

Reduction of C₁ Substrates to Hydrocarbons by the Homometallic Precursor and Synthetic Mimic of the Nitrogenase Cofactor

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S Supporting Information

ABSTRACT: Solvent-extracted nitrogenase cofactors can reduce C₁ substrates (CN⁻, CO and CO₂) to hydrocarbons in reactions driven by a strong reductant, SmI₂ ($E^{0'}$ = -1.55 V vs SCE). Here we show that a synthetic [Et₄N]₄[Fe₆S₉(SEt)₂] cluster (designated the Fe₆^{RHH}-cluster), which mimics the homometallic [Fe₈S₉C] core of the nitrogenase cofactor (designated the L-cluster), is capable of conversion of C₁ substrates into hydrocarbons in the same reactions. Comparison of the yields and product profiles between these homometallic clusters and their heterometallic counterparts points to possible roles of the heterometal, interstitial carbide and belt sulfur-bridged iron atoms in catalysis. More importantly, the observation that a “simplified”, homometallic cofactor mimic can perform Fischer–Tropsch-like hydrocarbon synthesis suggests future biotechnological adaptability of nitrogenase-based biomimetic compounds for recycling C₁ substrates into useful chemical and fuel products.

Metal clusters containing iron and sulfur represent a class of versatile catalytic moieties that are ubiquitous in all living organisms. In nature, the arguably most complex iron–sulfur clusters identified to date are utilized at the active sites of nitrogenases, a family of metalloenzymes that are capable of catalyzing the reduction of a variety of substrates, including dinitrogen (N₂), cyanide ions (CN⁻), carbon monoxide (CO) and carbon dioxide (CO₂), under ambient conditions.^{1–3} The molybdenum (Mo)- and vanadium (V)-dependent nitrogenases contain two homologous cofactors within their respective active sites: the iron–molybdenum cofactor (designated the M-cluster), which has a core composition of [MoFe₇(μ₂-S)₃(μ₃-S)₆(μ₆-C)] and an *R*-homocitrate ligand that is bound to the Mo center (Figure S1a);^{4,5} and the iron–vanadium cofactor (designated the V-cluster), which closely resembles the core composition and structure of the M-cluster except for the replacement of the heterometal by V (Figure S1b).^{6,7} In addition, an iron (Fe)-only precursor to the mature M-cluster (designated the L-cluster), which has an [Fe₈S₉C] core but is free of homocitrate, is found to be structurally homologous to both cofactors other than having a Fe atom in place of the heterometal (Figure S1c).^{8–10} All three cofactor

species can be extracted from their respective protein scaffolds into *N*-methylformamide (NMF) or dimethylformamide (DMF),^{6,9,11} allowing the properties and reactivities of the isolated clusters to be assessed without the interference of protein environment.

Previously, it was shown that short-chain hydrocarbons could be catalytically generated in aqueous buffers by reacting extracted nitrogenase cofactor species with C₁ substrates (CN⁻ or CO) in the presence of a one-electron reductant, europium(II) diethylenediaminepentaacetate ([Eu^{II}DTPA]³⁻; $E^{0'}$ = -1.14 V at pH 8).^{12,13} However, the reduction of CO was substoichiometric using [Eu^{II}DTPA]³⁻, and CO₂ hydrogenation could not be achieved with this reductant. Use of a stronger reductant, samarium(II) diiodide (SmI₂; $E^{0'}$ = -1.55 V vs SCE in tetrahydrofuran), in a buffered DMF solution provided sufficient reductive power to the cofactors to catalytically form CO and C₁–C₃ hydrocarbons from CO₂, and C₁–C₄ products from CN⁻ and CO.¹⁴ These Fischer–Tropsch-like reactions^{15,16} demonstrated the inherent catalytic properties of the M-, V- and L-clusters even when removed from the native protein matrix; more importantly, they presented an opportunity to recycle the C₁ carbon wastes and, in particular, the readily available greenhouse gas CO₂, into useful chemical and fuel products under ambient conditions.^{17–21} It is interesting to note a higher reactivity of the homometallic L-cluster with C₁ substrates than its Mo- and V-containing homologues. This observation suggested a non-essential function of the heterometal and homocitrate components in facilitating these hydrogenation reactions while raising a relevant question of whether this reduction chemistry could be accessed using synthetic Fe–S-containing clusters.

To address this question, a synthetic [Et₄N]₄[Fe₆S₉(SEt)₂] cluster (designated the Fe₆^{RHH}-cluster) was used for comparative turnover analysis with the L-cluster. First prepared by Holm and co-workers,^{22,23} this homometallic cluster has a unique [Fe₆(μ₂-S)₆(μ₃-S)₂(μ₄-S)]²⁻ core (Figure 1a) that bears topological similarities to the homometallic L-cluster (Figure 1b), as well as the heterometallic M- and V-clusters (Figure S1). A superposition of the structures of Fe₆^{RHH}- and L-clusters

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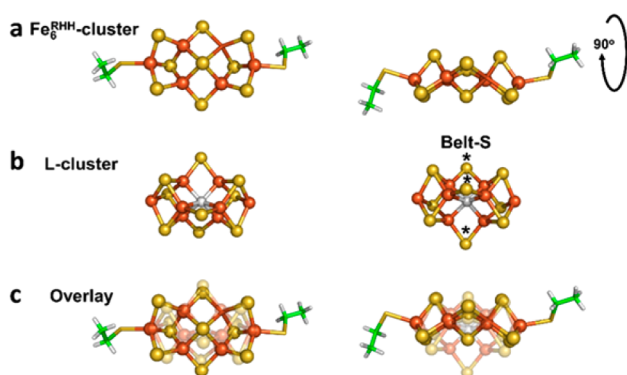


Figure 1. Structural models of (a) the synthetic Fe_6^{RHH} -cluster ($[\text{Fe}_6\text{S}_9(\text{SEt})_2]^{4+}$) and (b) the L-cluster ($[\text{Fe}_3\text{S}_9\text{C}]$) and (c) overlay of the two clusters in top (left) and side (right) views. PDB entry 3PDI⁸ and data from ref 15 were used to generate these models. Atoms are colored as follows: Fe, orange; S, yellow; C (in L-cluster), light gray; C (in Fe_6^{RHH}), green; H (in Fe_6^{RHH}), gray. The belt-S atoms are indicated by * in panel b.

reveals the presence of a unique μ_4 -S atom in the former at a similar position to that of the μ_6 -C⁴⁻ ion in the latter, as well as a similar face shared by the two clusters that consists of μ_3 -S and μ_2 -S atoms coordinated to Fe atoms (Figure 1c). The structural homology is further manifested by the presence of two pairs of μ_2 -S-bridged Fe atoms in the Fe_6^{RHH} -cluster that resemble the three pairs of Fe atoms bridged by the so-called “belt” μ_2 -S atoms in the L- and M-clusters (Figure 1b, *; also see Figure S1). Coordinated further to the interstitial μ_6 -C⁴⁻ ion, these three pairs of Fe atoms across the belt of the cofactor have been suggested as the sites of catalysis in models of nitrogenase cofactor reactivity.^{24,25} Recent structural and biochemical analyses of CO-bound conformations of M- and V-clusters have provided further support for this hypothesis, suggesting a potential mechanistic relevance of sulfur displacement to the activation of these Fe sites.^{7,26} By analogy, the two pairs of “interstitial” μ_4 -S-coordinated, μ_2 -S-bridged Fe atoms in the L-cluster (Figure 1a) could very well facilitate the binding and reduction of substrates like their counterparts in the nitrogenase cofactors. In support of this hypothesis, our recent study showed that the Fe_6^{RHH} -cluster could be inserted into a cofactor-deficient form of the catalytic component of Mo-nitrogenase, resulting in a semiartificial metalloenzyme that was capable of converting a C₁ substrate, CN⁻, to C₁–C₃ hydrocarbons in a $[\text{Eu}^{\text{II}}\text{DTPA}]^{3-}$ -driven reaction.²⁷

The observation that the synthetic Fe_6^{RHH} -cluster behaved similarly to the biogenic nitrogenase cofactor within the same protein matrix is important, as it suggests the possibility to use

the “bare” Fe_6^{RHH} -cluster in a similar manner to the extracted nitrogenase cofactors to enable the reduction of C₁ substrates in a reductant-driven reaction. Indeed, like the extracted L-cluster, the extracted Fe_6^{RHH} -cluster exhibited the ability to generate hydrocarbons from CN⁻, CO and CO₂ at ambient temperature and pressure. A variety of short-chain hydrocarbons were generated from the reduction of C₁ substrates upon addition of a tetrahydrofuran (THF) solution of SmI₂ to a DMF solution containing the Fe_6^{RHH} - or L-cluster and Et₃N-buffered triethylammonium ($[\text{Et}_3\text{NH}]^+$, pK_a = 9.0 in DMSO). Both Fe_6^{RHH} - and L-clusters generated CH₄, C₂H₄, C₂H₆, C₃H₆, C₃H₈, C₄H₈, C₄H₁₀, C₅H₁₀ and C₅H₁₂ as products of CN⁻ (Figure 2a) and CO (Figure 2b) reduction; and CO, CH₄, C₂H₄, C₂H₆, C₃H₆, C₃H₈, C₄H₈ and C₄H₁₀ as products of CO₂ reduction (Figure 2c). Gas chromatography–mass spectrometry (GC–MS) analysis of the reaction headspace revealed the expected mass shifts of C₁–C₅ products upon substitution of the corresponding ¹³C isotopologs for the C₁ substrates (Figure S2), confirming CN⁻, CO and CO₂ as the origins of the evolved hydrocarbons. Remarkably, the Fe_6^{RHH} - and L-clusters were capable of catalyzing the reduction of C₁ substrates with turnover numbers (TONs; calculated based on μmol total C in hydrocarbons/ μmol cluster) of 409 and 611, respectively, with CN⁻ as a substrate; 92 and 175, respectively, with CO as a substrate; and 14 and 30, respectively, with CO₂ as a substrate (Figure 3a,b).

The synthetic Fe_6^{RHH} -cluster displayed TONs that were 67%, 53% and 45%, respectively, of those determined for the biogenic L-cluster in reactions with CN⁻, CO and CO₂. Moreover, despite producing a similar array of hydrocarbon products from the respective C₁ substrates, the Fe_6^{RHH} -cluster displayed a different product profile than the L-cluster. Specifically, although a preference for CH₄ formation from C₁ substrates was clearly observed in the cases of both clusters, the L-cluster appeared to bias the reactions further toward the formation of a higher percentage of CH₄ than the Fe_6^{RHH} -cluster, whereas the Fe_6^{RHH} -cluster seemed to favor the formation of a higher percentage of longer-chain hydrocarbons than the L-cluster (Figure 3c). The reduced TON and altered product profile of the Fe_6^{RHH} -cluster may be attributed in part to the absence of two iron atoms that are bridged by a “belt” sulfur (Figure 1), which have been implicated in substrate binding and catalysis.² Consistent with this argument, a recent study revealed that a heterometallic mimic of the nitrogenase cofactor, designated the Mo-cluster ($[\text{Cp}^*\text{MoFe}_5\text{S}_9(\text{SH})]^{3-}$; Cp* = η^5 -pentamethylcyclopentadienyl), displayed a similar decrease in the TONs of CN⁻, CO- and CO₂-reduction relative to those of its biogenic counterpart, the M-cluster.²⁸

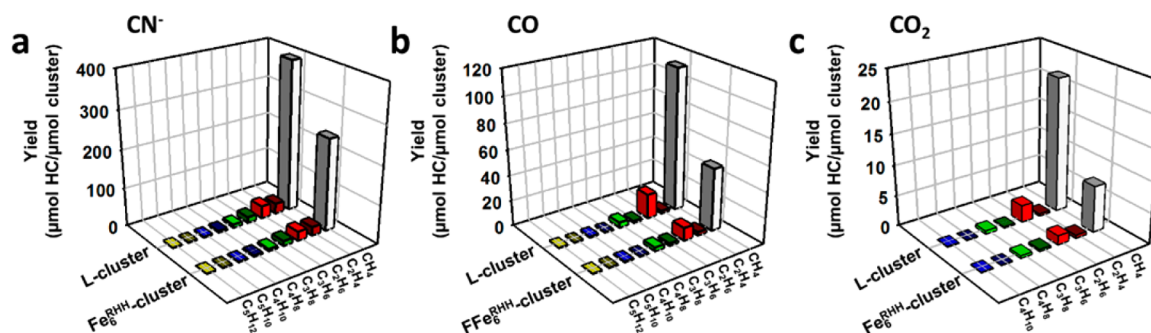


Figure 2. Yields of hydrocarbon formation by L- and Fe_6^{RHH} -clusters from the reduction of (a) CN⁻, (b) CO and (c) CO₂. See also Table S1.

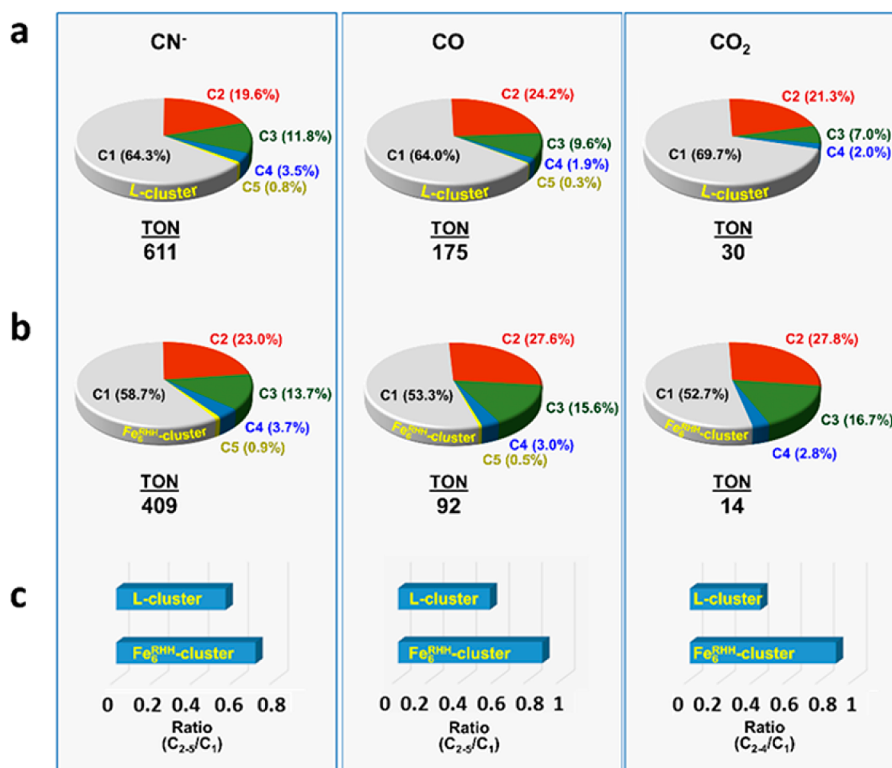


Figure 3. Product profiles of hydrocarbon formation by L- and Fe₆^{RHH}-clusters. The total amounts of hydrocarbons generated by (a) L- and (b) Fe₆^{RHH}-clusters from the reduction of CN⁻ (left), CO (middle) and CO₂ (right) were set as 100%, respectively, and the percentages of individual products were determined accordingly for each cluster. (c) C_{2,C1}/C₁ product ratios of L-cluster- and Fe₆^{RHH}-cluster-catalyzed reduction of CN⁻ (left), CO (middle) and CO₂ (right).

Like the Fe₆^{RHH}-cluster, the Mo-cluster represents one face of the cofactor with μ₃-S and μ₂-S atoms coordinated to Fe atoms (Figure S1d). The “collapsed” belt-sulfur region in both Fe₆^{RHH}- and Mo-clusters may very well account for the altered reactivities of these synthetic compounds, as a belt-sulfur-displacement mechanism has been proposed for substrate binding to the cofactor.^{7,26} Additionally, the “replacement” of the interstitial μ₆-C⁴⁻ ion by a unique μ₄-S atom in both synthetic clusters could also contribute to their modified reactivities given the suggested role of the μ₆-C⁴⁻ ion in stabilizing the cofactor and/or fine-tuning its reactivity.^{24,25}

The similar differences in TON and product profile when the respective pairs of the heterometallic and homometallic clusters are compared with each other (i.e., the biogenic M- vs L-cluster; or the synthetic Mo- vs Fe₆^{RHH}-cluster) point to a possible role of the heterometal in indirectly modifying the cluster properties and/or directly interacting with the substrates (Figure 4). Interestingly, although the M-cluster is more active than the L-cluster in CN⁻, CO- and CO₂-reduction, the Mo-cluster is less active than the Fe₆^{RHH}-cluster in CN⁻ and CO-reduction but more active than the latter in CO₂-reduction (Figure 4). One plausible explanation for such a discrepancy is the ligation of Mo atom to Cp* in the synthetic Mo-cluster, which renders the Mo atom coordinatively saturated and sterically encumbered. Consequently, the inaccessibility of the Mo center in the Mo-cluster could impact the interaction of Mo with certain substrates (such as CN⁻ and CO) while leaving the interactions of other substrates (such as CO₂) at locations other than Mo intact. Although the mechanistic details of the interactions between the nitrogenase cofactors/analogs with C₁ substrates are yet to be elucidated, the

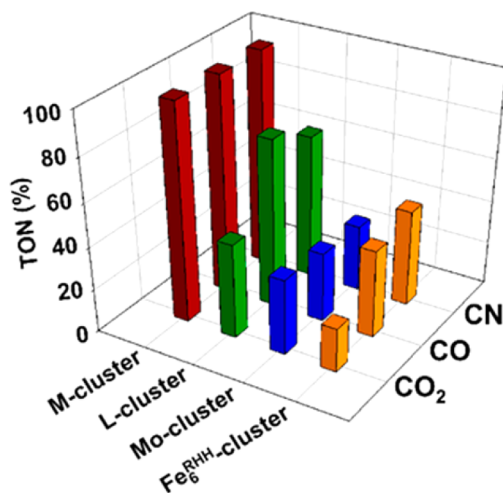


Figure 4. Comparison of the turnover numbers (TONs) of the M-, L-, Mo- and Fe₆^{RHH}-clusters from the reduction of CN⁻, CO and CO₂. For each C₁ substrate, the TON of the M-cluster was set at 100%, and the percentage TONs of the L-, Mo- and Fe₆^{RHH}-clusters were calculated accordingly.

observed substrate-reducing activity of the Fe₆^{RHH}-cluster is exciting, as it establishes this cluster as the first homometallic synthetic mimic of the nitrogenase cofactor that is capable of facilitating the conversion of C₁ substrates into hydrocarbons.

The fact that a synthetic Fe–S cluster can perform the reduction chemistry of C₁ substrates without the presence of the interstitial C⁴⁻ ion suggests the potential of Fe–S clusters, and perhaps even simpler ones, to react similarly with C₁

substrates. As such, the results of these experiments could have implications for the prebiotic evolution of organic molecules. Studies have found the presence of C₁–C₄ hydrocarbons of abiotic origin around undersea hydrothermal vents and attributed the chemistry of hydrocarbon formation to Fe-containing minerals.^{29,30} Others have shown that, under hydrothermal conditions, Fe–S-containing minerals can catalyze the formation of organic molecules in the presence of H₂ and CO₂.^{31,32} Although Sml₂ represents a reductant outside the normal biological redox range, catalysis by the Fe₆^{RHH}-cluster in this reductant supplies additional, albeit indirect, proof for the abiogenic hydrocarbon production from Fe–S clusters under unusual redox conditions in the primordial environment on Earth. In a practical vein, the observed activity of a synthetic Fe–S cluster that is simpler than the biogenic cofactor in composition points to the possibility to further simplify and/or modify the Fe–S core and fine-tune the reactivity of the synthetic cluster toward certain C₁ substrates. The current methods are limited by the O₂ sensitivity, the reaction scale, and the consumable reductant; however, optimizing these factors and extending this reactivity to an electrochemical context by replacing the electron source with an electrode may present an effective route to hydrocarbon products (including carbon fuels) without the need of a sacrificial reductant. Efforts are currently underway along these lines, which will hopefully assist in the development of bioinspired catalysts for recycling the C₁ carbon wastes back into useful chemical and fuel products.

■ ASSOCIATED CONTENT

📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b11633.

Procedures and additional data (PDF)

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Notes

The authors declare no competing financial interest.

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