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Catalytic Reduction of CN^-, CO, and CO_2 by Nitrogenase Cofactors in Lanthanide-Driven Reactions**

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Abstract: *Nitrogenase cofactors can be extracted into an organic solvent to catalyze the reduction of cyanide (CN^-), carbon monoxide (CO), and carbon dioxide (CO_2) without using adenosine triphosphate (ATP), when samarium(II) iodide (SmI_2) and 2,6-lutidinium triflate (Lut-H) are employed as a reductant and a proton source, respectively. Driven by SmI_2, the cofactors catalytically reduce CN^- or CO to C_1–C_4 hydrocarbons, and CO_2 to CO and C_1–C_3 hydrocarbons. The C–C coupling from CO_2 indicates a unique Fischer–Tropsch-like reaction with an atypical carbonaceous substrate, whereas the catalytic turnover of CN^-, CO, and CO_2 by isolated cofactors suggests the possibility to develop nitrogenase-based electrocatalysts for the production of hydrocarbons from these carbon-containing compounds.*

Nitrogenase is a uniquely versatile metalloenzyme that catalyzes the reduction of various substrates, such as nitrogen (N_2), carbon monoxide (CO), and cyanide (CN^-), at its cofactor site.[1–4] The molybdenum (Mo) and vanadium (V) nitrogenases, two homologous members of this enzyme family, contain homologous cofactors, the molybdenum–iron cofactor (designated the M-cluster) and the vanadium–iron cofactor (designated the V-cluster), respectively, at their respective active sites.[1,5] The M-cluster (Figure S1 A) is a $[MoFe_7S_9C]$ cluster that can be viewed as $[Fe_4S_3]$ and $[MoFe_3S_3]$ subclusters bridged by three equatorial μ_2 sulfides and one interstitial μ_6 carbide. In addition, this cofactor has an endogenous compound, homocitrate, attached to its Mo end.[6–8] The V-cluster (Figure S1 B) 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.[9,10] 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 as the L-cluster (Figure S1 C), this $[Fe_8S_9C]$ 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.[11–13] 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),[10] and directly used as a catalyst to reduce CN^- or CO to hydrocarbons in the presence of a strong reductant, europium(II) diethylenetriaminepentaacetate (Eu^{II}-DTPA).[14] Driven by Eu^{II}-DTPA ($E^{0\prime} = -1.14$ V at pH 8), both cofactors generate alkanes and alkenes of varying lengths as products of CN^- or CO reduction at comparable efficiencies. Additionally, they both display a strong preference of CN^- over CO as a substrate, which may originate from a stabilizing effect of CN^- on certain oxidation states of the two cofactors.[14] However, Eu^{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 one.[15] Moreover, this reductant does not support the reduction of CO_2 by the cofactors, an event that requires more reducing power than the reduction of CN^- or CO.[16] This observation prompts the questions of 1) whether CO and CO_2 can be catalytically turned over by these clusters in the presence of an appropriate reductant; and 2) if the L-cluster resembles the M- and V-clusters in the conversion of carbon-containing compounds to hydrocarbons.

The answer to both questions is yes. When Eu^{II}-DTPA is replaced by a stronger reductant, samarium(II) iodide (SmI_2),[17] the NMF-extracted M-, V-, and L-clusters are all capable of turning over CN^-, CO, and CO_2 under ambient conditions in organic solvents. Driven by SmI_2 ($E^{0\prime} = -1.55$ V in THF) and using protons supplied by 2,6-lutidinium triflate (Lut-H),[18] the three clusters not only reduce CN^- (Figure 1 A, upper part; Table S1) and CO (Figure 1 B, upper part; Table S1) to CH_4, C_2H_4, C_2H_6, C_3H_6, C_3H_8, 1-C_4H_8, and *n*-C_4H_{10}, but also reduce CO_2 to CO, CH_4, C_2H_4, C_2H_6, C_3H_6, and C_3H_8 (Figure 1 C, upper part; Table S1). Gas chromatography–mass spectrometry (GC-MS) analysis confirms CN^-, CO, and CO_2 as the carbon sources for the hydrocarbons generated in these reactions, as all products display the expected mass shifts upon substitution of $^{13}CN^-$, ^{13}CO, and $^{13}CO_2$, for $^{12}CN^-$ (Figure 1 A, lower part), ^{12}CO (Figure 1 B, lower part), and $^{12}CO_2$ (Figure 1 C, lower part), respectively. Activity analysis further demonstrates that all three clusters turn over CN^-, CO, and CO_2 catalytically (i.e., TON > 1) in the presence of SmI_2, with the M-, V-, and L-clusters showing TONs of 15, 13, and 13, respectively, for CN^- (Figure 2 A), 3.0, 2.7, and 4.5, respectively, for CO (Figure 2 B), and 1.4, 1.8,

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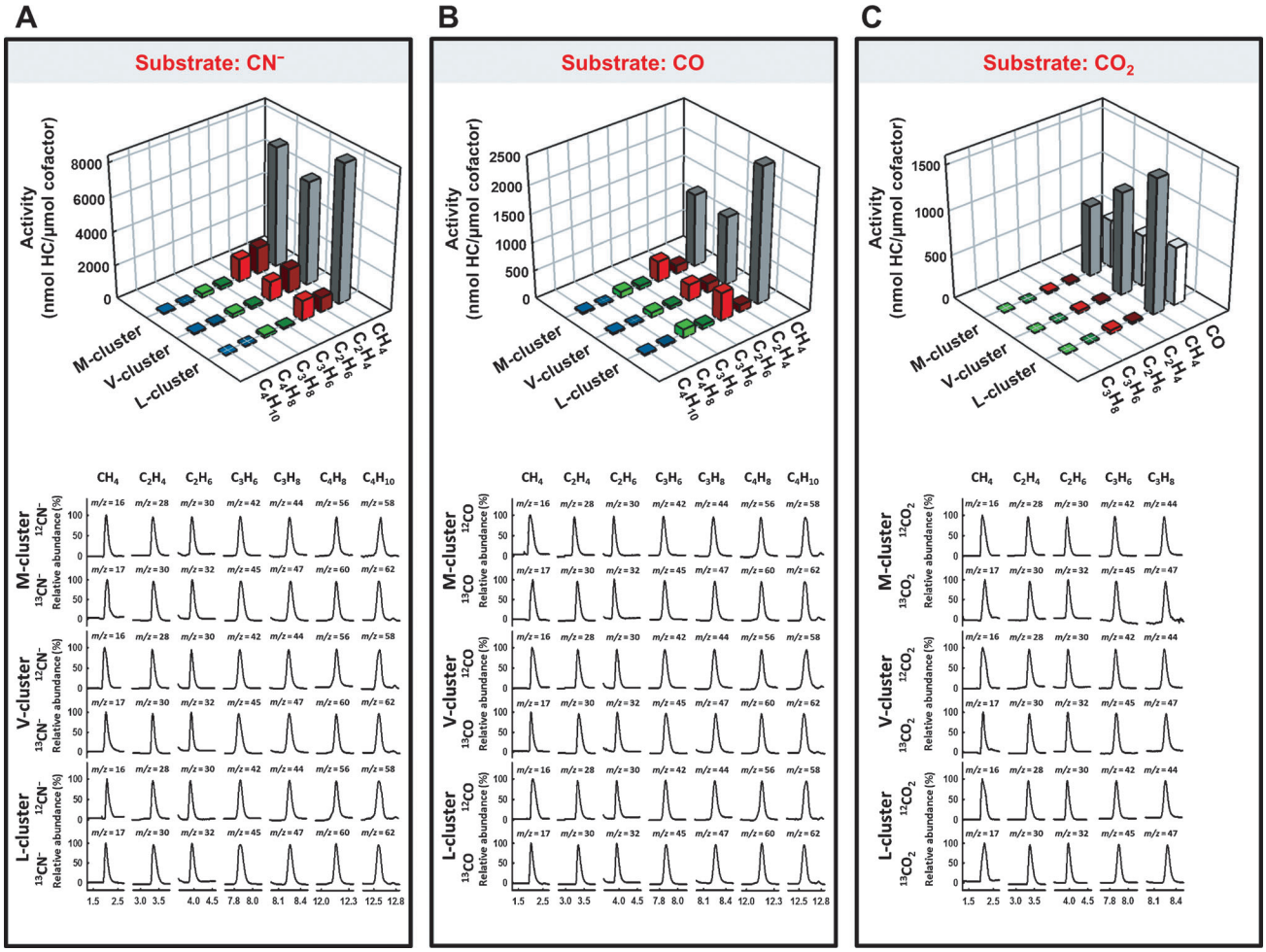

Figure 1. Reduction of CN^-, CO, and CO_2 by nitrogenase cofactors. Shown are the activity (upper part) and GC-MS (lower part) analyses of hydrocarbon (HC) formation in the reductions of A) CN^-, B) CO, and C) CO_2 by M-, V-, and L-clusters.

and 2.3, respectively, for CO_2 (Figure 2C). While the preference of CN^- as a substrate is preserved by all three clusters in reactions driven by SmI_2, the catalytic turnover of CO and 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 not observed, ATP-independent reaction that involves the conversion of CO_2 to hydrocarbons by these unique metal clusters in the isolated forms.

It should be noted that the ATP-dependent reduction of CO_2 was reported both for a variant of Mo-nitrogenase and for the wild-type V-nitrogenase;[19–21] however, CH_4 was detected as the sole hydrocarbon product in the case of the former,[20] whereas CH_4, C_2H_4, and C_2H_6 were detected only upon substitution of D_2O for H_2O in the case of the latter.[21] In comparison, the isolated cofactors are "pushed" by SmI_2 not only toward the formation of a C–C bond (i.e., hydrocarbons larger than C_1 products), but also toward the formation of longer carbon chains (i.e., up to C_3 products) from CO_2 (Figure 1C). The C_2 and C_3 hydrocarbons do not originate from the coupling between the CO_2-derived CO in the SmI_2-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_2 reduction (Figure S2). Furthermore, the reduction of CO_2 to CO and hydrocarbons is carried out by protons (H^+) and electrons in these reactions, and it is accompanied by the reduction of H^+ to hydrogen (H_2; 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 up to C_2 products from CN^- (Figure 2A) and CO (Figure 2B), with the C_1 (CH_4) and C_2 (C_2H_4, C_2H_6) products comprising a major portion (90.3–97.8%) of the product profiles of these reactions. The tendency toward the formation of small products is even more apparent in the cases of CO_2 reduction by these clusters, where the C_1 products (CO, CH_4) constitute the predominant portion (97.1–97.5%) of the product profiles (Figure 2C). In all these reactions, CH_4 is the singularly dominant hydrocarbon product, making up 58.2–78.1% of the total amount of

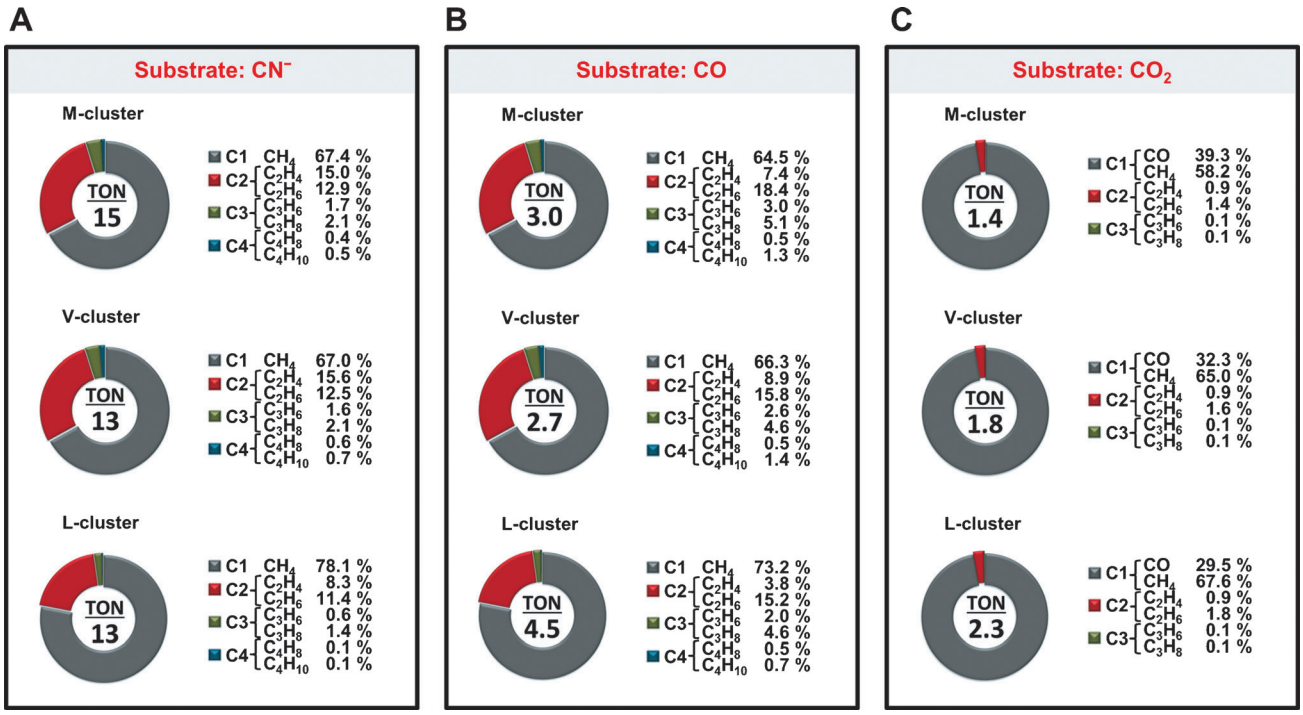

Figure 2. Product profiles of nitrogenase cofactors. Shown are the percentages of C_1, C_2, C_3, and C_4 products formed in the reductions of A) CN^-, B) CO, and C) CO_2 by M-, V-, and L-clusters. The turnover number (TON) was calculated based on the amount of carbon atoms (in nmol) that appeared in the hydrocarbon products relative to the amount of isolated cluster (in nmol) used in the reaction.

products. Such a strong shift toward CH_4 formation is not observed in the reduction of CO by the protein-bound M- or L-cluster,[2,3] where C_2H_4 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,[3] 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 (Figure 2B) and CO_2 (Figure 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_4 from the reduction of all three substrates and, in the reactions of CN^- (Figure 2A) and CO (Figure 2B) reduction, the increased formation of CH_4 by the L-cluster is accompanied by a decreased formation of C_2H_4, consistent with a preference of this cluster to reduce CN^- and CO all the way to CH_4 over the C–C coupling of these substrates into C_2H_4. Strikingly, an analogous reaction was shown to be enabled by iron sulfide (FeS), a simplest FeS unit; only in this case, methanethiol (CH_3SH) was generated as a product of CO_2 reduction in the presence of FeS and hydrochloric acid (HCl).[22] The increased formation of CH_4 by the L-cluster is not only interesting because of the value of CH_4 as a fuel source, but also important because of the all-iron composition of the L-cluster (see Figure 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^-, CO, and CO_2 to hydrocarbon products. The success in achieving the catalytic turnover of CO and CO_2 by these clusters in the presence of a stronger reductant, SmI_2, suggests the possibility to develop nitrogenase-based electrocatalysts for further improvement of catalytic efficiency and substrate range. On the other hand, 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, imply 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-type reaction with an atypical Fisher–Tropsch substrate, CO_2.[23] The formation of CO in this reaction is likely analogous to the reaction of reverse water–gas shift (i.e., $CO_2 + H_2 \rightarrow CO + H_2O$).[24] Only in this case, the expensive syngas, H_2, is replaced by H^+ (provided by Lut-H) and e^- (supplied by SmI_2), and it is further produced as an abundant side product of H^+ reduction (see Table S1). The formation of hydrocarbons also utilizes H^+ as a hydrogen source, and this reaction likely involves the direct C–C coupling from CO_2 or CO_2-derived intermediate(s) other than CO (see Figure S2). As such, the reduction of CO_2 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 the potential to serve as a blueprint for the future design of strategies to recycle CO_2 into useful carbon fuels.

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