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Widening the Product Profile of Carbon Dioxide Reduction by Vanadium Nitrogenase

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Abstract

Two V-nitrogenase-based reaction systems were previously shown to reduce CO₂ to hydrocarbons: (1) an enzyme-based system, which requires both components of V-nitrogenase for ATP-dependent reduction of CO₂ to $\leq$ C2 hydrocarbons; and (2) a cofactor-based system, which employs SmI₂ to supply electrons to the isolated V-cluster for ATP-independent reduction of CO₂ to $\leq$ C3 hydrocarbons. Here, we report ATP-independent reduction of CO₂ to hydrocarbons by a reaction system comprising Eu(II) DTPA and VFe protein. Combining features of both enzyme- and cofactor-based systems, this system demonstrates an improved activity of C-C coupling, as well as a widened product profile of $\leq$ C4 hydrocarbons. The C-C coupling does not route via CO₂-derived CO, and it is significantly enhanced in D₂O. These observations afford the initial insights into the characteristics of this unique reaction and provide a potential template for future design of catalysts to recycle the greenhouse gas CO₂ into useful products.

Keywords

V-nitrogenase; ATP-independent system; CO₂ reduction; C-C coupling reactions; hydrocarbon formation

Nitrogenase plays a key role in the global nitrogen cycle, catalyzing the ATP-dependent reduction of atmospheric dinitrogen (N₂) to bioavailable ammonia (NH₃) under ambient conditions.^[1,2] Other than N₂, this enzyme is also capable of reducing carbon monoxide (CO) and carbon dioxide (CO₂) to hydrocarbons;^[3–10] in particular, the “alternative” vanadium (V)-nitrogenase displays considerably higher activities of CO- and CO₂-reduction than the “conventional” molybdenum (Mo)-nitrogenase,^[5,7] generating specific interest in exploring the catalytic characteristics of V-nitrogenase in these reactions. The V-nitrogenase from *Azotobacter vinelandii* consists of two component proteins: the γ_2 -homodimeric iron (Fe) protein, which contains a subunit-bridging [Fe₄S₄] cluster and a nucleotide-binding site per subunit;^[11,12] and the $\alpha_2\beta_2\delta_4$ -octameric vanadium-iron (VFe) protein, which contains a

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P*-cluster (a [Fe₄S₄]-like cluster pair) at the α/β -subunit interface and a V-cluster (a [VFe₇S₉] cofactor) within each α -subunit (Figure 1a).^[13,14] Two V-nitrogenase-based reaction systems were previously shown to reduce CO₂ to hydrocarbons.^[7,8] One of them utilized the complete enzymatic system of V-nitrogenase, namely, the Fe protein and the VFe protein, to enable ATP-dependent electron transfer from the [Fe₄S₄] cluster of the Fe protein to the V-cluster of the VFe protein for the reduction of CO₂ to hydrocarbons in an aqueous buffer (Figure 1a);^[7] the other employed the isolated cofactor of V-nitrogenase, namely, the V-cluster, to directly receive electrons from a strong reductant, samarium (II) iodide (SmI₂; $E^{0'} = -1.5$ V), for ATP-independent reduction of CO₂ to hydrocarbons in an organic solvent (Figure 1c).^[8] While hydrocarbons of up to C₂ and C₃ in length were generated as the respective products of CO₂ reduction by the enzyme- and cofactor-based reactions, the former consumed ATP as an energy source; whereas the latter was hampered by the instability of the V-cluster in the isolated state. These observations have prompted attempts to combine the desirable features of the enzyme- and cofactor-based reactions into an ATP-independent, reductant-driven reaction system, which utilizes the VFe protein-bound V-cluster as a stand-alone catalyst for improved production of hydrocarbons from CO₂ reduction (Figure 1b).

One such system could be generated by combining europium (II) DTPA [Eu(II) DTPA; $E^{0'} = -1.1$ V]^[15,16] with VFe protein in an aqueous buffer. Driven directly by Eu(II) DTPA, the VFe protein was capable of catalyzing ATP-independent reduction of CO₂ to CO (Figure 2a) and CH₄ (Figure 2b). The activity of the VFe protein to convert CO₂ to CO was consistently higher in D₂O (Figure 2a, ○) than that in H₂O (Figure 2a, ▽). In contrast, the activity of CH₄ formation by VFe protein could not be detected in the presence of D₂O (Figure 2b, ○); yet, it increased from 0 to 30 nmol/μmol protein over a time period of 180 minutes upon substitution of H₂O for D₂O (Figure 2b, ▽). Interestingly, when CO was supplied in a concentration simulating that achieved in from CO₂ reduction by VFe protein at 180 minutes (*see* Figure 2a, *arrows*), no CD₄ could be detected as a product in the presence of D₂O (Figure 2b, •); whereas the activity of CH₄ formation by VFe protein increased from 0 to a maximum of 18 nmol/μmol protein within the first 30 minutes of the reaction in the presence of H₂O (Figure 2b, ▽). Such an increase in activity approximated the increase of 12 nmol/μmol protein between 10 and 30 minutes when CO₂ was directly supplied to the reaction, following a delay of 10 minutes in which no CH₄ could be detected (Figure 2b, ▼). Beyond 30 minutes, however, the activity of CH₄ formation by VFe protein plateaued when CO was supplied as a substrate (Figure 2b, ▼); whereas it continued to increase when CO₂ was supplied as a substrate (Figure 2b, ▽). The similarity between the initial phases of CO- and CO₂-based CH₄ formation by VFe protein (*see* Figure 2b, *inset*) suggests that a portion of CH₄ is formed via CO₂-derived CO, particularly given the 10-minute delay in the CO₂-based reaction that could correlate with the time required to accumulate a sufficient amount of CO from CO₂ reduction. The disparity between the two reactions beyond 30 minutes, on the other hand, implies that a certain portion of CH₄ is generated directly from CO₂ reduction, which could account for the different lineshapes of the time courses of CH₄ formation, as well as the total amounts of CH₄ generated in the CO- and CO₂-based reactions.

More excitingly, other than reducing CO₂ to the C1 hydrocarbon product (*i.e.*, CH₄), the VFe protein could use CO₂ as a substrate for ATP-independent C-C coupling into hydrocarbon products of up to C4 in length (Figure 3). Contrary to the formation of CH₄, there was a dramatic increase in the activities of C2-C4 hydrocarbon formation by VFe protein upon substitution of D₂O for H₂O. In the presence of D₂O, formation of C₂D₄, C₂D₆, C₃D₆, C₃D₈, C₄D₈ and C₄D₁₀ by VFe protein reached 18, 12, 14, 5, 4.5 and 2.0 nmol/μmol protein, respectively, over a time period of 300 minutes (Figure 3a-f, ○); whereas in the presence of H₂O, formation of C₂H₄, C₂H₆, C₃H₆, C₃H₈, C₄H₈ and C₄H₁₀ started to plateau between 120 and 180 minutes at 6, 5, 3, 1.7, 0.8 and 0.6 nmol/μmol protein, respectively (Figure 3a-f, ▽). When CO was supplied in a concentration mimicking that achieved from CO₂ reduction by VFe protein at 180 minutes (*see* Figure 2, *arrows*), little or no C2-C4 product could be detected in the presence of either D₂O (Figure 3a-f, •) or H₂O (Figure 3a-f, ▼), suggesting that these products were formed directly from CO₂ reduction instead of indirectly from CO₂-derived CO. GC-MS analysis further confirmed the source of carbon in the C1-C4 hydrocarbons as that from CO₂, showing mass shifts of +1, +2, +3 and +4, respectively, of C1, C2, C3 and C4 products upon substitution of ¹³CO₂ for ¹²CO₂ in H₂O-based reactions (Figure 4a *vs.* b). In addition, it demonstrated the source of hydrogen in these products as that from H₂O, displaying mass shifts of +4, +6 and +8, respectively, of products containing 4, 6 and 8 hydrogen atoms upon substitution of D₂O for H₂O in ¹²CO₂-based reactions (Figure 4a *vs.* c); and additional mass shifts of +2, +3 and +4, respectively, of C2, C3 and C4 products upon further substitution of ¹³CO₂ for ¹²CO₂ in these reactions (Figure 4c *vs.* d). In the cases of C₄H₁₀ and C₄D₁₀, the base peaks were monitored to circumvent the problem of detection limit, showing masses of 43.087, 46.064, 50.098 and 58.108, respectively, that represent the predominant fragment ions formed in the cases of ¹²C₄H₁₀, ¹³C₄H₁₀, ¹²C₄D₁₀ and ¹³C₄D₁₀ (Figure 4).

The total activity of C-C coupling (*i.e.*, formation of ≥C2 products) by VFe protein in the ATP-independent, Eu(II) DTPA-driven reaction (designated system II) is higher than that by V-cluster in the ATP-independent, SmI₂-driven reaction (designated system III) (Figure 5a, *green vs. yellow*) and that by V-nitrogenase in the ATP-dependent reaction (designated system I) in D₂O (Figure 5a, *green vs. grey*) or H₂O (Figure 5a, *red vs. black*). Moreover, compared to system I and system III, there is a greater tendency of system II to reduce CO₂ to longer carbon chains and generate a broader product profile both in D₂O (Figure 5b, *green*) and in H₂O (Figure 5b, *red*). Given the absence of Fe protein from system II, the active V-cluster site is likely more “open” in this system than that in system I (*see* Figure 1), which could explain the improved ability of system II to extend the carbon chain and generate larger hydrocarbon products. The protein scaffold that houses the V-cluster in system II, on the other hand, likely provides stability to the cluster while modulating its redox potential in the same time, which may account for a higher activity accomplished by a weaker reductant (*i.e.*, Eu(II) DTPA) in the case of system II as compared to a lower activity accomplished by a stronger reductant (*i.e.*, SmI₂) in the case of system III (Figure 5a, *green vs. yellow*). Apart from the composition-dependent differences, there is a strong deuterium effect on the activity of protein-enabled CO₂ reduction in an aqueous buffer, as both system I and system II displayed a dramatic increase of CO₂-reducing activity upon substitution of D₂O for H₂O (Figure 5, *green vs. red; grey vs. black*) While systematic studies are required

to elucidate the mechanism of CO₂ reduction by V-nitrogenase, these observations afford the initial insights to the characteristics of this unique reaction and provide a potential template for future design of nitrogenase-based catalysts to recycle the greenhouse gas CO₂ into useful hydrocarbon products.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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References

1. Burgess BK, Lowe DJ. Chem. Rev. 1996; 96:2983–3012. [PubMed: 11848849]
2. Rees DC, Tezcan FA, Haynes CA, Walton MY, Andrade S, Einsle O, Howard JB. Philos. Trans. A. Math. Phys. Eng. Sci. 2005; 363:971–984. [PubMed: 15901546]
3. Lee CC, Hu Y, Ribbe MW. Science. 2010; 329:642. [PubMed: 20689010]
4. Lee CC, Hu Y, Ribbe MW. Angew. Chem. Int. Ed. Engl. 2011; 50:5545–5547. [PubMed: 21538750]
5. Hu Y, Lee CC, Ribbe MW. Science. 2011; 333:753–755. [PubMed: 21817053]
6. Lee CC, Hu Y, Ribbe MW. Angew. Chem. Int. Ed. Engl. 2012; 51:1947–1949. [PubMed: 22253035]
7. Rebelein JG, Hu Y, Ribbe MW. Angew. Chem. Int. Ed. Engl. 2014; 53:11543–11546. [PubMed: 25205285]
8. Lee CC, Hu Y, Ribbe MW. Angew. Chem. Int. Ed. Engl. 2015; 54:1219–1222. [PubMed: 25420957]
9. Yang ZY, Dean DR, Seefeldt LC. J. Biol. Chem. 2011; 286:19417–19421. [PubMed: 21454640]
10. Yang ZY, Moure VR, Dean DR, Seefeldt LC. Proc. Natl. Acad. Sci. U. S. A. 2012; 109:19644–19648. [PubMed: 23150564]
11. Eady RR. Chem. Rev. 1996; 96:3013–3030. [PubMed: 11848850]
12. Hales BJ. Adv. Inorg. Biochem. 1990; 8:165–198. [PubMed: 2206026]
13. Lee CC, Hu Y, Ribbe MW. Proc. Natl. Acad. Sci. U. S. A. 2009; 106:9209–9214. [PubMed: 19478062]
14. Fay AW, Blank MA, Lee CC, Hu Y, Hodgson KO, Hedman B, Ribbe MW. J. Am. Chem. Soc. 2010; 132:12612–12618. [PubMed: 20718463]
15. Vincent KA, Tilley GJ, Quammie NC, Streeter I, Burgess BK, Cheesman MR, Armstrong FA. Chem. Commun. (Camb.). 2003; 21:2590–2591. [PubMed: 14594295]
16. Danyal K, Inglet BS, Vincent KA, Barney BM, Hoffman BM, Armstrong FA, Dean DR, Seefeldt LC. J. Am. Chem. Soc. 2010; 132:13197–13199. [PubMed: 20812745]

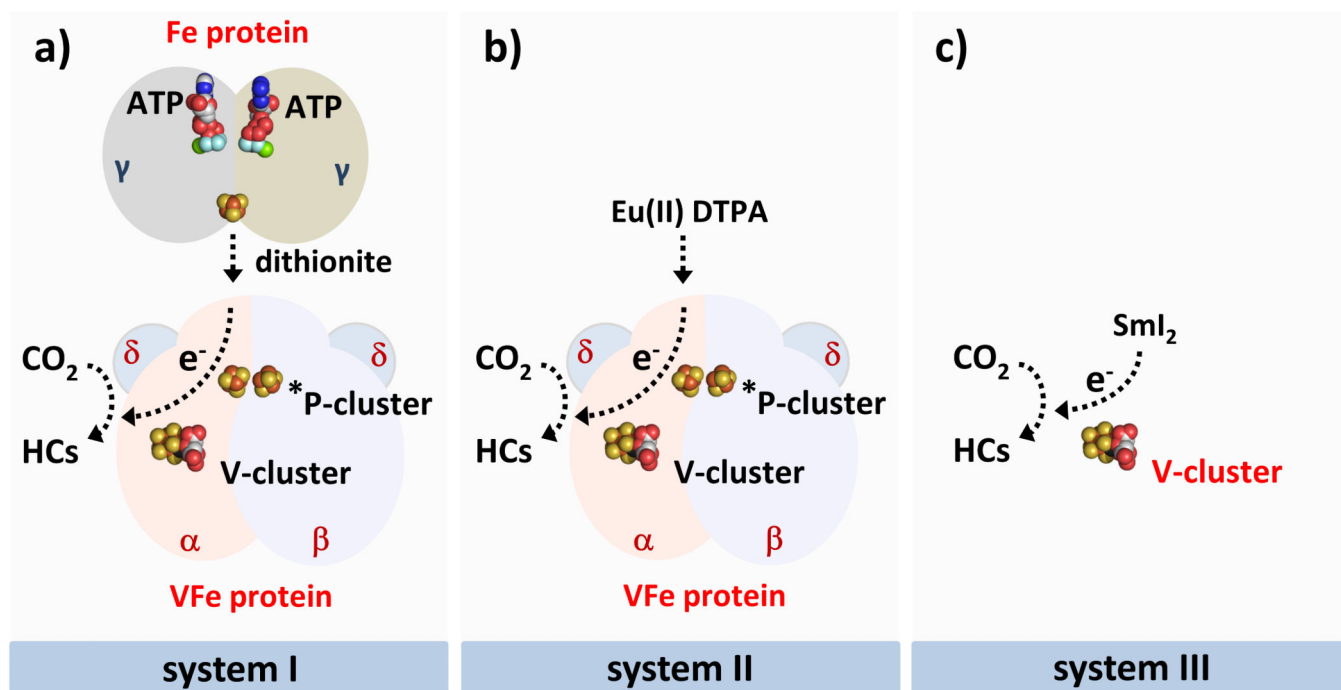


Figure 1.

Three CO_2 -reducing reaction systems based on V-nitrogenase: a) V-nitrogenase plus dithionite (designated system I), which permit ATP-dependent transfer of electrons from the $[\text{Fe}_4\text{S}_4]$ cluster of Fe protein to the V-cluster (or cofactor) of VFe protein in an aqueous buffer; b) VFe protein plus Eu(II) DTPA (designated system II), which permit ATP-independent electron transfer from Eu(II) DTPA to the V-cluster of VFe protein in an aqueous buffer; and c) V-cluster plus SmI_2 (designated system III), which permit ATP-independent electron transfer from SmI_2 to the isolated V-cluster in an organic solvent. For the purpose of simplicity, only one half of the VFe protein is shown. HCs, hydrocarbons.

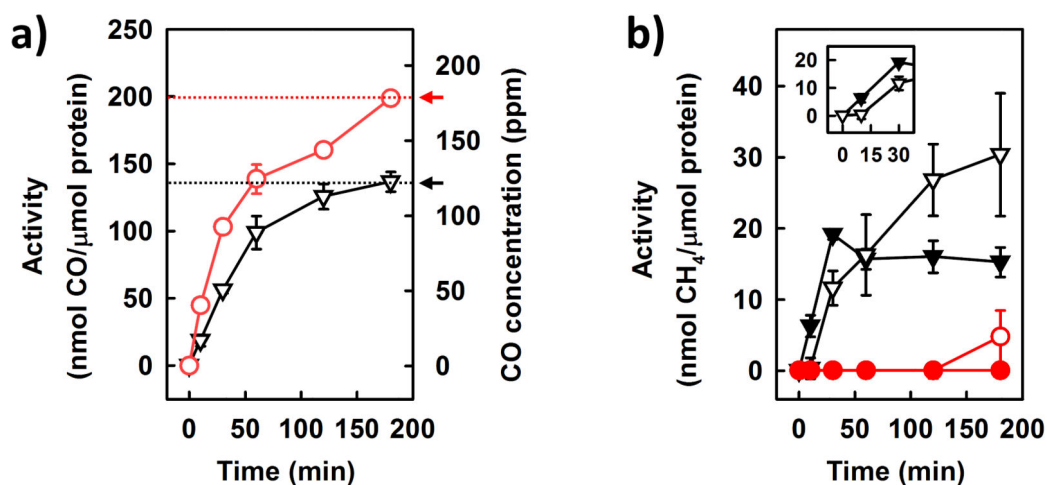


Figure 2.

ATP-independent reduction of CO₂ to C1 products by VFe protein. a) Time-dependent formation of CO from CO₂-reduction in H₂O (▽) or D₂O (○). b) Comparison of time-dependent formation of CH₄ from CO₂-reduction in H₂O (▽) or D₂O (○) with that from CO-reduction in H₂O (▼) or D₂O (●). The amounts of CO supplied (in b) were 136 ppm (▼) and 178 ppm (●), respectively, which simulated the amounts of CO formed in H₂O (▽) and D₂O (○), respectively, from CO₂-reduction at 180 min (*see a, arrows*). The inset (in b) is a blown-up of the first 30 min of CH₄ formation in H₂O where CO (▼) or CO₂ (▽) was supplied as a substrate. Data are presented as mean ± SD (*N* = 3) after background correction.

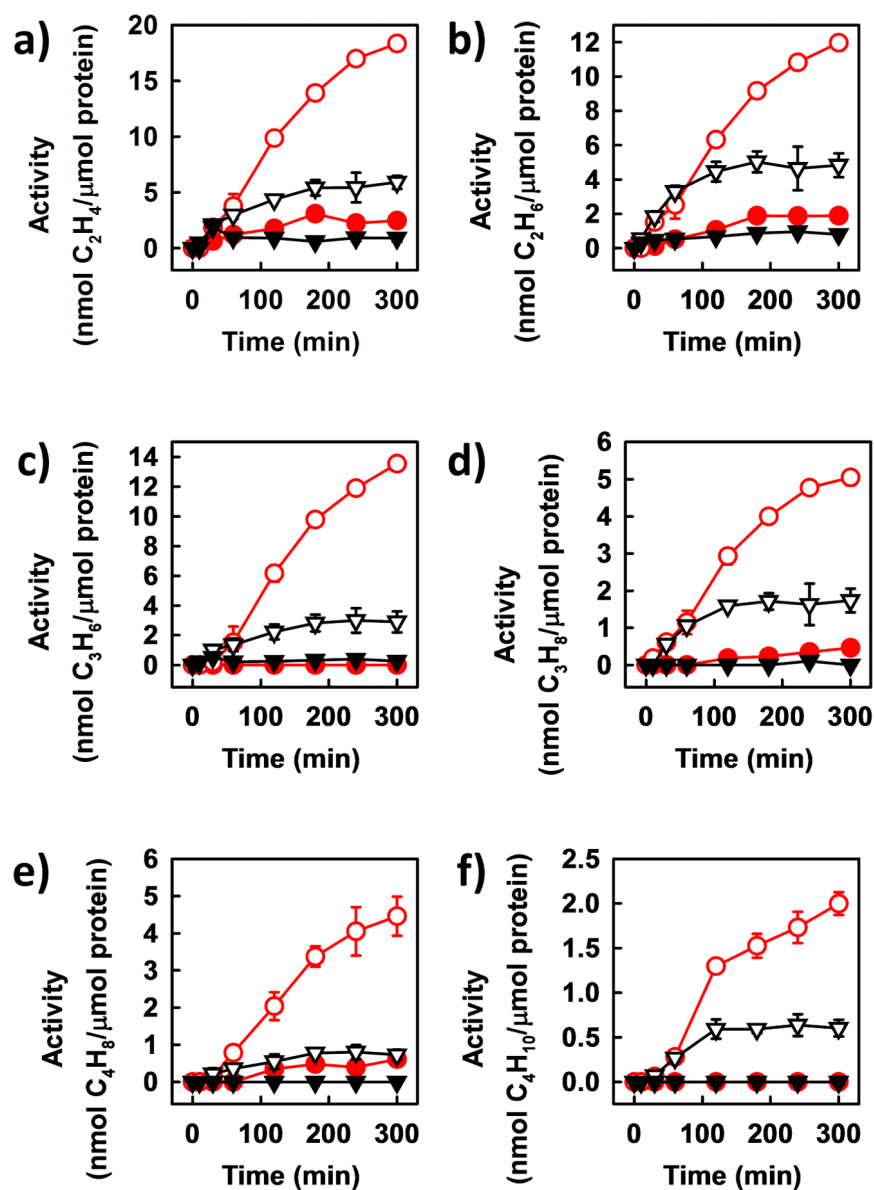
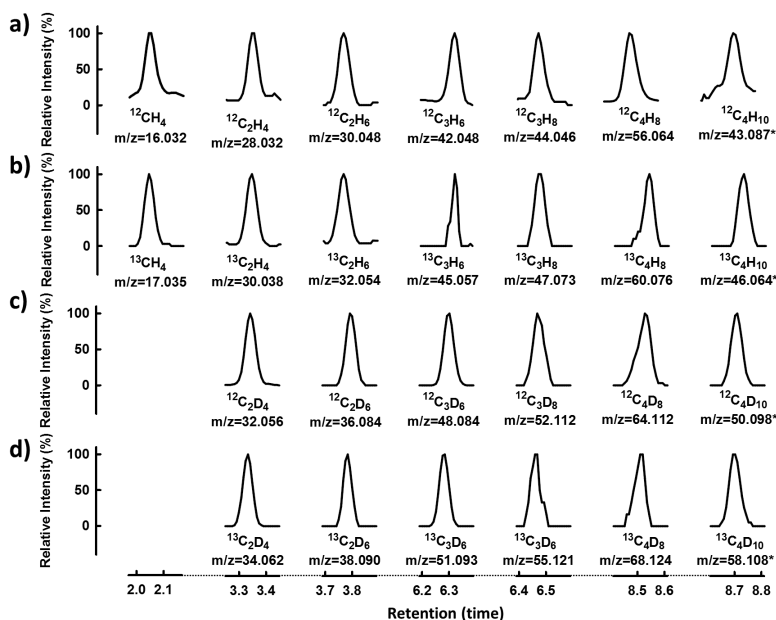


Figure 3.

ATP-independent reduction and coupling of CO₂ into C₂-C₄ products by VFe protein. Comparison of time-dependent formation of a) C₂H₄, b) C₂H₆, c) C₃H₆, d) C₃H₈, e) C₄H₈ and f) C₄H₁₀ from CO₂-reduction in H₂O (∇) or D₂O ($\circ$) with those from CO-reduction in H₂O ($\blacktriangledown$) or D₂O ($\bullet$). The amounts of CO supplied (in a-f) were 136 ppm ($\blacktriangledown$) and 178 ppm ($\bullet$), respectively, which simulated the amounts of CO formed in H₂O (∇) and D₂O ($\circ$), respectively, from CO₂-reduction by VFe protein at 180 min (see Figure 2a, arrows). Data are presented as mean $\pm$ SD ($N=3$) after background correction.

**Figure 4.**

GC-MS analyses of hydrocarbons generated by VFe protein in the ATP-independent reaction of CO_2 -reduction. The products were generated in H_2O when a) $^{12}\text{CO}_2$ or b) $^{13}\text{CO}_2$ was supplied as the substrate; or in D_2O when c) $^{12}\text{CO}_2$ or d) $^{13}\text{CO}_2$ was supplied as the substrate. The mass-to-charge (m/z) ratios at which the products were traced are indicated. In the cases of C_4H_{10} and C_4D_{10} (a-d), the base peaks (*), or the tallest peaks representing the most common fragment ions of these species, were monitored to overcome the problem of detection limit.

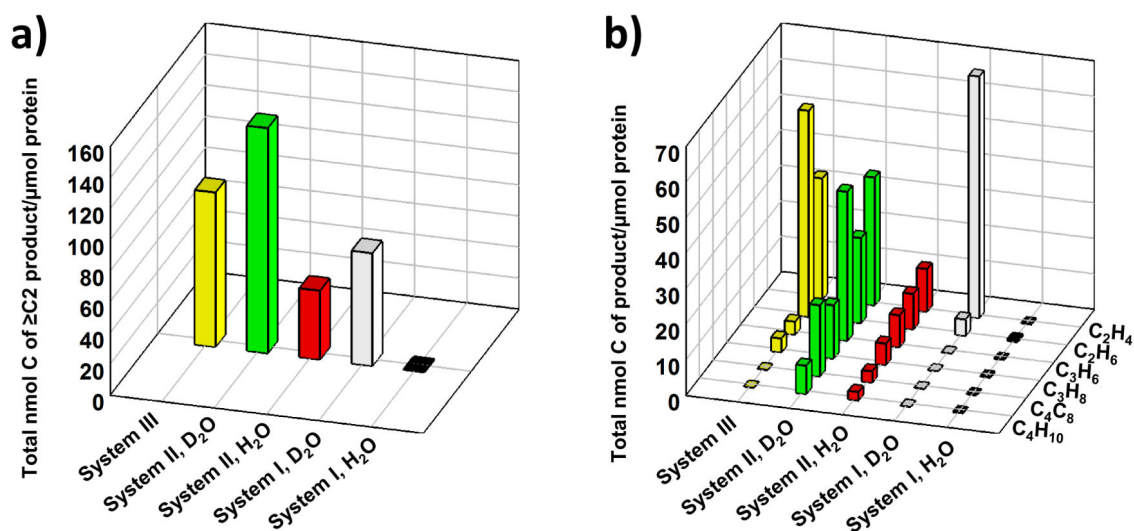


Figure 5. C-C coupling from CO_2 reduction by three V-nitrogenase-based systems. a) Total and b) individual activities of C-C coupling by system I, II and III. Activities were calculated based on the sums of carbons that appeared in all $\geq C_2$ hydrocarbon products (in a) or in each individual $\geq C_2$ hydrocarbon product (in b). For compositions of system I, II and III, please refer to Figure 1 and text. Data of system I and III were taken from Ref. 8 and Ref. 7, respectively.