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Tracing the Interstitial Carbide of the Nitrogenase Cofactor during Substrate Turnover

Jared A. Wiig,† Chi Chung Lee,† Yilin Hu,* and Markus W. Ribbe*

Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92697-3900, United States

Supporting Information

ABSTRACT: The fate of the interstitial atom of the nitrogenase cofactor during substrate turnover has remained a topic of interest since the discovery of this atom more than a decade ago. In this study, we labeled the interstitial carbide atom with ^{14}C and ^{13}C isotopes and traced the fate of the isotope under turnover conditions. Our results show that the interstitial carbide cannot be exchanged upon turnover, nor can it be used as a substrate and incorporated into the products. These observations point to a role of the interstitial carbide in stabilizing the cofactor structure, although a function of this atom in indirectly modulating the reactivity of the cofactor or directly interacting with the substrate cannot be excluded.

Molybdenum (Mo) nitrogenase catalyzes the reduction of a wide range of substrates, such as N_2, C_2H_2, and CO, at its iron–molybdenum cofactor (FeMoco) site.[1,2] Arguably one of the most complex metalloclusters in biological systems, the FeMoco consists of two partial cubanes, a [$MoFe_3S_3$] subcluster and a [Fe_4S_3] subcluster, that are bridged by three μ_2-sulfides and one μ_6-carbide (Figure 1).[3,4] It also has an endogenous organic compound, homocitrate, attached to its Mo end (Figure 1). The discovery of a μ_6-coordinated carbide atom at the center of the metal–sulfur core of FeMoco has raised the interesting question of whether this interstitial atom participates in the substrate turnover of nitrogenase.[4] However, there has been no effective means to monitor the flow of carbide until recently, when the methyl group of *S*-adenosyl-L-methionine (SAM) was identified as the source of this atom.[5,6] Such a finding permits specific labeling of the interstitial carbide[7] using [^{14}C-methyl]- or [^{13}C-methyl]SAM and subsequent tracing of the isotope in either the cofactor sample or the reaction products under turnover conditions.

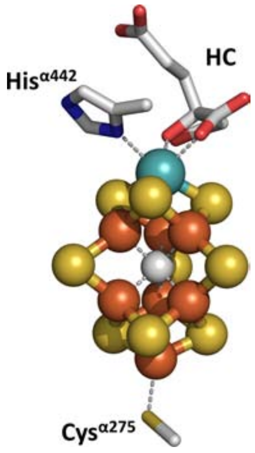

Figure 1. Structure of the FeMoco, [$MoFe_7S_9C$(homocitrate)]. The cluster is shown as a ball-and-stick model, with the atoms colored as follows: Fe, orange; S, yellow; Mo, cyan; O, red; C, gray; N, blue. PYMOL was used to generate this figure using PDB entry 3U7Q.

The fate of the interstitial carbide was first examined by labeling it with ^{14}C and determining whether the ^{14}C label disappeared from the cofactor upon turnover. C_2H_2 and N_2 were chosen as the substrates for this experiment, as they would be turned over rapidly by the Mo nitrogenase, thereby enabling a fast exchange of the interstitial carbide upon turnover. In addition, both substrates were allowed to undergo an extended turnover process in large molar excess with respect to the interstitial carbide, further ensuring the observation of a possible "dilution" of the ^{14}C label in the cofactor during turnover. However, even under these conditions, the intensity of the ^{14}C label in the cofactor sample remained unchanged after 3 h of turnover with C_2H_2 and N_2, suggesting that the interstitial carbide was not exchanged in these reactions (Figure 2).

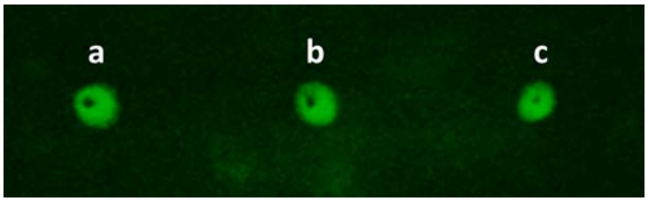

Figure 2. Intensities of ^{14}C labels in MoFe protein samples without turnover (A) or upon turnover of C_2H_2 (B) or N_2 (C). Shown is the autoradiography-detected radiation of the ^{14}C label in the MoFe protein-bound FeMoco. All of the MoFe protein samples contained equivalent amounts of ^{14}C-labeled FeMoco.

The flow of the interstitial carbide was further traced by labeling it with ^{13}C and determining whether the ^{13}C label appeared in the products upon turnover. CO was chosen as the substrate for this experiment, as it would be turned over very slowly by the Mo nitrogenase, thereby preventing a quick dilution of the ^{13}C label in the products in the case of fast-turnover substrates. In addition, the total amount of carbon in the products was kept at a submolar ratio with respect to the

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amount of the interstitial carbide, further facilitating the enrichment of carbide in the hydrocarbon products. However, contrary to the observation of labeled products when ^{13}CO was turned over by the unlabeled cofactor, no labeled product could be detected when ^{12}CO was turned over by the ^{13}C-labeled cofactor, suggesting that the interstitial carbide was not exchanged into the hydrocarbon products upon CO reduction (Figure 3).

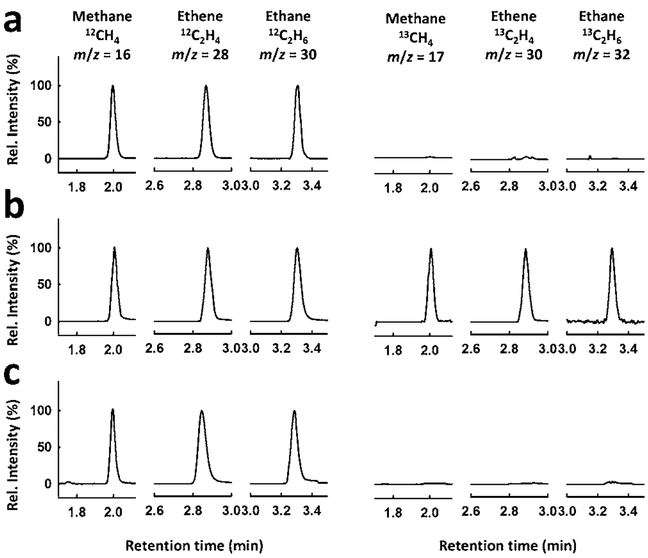

Figure 3. GC−MS analysis of hydrocarbon products generated from the turnover of (A) ^{12}CO by unlabeled FeMoco, (B) ^{13}CO by unlabeled FeMoco, and (C) ^{12}CO by ^{13}C-labeled FeMoco. The relative intensity of each hydrocarbon product traced at a given mass was arbitrarily set at 100%.

The question of whether the interstitial atom is involved in substrate turnover was tackled even before this atom was identified as a carbide ion, and early electron−nuclear double resonance (ENDOR)/electron spin echo envelope modulation (ESEEM) analyses demonstrated that this atom was not an exchangeable nitrogen atom.[8] Here we have provided direct evidence that the interstitial carbide can neither be exchanged during turnover nor used as a substrate and incorporated into the products. These results point to a role of this interstitial atom in stabilizing the structure of the cofactor, providing a certain "rigidity" to the metal−sulfur core through symmetrical coordination of this atom to the six core Fe atoms of the cofactor (Figure 1).

Interestingly, previous density functional theory calculations indicated that a more stable structure of FeMoco could be achieved by having an interstitial nitrogen or oxygen species rather than an interstitial carbon species.[9,10] Consistent with these calculations, a recent study of N_2 activation on iron metallaboratranes suggested that the presence of a carbide atom in the center of the FeMoco could allow variations of the Fe−C bond distances and adjustments of the overall geometry of the metal−sulfur core during the substrate turnover process.[11] Thus, a possible function of this interstitial atom in nitrogenase catalysis—be it indirect in tuning the reactivity of the cofactor or direct in interacting with the substrates—cannot be excluded. The exact role of the interstitial carbide in nitrogenase mechanism merits further investigation.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Materials and Methods. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

yilinh@uci.edu; mribbe@uci.edu

Author Contributions

[†]J.A.W. and C.C.L. contributed equally.

Notes

The authors declare no competing financial interest.

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