text{CO}_2$ , respectively, for  $^{12}\text{CN}^-$  (Fig. 1A, lower),  $^{12}\text{CO}$  (Fig. 1B, lower) and  $^{12}\text{CO}_2$  (Fig. 1C, lower). Activity analysis further reveals that all three clusters turn over  $\text{CN}^-$ , CO and  $\text{CO}_2$  catalytically (*i.e.*,  $\text{TON} > 1$ ) in the presence of  $\text{SmI}_2$ , with the M-, V- and L-clusters showing TONs of 15, 13 and 13, respectively, for  $\text{CN}^-$  (Fig. 2A); 3.0, 2.7 and 4.5, respectively, for CO (Fig. 2B); and 1.4, 1.8 and 2.3, respectively, for  $\text{CO}_2$  (Fig. 2C). While the preference of  $\text{CN}^-$  as a substrate is preserved by all three clusters in reactions driven by  $\text{SmI}_2$ , the observation of the catalytic turnover of CO and  $\text{CO}_2$  by these clusters in the

presence of this reductant is particularly exciting, as it not only illustrates the impact of redox potential on the catalytic efficiency and substrate range of nitrogenase cofactors, but also defines a previously-unobserved, ATP-independent reaction that involves the conversion of CO<sub>2</sub> to hydrocarbons by these unique metal clusters in the isolated forms.

It should be noted that the ATP-dependent reduction of CO<sub>2</sub> was reported both for a variant of Mo-nitrogenase and for the wild-type V-nitrogenase;<sup>[19–21]</sup> however, CH<sub>4</sub> was detected as the sole hydrocarbon product in the case of the former,<sup>[20]</sup> whereas C<sub>2</sub> alkanes and alkenes were detected only upon substitution of D<sub>2</sub>O for H<sub>2</sub>O in the case of the latter.<sup>[21]</sup> In comparison, the isolated cofactors are “pushed” by SmI<sub>2</sub> not only toward the formation of C-C bond (*i.e.*, >C1 products), but also toward the formation of longer carbon chains (*i.e.*, up to C3 products) from CO<sub>2</sub> (Fig. 1C). The C<sub>2</sub> and C<sub>3</sub> hydrocarbons do not originate from the coupling between the CO<sub>2</sub>-derived CO in the SmI<sub>2</sub>-driven reactions, as these products cannot be detected if CO is supplied directly as a substrate at the same concentration as the maximum amount of CO generated from CO<sub>2</sub> reduction (Fig. S2). Further, the reduction of CO<sub>2</sub> to CO and hydrocarbons is carried out by protons (H<sup>+</sup>) and electrons in these reactions, and it is accompanied by the reduction of H<sup>+</sup> to hydrogen (H<sub>2</sub>) (Table S1).

Interestingly, the activities of the three clusters seem to be “normalized” upon isolation from their respective protein environments. In addition to turning over each substrate with comparable TONs, these clusters also generate the same range of products at similar percentages from the same substrate. All of them display a strong tendency toward the formation of C<sub>2</sub> products from CN<sup>-</sup> (Fig. 2A) and CO (Fig. 2B), with the C<sub>1</sub> (CH<sub>4</sub>) and C<sub>2</sub> (C<sub>2</sub>H<sub>4</sub>, C<sub>2</sub>H<sub>6</sub>) products comprising a major portion (90.3–97.8%) of the product profiles of these reactions. The tendency toward formation of small products is even more apparent in the cases of CO<sub>2</sub> reduction by these clusters, where the C<sub>1</sub> products (CO, CH<sub>4</sub>) constitute the predominant portion (97.1–97.5%) of the product profiles (Fig. 2C). In all these reactions, CH<sub>4</sub> is the singularly dominant hydrocarbon product, which consists of 58.2–78.1% of the total amount of products. Such a strong shift toward CH<sub>4</sub> is not observed in the reaction of CO reduction by the protein-bound M- or L-cluster,<sup>[2,3]</sup> where C<sub>2</sub>H<sub>4</sub> is produced as the major product along with a more evenly distributed product profile toward longer hydrocarbons. Moreover, the “normalization” of the isolated M- or L-cluster in the reaction efficiency and product distribution of CO reduction contrasts the approximately 700-fold activity difference and a significant disparity in product formation between their protein-bound counterparts,<sup>[3]</sup> highlighting the impact of protein environment on the reactivities of nitrogenase cofactors.

Apart from the protein environment, variations of the cofactor composition, particularly those at the “heterometal end”, seem to play a role in modulating the catalytic properties of these clusters. A good example in this regard is the higher TONs of CO (Fig. 2B) and CO<sub>2</sub> (Fig. 2C) by the L-cluster, an all-iron form of the cofactor, than those by the M- and V-clusters. Moreover, among the three clusters, the L-cluster forms the highest percentage of CH<sub>4</sub> from the reduction of all three substrates and, in the reactions of CN<sup>-</sup> (Fig. 2A) and CO (Fig. 2B) reduction, the increased formation of CH<sub>4</sub> by L-cluster is accompanied by a decreased formation of C<sub>2</sub>H<sub>4</sub>, consistent with a preference of this cluster to reduce CN<sup>-</sup> and CO all the way to CH<sub>4</sub> over the C-C coupling of these substrates into C<sub>2</sub>H<sub>4</sub>. Strikingly, an

analogous reaction was shown to be enabled by iron sulfide (FeS), a simplest FeS unit; only in this case, methanethiol (CH<sub>3</sub>SH) was generated as a product of CO<sub>2</sub> reduction in the presence of FeS and hydrochloric acid (HCl).<sup>[22]</sup> The increased formation of CH<sub>4</sub> by L-cluster is not only interesting because of the value of CH<sub>4</sub> as a fuel source, but also important because of the all-iron composition of the L-cluster (*see* Fig. S1), which may simplify the task of synthesizing biomimetic nitrogenase “cofactors” by omitting the need to incorporate heterometal and homocitrate.

Together with the M- and V-clusters, the L-cluster forms a group of homologous, high-nuclearity metal-sulfur clusters that are capable of catalyzing the unique conversion of CN<sup>-</sup>, CO and CO<sub>2</sub> to hydrocarbon products. The success in achieving the catalytic turnover of CO and CO<sub>2</sub> by these clusters in the presence of a stronger reductant, SmI<sub>2</sub>, suggests the possibility to develop nitrogenase-based electrocatalysts for further improvement of catalytic efficiency and substrate range; whereas the observation of the differences between the activities of the protein-bound and NMF-extracted clusters, as well as the differences between the activities of the isolated clusters, implies the potential to alter the product profiles of these reactions by varying the compositions of the clusters and attaching the clusters to artificial matrices for further modulation of their catalytic properties. Perhaps most excitingly, these studies have led to the identification of a room-temperature, Fisher-Tropsch (F-T) type reaction with an atypical F-T substrate, CO<sub>2</sub>.<sup>[23]</sup> The formation of CO in this reaction is likely analogous to the reaction of reverse water-gas shift (*i.e.*, CO<sub>2</sub> + H<sub>2</sub> → CO + H<sub>2</sub>O).<sup>[24]</sup> Only in this case, the expensive syngas, H<sub>2</sub>, is replaced by H<sup>+</sup> (provided by LutH) and e<sup>-</sup> (supplied by SmI<sub>2</sub>), and it is further produced as an abundant side product of H<sup>+</sup> reduction (*see* Table S1). The formation of hydrocarbons also utilizes H<sup>+</sup> as a hydrogen source, and this reaction likely involves direct C-C coupling from CO<sub>2</sub> or CO<sub>2</sub>-derived intermediate(s) other than CO (*see* Fig. S2). As such, the reduction of CO<sub>2</sub> to CO and hydrocarbons by M-, V- and L-clusters not only defines two unique reactions that are related to two important industrial processes, but also bears potential to serve as a blueprint for future design of strategies to recycle CO<sub>2</sub> into the useful carbon fuels.

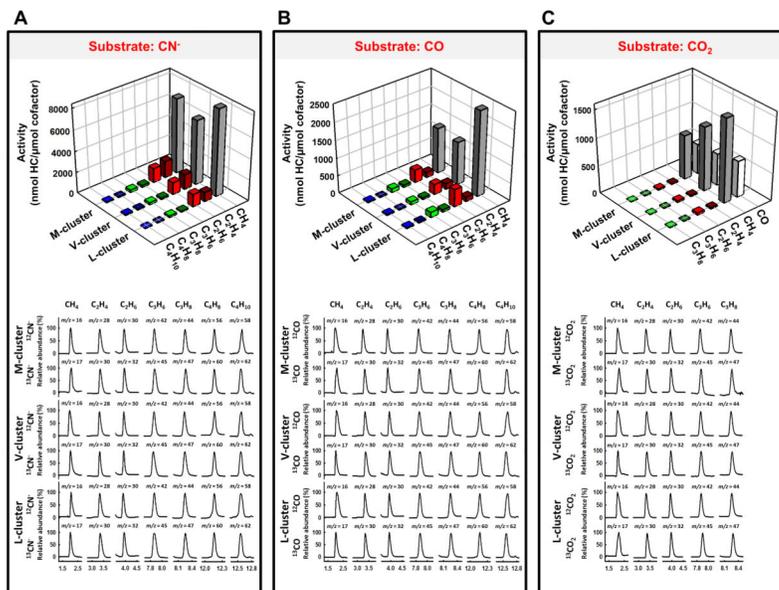
## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

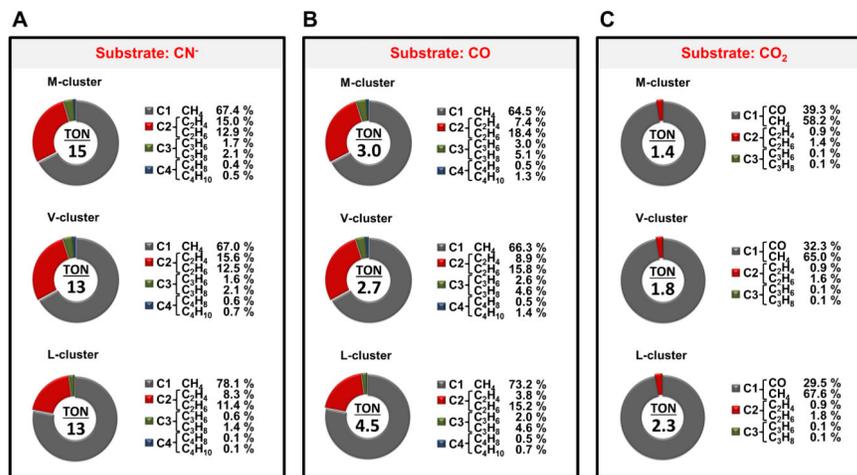
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**Figure 1.** Reduction of  $\text{CN}^-$ ,  $\text{CO}$  and  $\text{CO}_2$  by nitrogenase cofactors. Shown are the activity (*upper*) and GC-MS (*lower*) analyses of hydrocarbon (HC) formation in the reactions of (A)  $\text{CN}^-$ , (B)  $\text{CO}$  and (C)  $\text{CO}_2$  reduction by M-, V- and L-clusters.



**Figure 2.** Product profiles of nitrogenase cofactors. Shown are the percentages of C1, C2, C3 and C4 products formed in the reactions of (A) CN<sup>-</sup>, (B) CO and (C) CO<sub>2</sub> reduction by M-, V- and L-clusters. TON, or turnover number, was calculated based on the nmole of carbon that appeared in the hydrocarbon products per nmole of isolated cluster used in the reaction.