

Deciphering the metal ion environment in formate dehydrogenases: Insights from EPR, isotopic enrichment and DFT calculations in a W and Se dependent enzyme

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Mononuclear molybdenum and tungsten enzymes are found in virtually all living organisms. In prokaryotes, most of these enzymes harbour a large Mo/W-*bis* pyranopterin guanosine dinucleotide cofactor in which the metal ion is coordinated by the four sulfur atoms of the two pterin rings, by an amino-acid of the polypeptide chain (Ser, Asp, Cys/SeCys) and by a sixth ligand that can be oxygen or sulfur. The enzymes of this family catalyse a wide diversity of redox reactions involved in major biogeochemical cycles, and among them, formate dehydrogenases (Fdh) attract much attention due to their activity of CO₂ reduction to formate. During catalysis, the metal ion cycles between the +IV and +VI redox states, the intermediate Mo(V) and W(V) states being EPR-active (S=1/2). In the various Fdh's investigated so far, several Mo(V) and W(V) species have been identified, but in spite of numerous crystallographic and spectroscopic studies their structure and catalytic relevance is still largely debated ¹. To address these questions, we have developed an approach based on the combination of EPR spectroscopy, selective isotopic enrichment, hyperfine interaction analysis and DFT calculations ².

In this work this strategy is applied to identify the W(V) species in the recently characterized W-Fdh from the bacterium *Desulfovibrio vulgaris* Hildenborough which exhibits remarkable properties of CO₂ reduction activity and O₂ tolerance ³. Surprisingly, two different W(V) species were obtained, depending on the nature of the reductant used. By combining g-tensor analysis and DFT calculations of model compounds, we show that in these two species the W(V) ion has the same 6-ligands coordination sphere and that the magnetic parameter differences result from small variations of the twist angle between the four sulfur ligands of the two pterin rings. In addition, by using a specific ⁷⁷Se enrichment of the enzyme, we demonstrate that the conserved SeCys is a direct ligand of the W atom in these W(V) intermediate species. These results suggest the stability of the six-ligand coordination of the W ion along the catalytic cycle and the implications on the Fdh's catalytic mechanism will be discussed.

¹ Grimaldi, S. *et al.*, In *Molybdenum and Tungsten Enzymes*, RSC, 2016, 68-120.

² Rendon J. *et al.*, *Inorg. Chem.* **2017**, 56, 4423-4435.

³ Oliveira, A. R. *et al.*, *ACS Catalysis* **2020**, 10, 3844–3856, 2020.