An integrative approach for the characterization of metalloenzymes from an anaerobic gas converter

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Abstract

Metalloenzymes are key elements of the metabolism in anaerobic microbes. These proteins catalyze some of the most remarkable and challenging chemical transformations in biological systems and despite intense efforts of the scientific community the mechanism of many of these enzymes is still poorly de?ned. The organometallic active sites of these enzymes are at the interface between biology and chemistry and a multidisciplinary approach is crucial for proper characterization of these biocatalysts.

The acetogenic bacterium *Clostridium autoethanogenum* is a gas-converting microbe used for mitigation of industrial waste gasses and grows chemolithotrophically on pure carbon monoxide (CO). The metabolism is initiated by CO conversion by the bifunctional CO dehydrogenase/acetyl-coenzyme A complex. This heterotetrameric enzyme catalyzes both the oxidation of CO, deriving electron for the cell, and its fixation into acetyl-CoA, the turntable of carbon and energy metabolism. The structural characterization of the enzyme by a combination of X-ray crystallography, cryo-electron microscopy and computational biology revealed structural rearrangements and enzyme flexibility to enhance substrate diffusion through a wide gas tunneling system, maximizing reaction turnovers. The resulting enzyme is a powerful CO conversion machinery, providing electrons for reduction reactions such as the thermodynamically challenging acetate reduction to acetaldehyde, the first step of ethanol production in the bacterium. The reaction is catalyzed by the tungsten-dependent aldehyde:ferredoxin oxidoreductase, an atypical enzyme harboring a pterin-based active site. Only a reduced number of these enzymes has been characterized and the structure of their cofactor is still unclear. We used a combination of X-ray crystallography and *in cristallo* Resonance Raman spectroscopy to dissect the active site and the tungsten coordination, providing the most complete description of the architecture of this cofactor and new insights on the reaction mechanism of these enzymes.

This work illustrates the importance of a multidisciplinary approach to dissect the architecture and reaction mechanism of metalloenzymes.