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YedY - a mononuclear molybdenum enzyme with a redox-active ligand?

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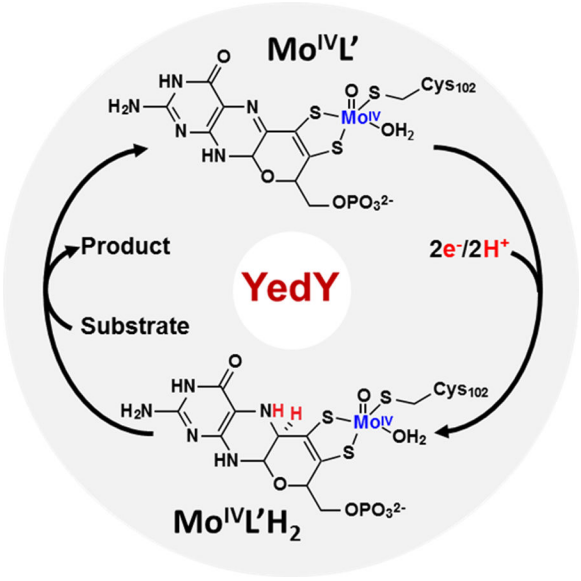
Graphical Abstract

A recent electrochemical investigation suggests that the mononuclear molybdenum enzyme, YedY, utilizes redox-active ligands during catalysis.



Keywords

YedY; molybdenum; pterin; electrochemistry; cyclic voltammetry; FTacV

Molybdenum (Mo) plays a unique role in bioinorganic chemistry. It is the only 2nd row transition metal that has known biochemical functions and is required in trace amounts by

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almost all forms of life.[1] A great number of putative Mo-containing proteins have been identified through genomic and proteomic analysis, of which only about 50 have been isolated and characterized.[1, 2] Apart from the Mo-nitrogenase, which contains a [$MoFe_7S_9C$] metal core,[3] all currently known Mo-containing enzymes house a type of Mo-pyranopterin cofactor and can be divided into three large and diverse families: the xanthine oxidase family, the DMSO reductase family, and the sulfite oxidase family.[1, 2] The Mo-containing active sites of these three family members in the oxidized state are shown in Figure 1. Members of xanthine oxidase family have active sites similar to those of the sulfite oxidase family: the former contains a [$LMo^{IV}O(S)(OH)$] core (L = pyranopterin dithiolate), whereas the latter contains a [$LMo^{IV}O_2$(S-Cys)] center.[1, 4] The DMSO reductase family contains two pyranopterin moieties per Mo, and the active sites typically consist of [$L_2Mo^{IV}O(R)$], where R can be OH, O-Ser, or other amino acid ligands (Figure 1). It should be noted that these Mo-active sites can function with various ligand substitutions; for example, the Mo=S moiety of the xanthine oxidase family can be substituted with Mo=Se, while the Mo=O of the DMSO reductase family can be replaced by Mo=S or even Mo=Se.[1, 4]

The diversity found within the active sites of these Mo-enzymes is reflected by their varied functions. Typically, Mo-enzymes are hydroxylases and oxotransferases, catalyzing a large number of reactions, such as aldehyde oxidation, carbon monoxide oxidation, nitrate reduction, and biotin-*S*-oxide reduction.[1, 4] Notably, despite the structural diversities, the three families of Mo-enzyme share a common type of reaction mechanism in that the enzymes utilize the $Mo^{VI/IV}$ redox couple to either oxidize or reduce substrates. In the case of sulfite oxidase, SO_3^{2-} is oxidized to SO_4^{2-} concomitant with the reduction of the Mo^{VI} center to Mo^{IV} (Figure 2).[1, 2, 5] The reduced active site is then cycled back to the catalytically accessible Mo^{VI} form via a two-electron oxidation step. Conversely, in the case of DMSO reductase, it is proposed that the enzyme cycles between the Mo^{IV} and the mono-oxo Mo^{VI} form. The former is responsible for abstracting an oxygen atom from substrate, while the latter is returned to the active reduced state following the reduction by two electrons and two protons (Figure 2).[1, 2]

YedY as a unique Mo-containing enzyme

YedY, a protein of unknown function, is widely distributed among Gram-negative bacteria.[6] The crystal structure of YedY from *Escherichia coli* has been solved and, based on its active site architecture, this Mo-enzyme has been placed within the sulfite oxidase family.[6] As shown in Figure 1, the Mo binding domain of YedY closely resembles those of sulfite oxidases with regard to the square-pyramidal coordination geometry of the Mo center and the homologous Cys residue that coordinates to Mo. However, there are also striking differences that distinguish YedY from this enzyme family. First, the as-isolated YedY exists in the Mo^V oxidation state as opposed to the Mo^V oxidation state that is more commonly adopted by sulfite oxidases.[7, 8] Second, XAS analysis has suggested that the Mo center of YedY consists of one oxo- and one hydroxo-coordination instead of the two terminal oxo-ligands that are commonly found in members of the sulfite oxidase family.[9, 10]

It is also suggested that a long O/N coordination from either Asn or Glu to Mo might be present in this state. Third, the Mo-center of YedY uses a less reduced form of the pyranopterin (designated dihydro pyranopterin), in which the tetrahydropyranopterin moiety is oxidized by $2e^-$ and $2H^+$ (Figure 1). [9–11] Remarkably, YedY is the only known Mo-containing enzyme that has this form of pyranopterin.

The physiological substrate of YedY remains unclear at present. Despite being a member of the sulfite oxidase family, YedY does not catalyze sulfite oxidation or similar reactions; instead, it reduces S- or N-oxides via DMSO reductase chemistry.[1, 6] Indeed, the activity of YedY is often assayed by DMSO or TMAO reduction. Intriguingly, while the Mo^V active site of YedY can be readily reduced to Mo^{IV}, the oxidized Mo^{VI} state cannot be accessed.[7, 8] This poses a perplexing question: how does YedY reduce DMSO with two electrons given that the enzyme can only access the $Mo^{IV/V}$ couple?

Investigation of the unusual electron transfer events using FTacV

To thoroughly elucidate the electron transfer process mediated by YedY during catalysis, Adamson *et al.* performed a series of in-depth voltammetric studies on protein thin films.[8] In conventional direct current cyclic voltammetry (dcV) on protein film electrodes, one major drawback is the large non-Faradaic background current that overwhelms the current response, making background subtraction from the voltammogram tedious and somewhat unreliable. Additionally, redox processes with fast kinetics are often not accurately measured with dcV methods. The authors employed Fourier transformed alternating current voltammetry (FTacV) to overcome these limitations.[8, 12] Both dcV and FTacV use a linear voltage-time sweep and measure the current-time response, but the latter superimposes a large-amplitude sine wave of frequency f on the dc ramp. At $f = 0$, FTacV gives the same catalytic information as a typical dcV experiment, while as f increases, the higher-order harmonic signals provide additional information on the fast, non-catalytic redox processes with minimal background interference.[12]

Using both dcV and FTacV, the authors showed that two main redox events occurred in YedY.[8] The first electrochemical feature at around +170 mV vs SHE was determined to be a relatively slow process ($k^0_{app} = 3 – 6\ s^{-1}$) and is best modeled as a $1H^+ + 1e^-$ reaction (Figure 3). Spectroelectrochemical analysis of the as-isolated YedY showed that electronic absorption transition signals associated with Mo^V center disappeared as the potential was lowered beyond this potential, suggesting that this first electron transfer event most likely involves the reduction of Mo^V to Mo^{IV} concomitant with protonation of Mo^V–OH that gives rise to Mo^{IV}–OH_2.[8–10] Moreover, the determined slow electron transfer rate is in agreement with a metal-based redox reaction, which requires structural rearrangement as the oxidation state and coordination environment of the metal center change. At around −250 mV, a second electrochemical feature was found and identified to be a fast process that involved the transfer of two electrons (Figure 3). While this information could also be gleaned from traditional dcV methods, simulation of the FTacV data revealed that the electron transfer rates approached approximately 20000 s^{-1} (Figure 3), nearly four orders of magnitude faster than the first event (i.e., the Mo-centered reduction). Based on this observation, the authors concluded that the redox event was far too fast to be occurring at the

metal center and excluded a conventional Mo-based $2H^+ + 2e^-$ reduction mechanism that was previously proposed for DMSO reductase (Figure 2). Given the unusual presence of the dihydropyranopterin ligand, the authors assigned this event to the reduction of the dihydro- to the more common tetrahydropyranopterin form. Such an assignment of the reduced species would be consistent with earlier redox studies of simple pterin units that demonstrated the feasibility of this dihydro- to tetrahydro- reduction,[13] leading to the proposal that the as-isolated YedY was reduced by $3e^-$ and $3H^+$ to a fully reduced, catalytically competent state.

Significance and future outlook

The utility of FTacV is highlighted through the demonstration of the catalytic relevance of the fully-reduced YedY, as it not only permits determination of the potential at which the active site is accessible for substrate as approximately −0.3V vs SHE (Figure 3, blue box), but also demonstrates that the electron transfer events at +170 mV and −250 mV vs SHE remain unchanged. In combination with the outcome of the pH-dependence studies, these data indicate that the fully-reduced YedY is the catalytically competent species that allows substrate reduction to occur. On first examination, this conclusion may not be too surprising given the similarity of the ($3e^- + 3H^+$)-reduced Mo center of YedY and the typical Mo^{IV} centers of sulfite oxidases (Figure 2 and 3).[1, 8, 9] However, the inability of YedY to access the Mo^{VI} state would imply that the enzyme utilizes the Mo^{IV}L'/MoIVL'H_2 couple (where L' denotes the redox-active ligand dihydropyranopterin) instead of the canonical $Mo^{IV/VI}$ couple that is utilized by all other known Mo enzymes. While structural and kinetic investigations are required to confirm the proposed mechanistic model for YedY, the study by Adamson *et al.* raises the intriguing question of whether the participation of a redox-active ligand is also involved in the catalytic mechanisms of other Mo enzymes or enzymes containing other metals and, therefore, is of broad interest to the general field of redox chemistry catalyzed by biological systems.

Acknowledgments

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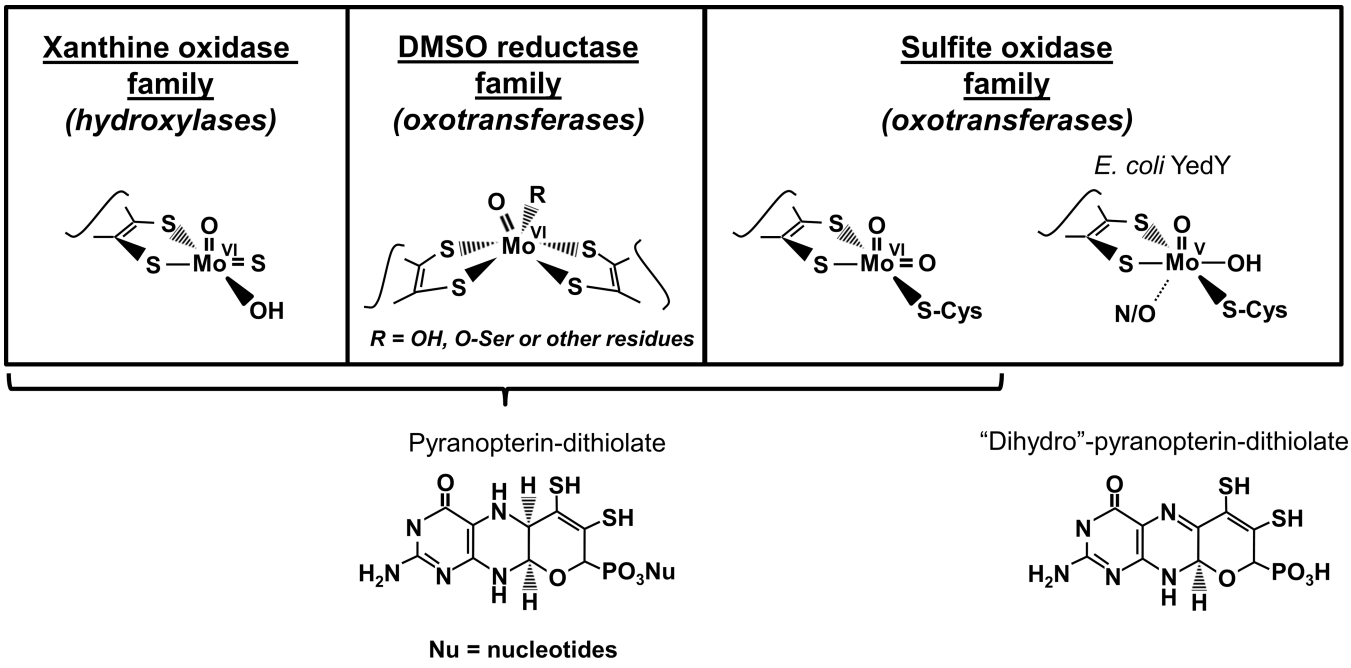

Figure 1.

The three families of Mo-pyranopterin-containing enzymes and YedY. The active sites of all enzymes are shown with the pyranopterin dithiolate ligand abbreviated as a wavy line. The "normal" pyranopterin cofactor that is common for all three families and the "dihydro"-pyranopterin moiety that is unique to YedY are shown at the bottom.

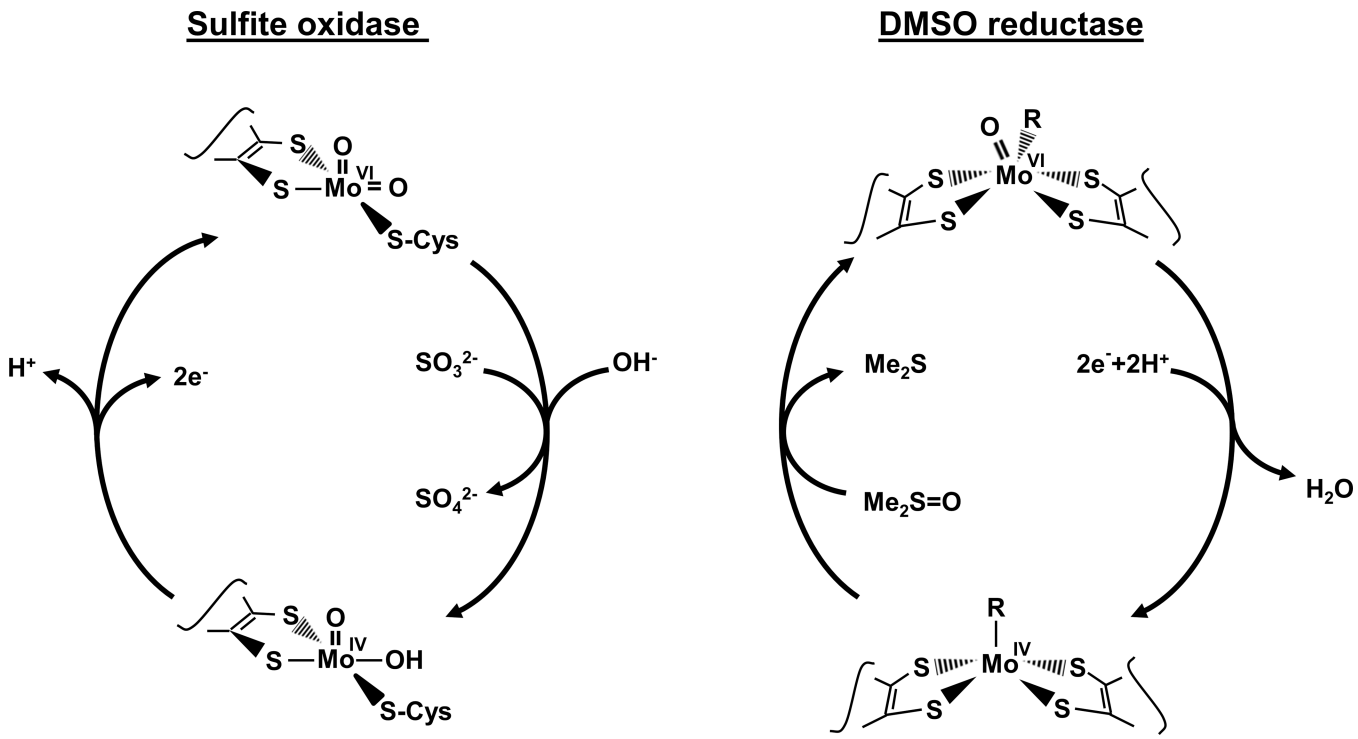

Figure 2.
The proposed catalytic cycles of sulfite oxidase and DMSO reductase.

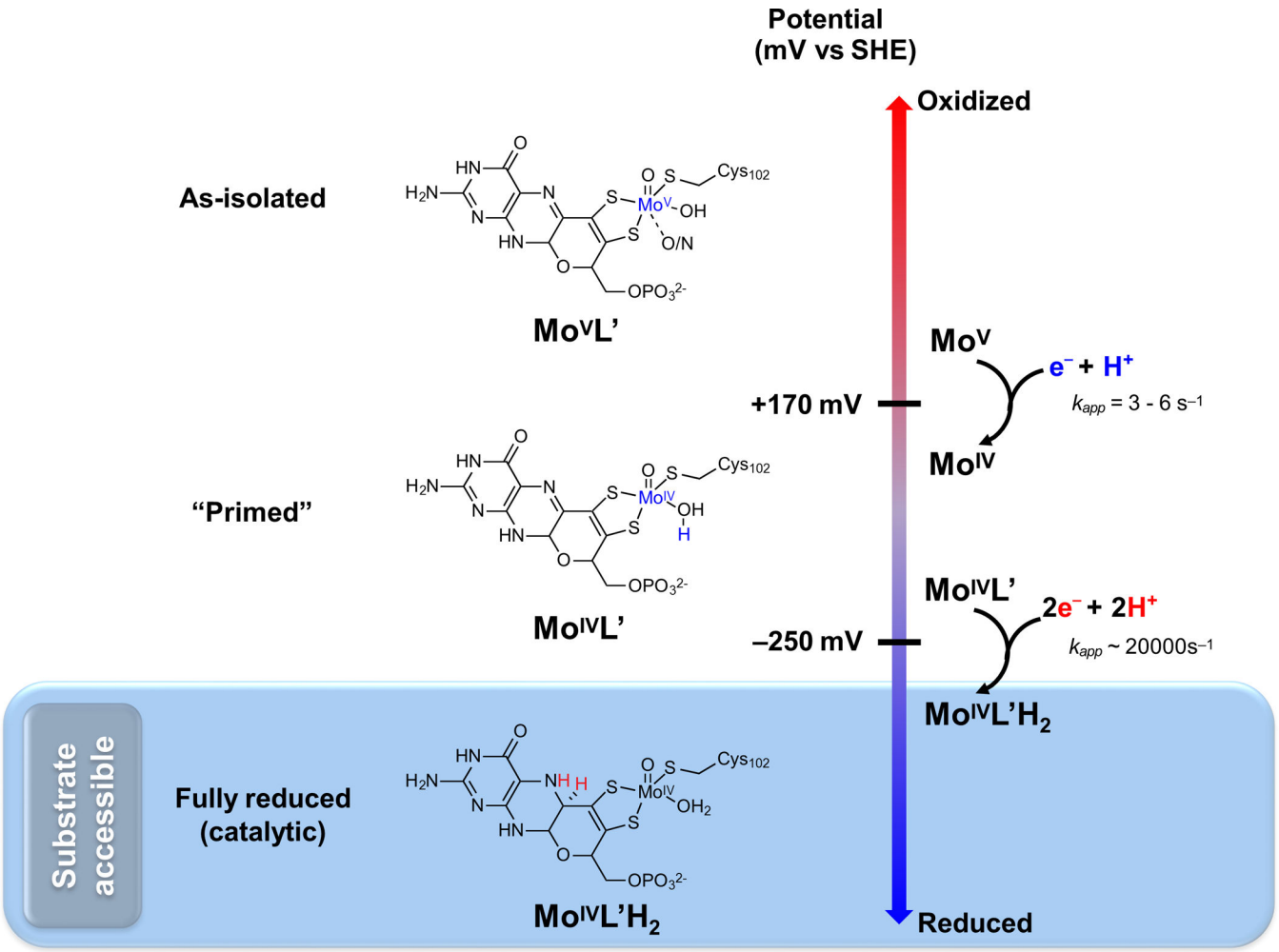

Figure 3.

Summary of the outcome of the electrochemical investigation of YedY by Adamson et al.[8]