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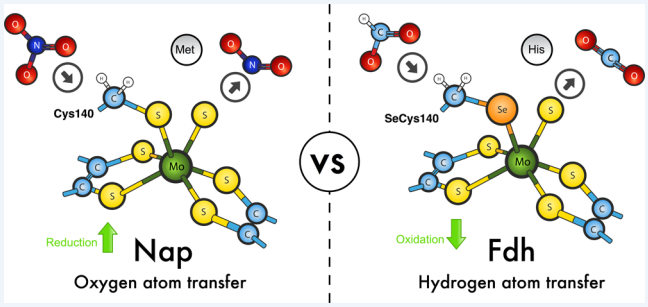
Periplasmic Nitrate Reductase and Formate Dehydrogenase: Similar Molecular Architectures with Very Different Enzymatic Activities

Nuno M. F. S. A. Cerqueira,[*,†] Pablo J. Gonzalez,[‡,§] Pedro A. Fernandes,[†] José J. G. Moura,[*,‡] and Maria João Ramos[†]

[†]REQUIMTE/UCIBIO, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal

[‡]REQUIMTE/UCIBIO, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal

CONSPECTUS: It is remarkable how nature has been able to construct enzymes that, despite sharing many similarities, have simple but key differences that tune them for completely different functions in living cells. Periplasmic nitrate reductase (Nap) and formate dehydrogenase (Fdh) from the DMSOr family are representative examples of this. Both enzymes share almost identical three-dimensional protein foldings and active sites, in terms of coordination number, geometry and nature of the ligands. The substrates of both enzymes (nitrate and formate) are polyatomic anions that also share similar charge and stereochemistry. In terms of the catalytic mechanism, both enzymes have a common activation mechanism (the sulfur-shift mechanism) that ensures a constant coordination number around the metal ion during the catalytic cycle. In spite of these similarities, they catalyze very different reactions: Nap abstracts an oxygen atom from nitrate releasing nitrite, whereas FdH catalyzes a hydrogen atom transfer from formate and releases carbon dioxide.



In this Account, a critical analysis of structure, function, and catalytic mechanism of the molybdenum enzymes periplasmic nitrate reductase (Nap) and formate dehydrogenase (Fdh) is presented.

We conclude that the main structural driving force that dictates the type of reaction, catalyzed by each enzyme, is a key difference on one active site residue that is located in the top region of the active sites of both enzymes.

In both enzymes, the active site is centered on the metal ion of the cofactor (Mo in Nap and Mo or W in Fdh) that is coordinated by four sulfur atoms from two pyranopterin guanosine dinucleotide (PGD) molecules and by a sulfido. However, while in Nap there is a Cys directly coordinated to the Mo ion, in FdH there is a SeCys instead. In Fdh there is also an important His that interacts very closely with the SeCys, whereas in Nap the same position is occupied by a Met. The role of Cys in Nap and SeCys in FdH is similar in both enzymes; however, Met and His have different roles. His participates directly on catalysis, and it is therefore detrimental for the catalytic cycle of FdH. Met only participates in substrate binding. We concluded that this small but key difference dictates the type of reaction that is catalyzed by each enzyme. In addition, it allows explaining why formate can bind in the Nap active site in the same way as the natural substrate (nitrate), but the reaction becomes stalled afterward.

INTRODUCTION

Molybdenum ($_{42}$Mo) is the only second row transition metal with a defined biological role. Together with tungsten, they are the heaviest elements used by living organisms. Both Mo and W exhibit multiple oxidation states within a biologically relevant range of electrochemical potentials and are capable of catalyzing different reactions important for the geochemical cycles of the most abundant elements of the earth (N, S, C, Cl, among others).

The DMSO reductase (DMSOr) family, that includes Nap and Fdh, shows a high variability in the active site composition. Four sulfur atoms from the dithiolene moieties of two pyranopterin guanosine dinucleotide (PGD) molecules coordinate the metal ions (Figure 1). In addition, the metal ion can coordinate two extra ligands, resulting in a distorted trigonal-prismatic geometry. The fifth and sixth ligands vary for the different enzymes within the family, and are thought to be essential to determine the redox properties of the active site and its reactivity/specificity. According to the identity of these last ligands, the family can be further split into three subfamilies. In subfamily I, that includes Nap and Fdh, Mo/W coordinates a cysteine (Nap) or a selenocysteine (Fdh) and a sulfido. In subfamily II, one or two oxygen atoms from the side chain of an aspartate residue complete the coordination sphere of the metal ion. In subfamily III a serine hydroxyl

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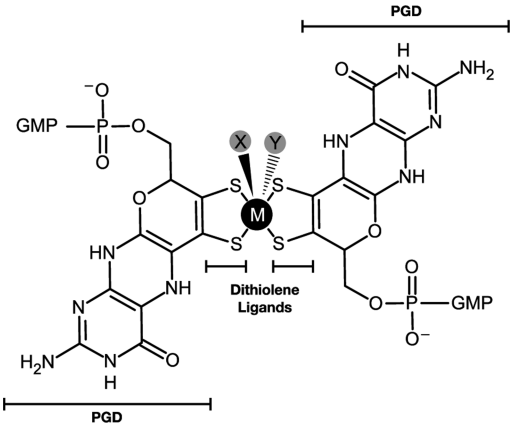

Figure 1. Molybdenum cofactor present in the enzymes of the DMSO reductase family (periplasmic nitrate reductase (Nap), formate dehydrogenase (Fdh), respiratory nitrate reductase (Nar), dimethyl sulfoxide reductase (DMSOr), among others). M = Mo, W; X = cysteine (Nap), selenocysteine (Fdh), aspartate (subfamily II, e.g., Nar), and serine (subfamily III, e.g., DMSOr). Y = sulfido (Nap and Fdh), oxo/hydroxy/water (subfamily II and III). In Nar, Y can also be an O atom from a bidentated aspartate residue. PGD and GMP stand for pyranopterin guanosine dinucleotide and guanosine monophosphate, respectively.

occupies the fifth coordination position of the metal ion. Most of these enzymes catalyze similar O atom transfer (OAT) reactions. However, Nap and Fdh are an example of enzymes sharing almost identical active sites but catalyzing very different reactions. Nap performs an OAT while Fdh catalyzes a H atom transfer (HAT). The comparison of these two enzymes at a molecular level serves to study how key changes in the amino acid sequence can tune the properties of the metal ion to modify its reactivity.

In this Account, we focus on two specific enzymes: the Mo-Nap from *Desulfovibrio desulfuricans* (*Dd*) ATCC 27774 (pdb code 2JIO[1]) and the Mo-Fdh-H from *Escherichia coli* (*Ec*) K12 (pdb codes 2IV2[2] and 1FDO[3]). The crystallographic structures of these two enzymes have been solved by X-ray crystallography, sharing very similar overall folds and active site arrangements.

Based on the available literature, we propose to review the structure, function, and, in more detail, the catalytic mechanism of Nap and Fdh and highlight the key features that make them apparently similar, as well as those that allow them to play different biological roles.[4−10]

ANALYSIS OF PRIMARY SEQUENCES AND STRUCTURES OF *Dd* Nap AND *Ec* Fdh-H

The amino acid sequences of *Dd* Nap and *Ec* Fdh-H share a low percentage of sequence identity (28.3%), but high structural similarities. The RMSD between the backbone atoms is only 2.08 Å. A closer inspection of the X-ray structures shows that the core catalytic regions of both proteins present a high degree of structural conservation. This core region contacts directly with the metal cofactor and, in both enzymes, the cofactors can be almost superimposed (RMSD ∼ 1.0 Å). From a structural point of view, the deviations in the structures of Nap and Fdh are found mainly in the loops located at the protein surface and around the highly conserved core catalytic region.

In both enzymes, the active site is deeply buried and centered on the Mo atom, which is hexacoordinated to four sulfur atoms of two PGDs, one inorganic sulfur, and one S (Nap) or Se (Fdh) atom from the side chain of a Cys and SeCys.[11] According to the *Dd* Nap X-ray structures (resolution of 2.20 Å, pdb code 2JIO[1]) and *Cupriavidus necator* H16 (resolution of 1.5 Å, pdb code 3ML1[12]), the inorganic sulfido ligand is partially bound to the cysteine sulfur, producing a bidentate persulfido ligand that stabilizes the hexacoordinated pseudo-trigonal prismatic geometry around the metal. The same situation is found in the X-ray structures of the *Ec* Fdh-H[3] and in the W-Fdh from *Desulfovibrio gigas*,[13] although the cysteine is replaced by a selenocysteine.

In the active site second shell region, there are other key residues that differentiate Nap from Fdh (Figure 2). In *Ec* Fdh-

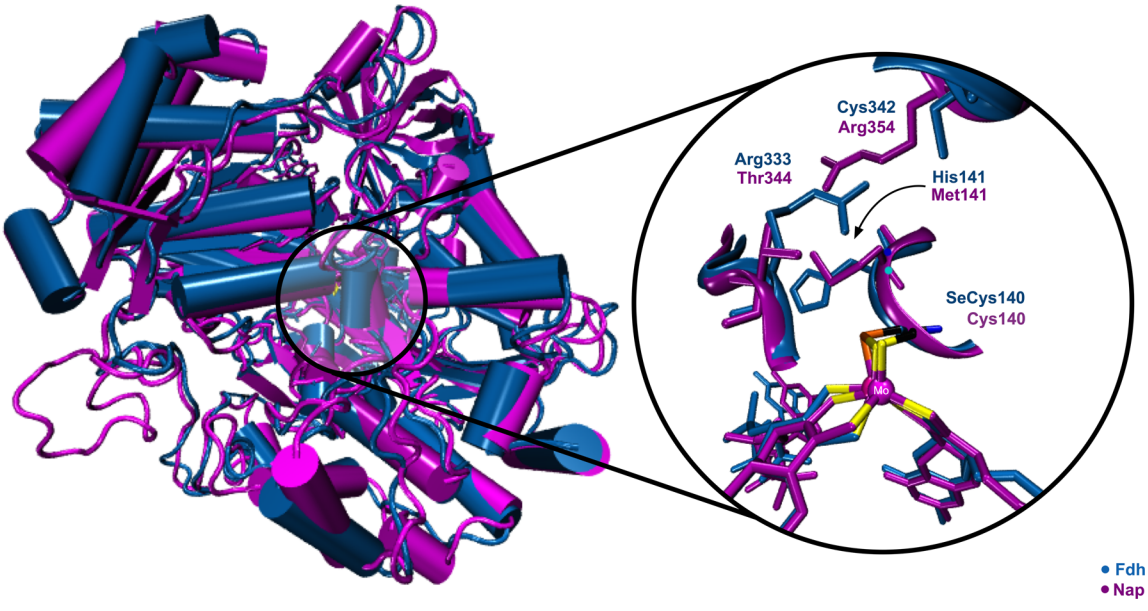

Figure 2. Superposition of the X-ray crystallography structures of *Dd* Nap (pdb code: 2JIO[1]) and *Ec* Fdh-H (pdb code 1FDO[3]) represented in magenta and blue, respectively.

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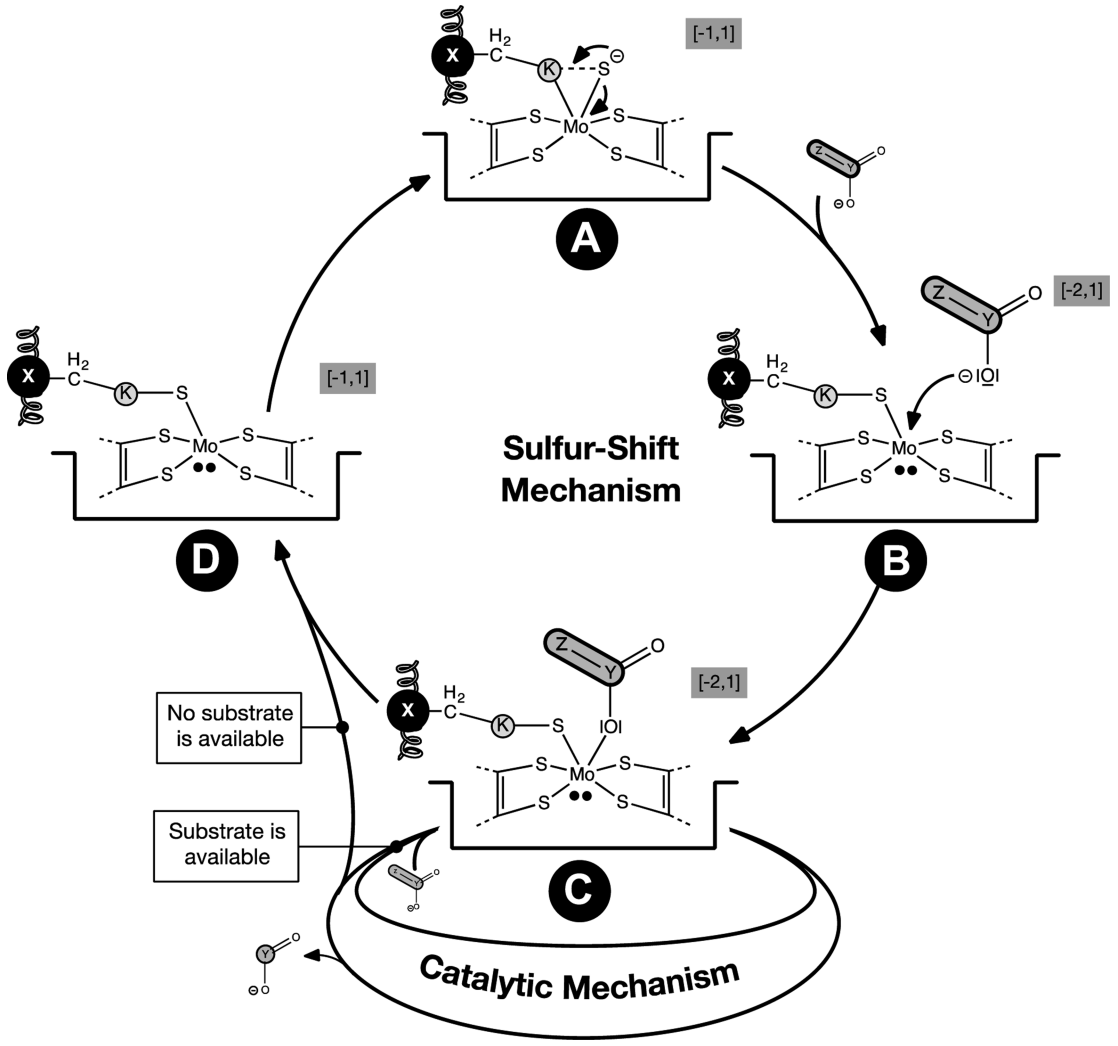

Figure 3. Sulfur-shift mechanism in nitrate reductase (Nap) and in formate dehydrogenase (Fdh) (in Nap, X = SCys, K = S, Y = N, and Z = O; in Fdh, X = SeCys, K = Se, Y = C, and Z = H).

H, there is an important histidine (His141) that interacts very closely with SeCys140 and has a key role in catalysis.[14] In Nap, the same position is occupied by Met141. This residue is not involved in catalysis, but was shown to participate in substrate binding.[15]

Above the region of the metal center, both enzymes present an arginine residue (Arg354 in Nap and Arg333 in Fdh-H) that is proposed to be key for stabilization and substrate binding. These residues occupy similar spatial positions, suggesting a common role. Since the substrates of both enzymes (nitrate and formate) are negatively charged, it was suggested that the side chain of these residues would interact electrostatically with the substrates, compensating for the negative charge and favoring their interaction with the negatively charged active site.[16]

■ THE CATALYTIC MECHANISMS OF Nap AND Fdh

The high degree of structural conservation observed in Nap and Fdh structures could suggest that both enzymes share similar catalytic activities. But this is far from the truth, since both enzymes catalyze very different reactions. Recent studies have shown that the catalytic mechanisms are different and involve different oxidation states of the molybdenum ion during the course of the reactions.[17,18] Nevertheless, the same studies have revealed also that both enzymes share some common processes in the initial stages of the enzymatic mechanism as it is discussed in the next sections.

The Sulfur-Shift Mechanism

Full coordination spheres in active sites metal ions are not prone to allow the binding of substrates or any other ligand in general. In most cases, a change of the metal ion oxidation state is needed to release a ligand and/or change the first-coordination shell. Transition metals with incomplete coordination spheres or with labile groups that can be replaced competitively by other ligands can easily coordinate substrates and catalyze reactions leading to the formation of products, creating pathways energetically favorable in the context of biological reactions. There are many examples in the literature where enzyme activity is controlled by changes in the coordination sphere of the catalytic metal site.[4,10] The conversion of inactive forms into active forms can be triggered by changes in coordination number (accompanied by conformational changes) creating free coordination sites available for the substrates. These aspects have been observed in the computational studies devoted to Nap and Fdh.[16−20] When substrate is not present at the active site, the Mo ion is hexacoordinated and no free position is available to bind the

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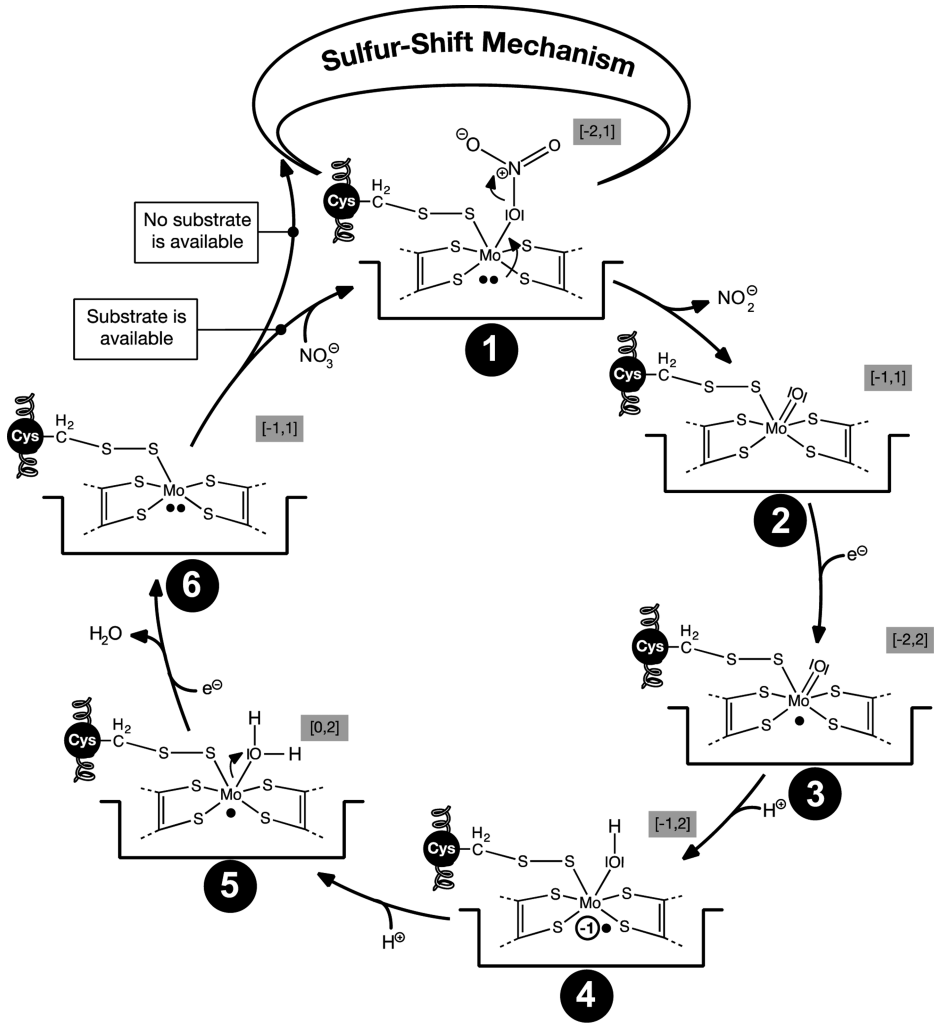

Figure 4. Proposed catalytic mechanism for nitrate reductase.

substrate, similarly to what is observed in the X-ray structure 2JIO.[1] Assuming that the Mo ion is in oxidation state VI, the total charge and spin multiplicity of the model system is −1 and 1, respectively (Figure 3: A). This negatively charged active site prompted several authors to propose that the catalytic mechanism of both Nap and Fdh should occur at a *second-shell*, as the approach and binding of negative substrates to the also (overall) negative cofactor would be extremely unfavorable from an energy point of view. However, recently it was shown that the mechanism occurs through a *first-shell* type of mechanism, in which the substrates bind and react directly with the Mo ion of both enzymes.[16−19,21] This is only possible due to a rearrangement on the coordination sphere around the Mo ion that occurs when the substrate comes close to metal ligands. This rearrangement was named "sulfur-shift mechanism" (Figure 3).

The sulfur-shift mechanism is defined as a change in the Mo ion coordination, which involves a first-to-second shell displacement (shift) of one of the metal ligands (the sulfur or selenium from the Cys or SeCys chain), resulting in a free coordination position that is used by the enzyme to bind the substrate with a low energy cost (Figure 3: A → B).[16]

The next step of the catalytic cycle is still similar for both enzymes, as Mo coordinates an oxygen atom from the substrates (Figure 3: B → C). From this point on, the mechanisms of Nap and Fdh diverge and follow different pathways.

Once the catalytic reactions are complete and the products of the reactions released, the enzymes arrived to a state in which a free coordination position is available at the Mo ion. From this point, two pathways can be followed. If a new substrate molecule is available at the active site, it can bind the metal ion occupying the free coordination position, and the catalytic process continues. However, if no substrate is readily available at the active site, the inverse of the sulfur-shift mechanism occurs, and the metal site returns to the stable hexacoordinated form (Figure 3: D → A).

The energetic profile of this mechanism is very favorable as the reactions are almost thermoneutral and involve low activation energies, allowing the enzyme to exchange easily between the ready and unready forms. Interestingly, the sulfur-shift mechanism, first predicted from computational means,[16] was later supported by X-ray crystallography data.[12]

This mechanism is very similar to the well-known carboxylate-shift mechanism that is found in other enzymes, as for example Zn-containing hydrolases.[22] Both sulfur- and carboxylate-shift mechanisms present an efficient way to modify the transition metal coordination number, allowing a constant number of ligands to the metal ion throughout the catalytic cycle. The main difference between these mechanisms is that

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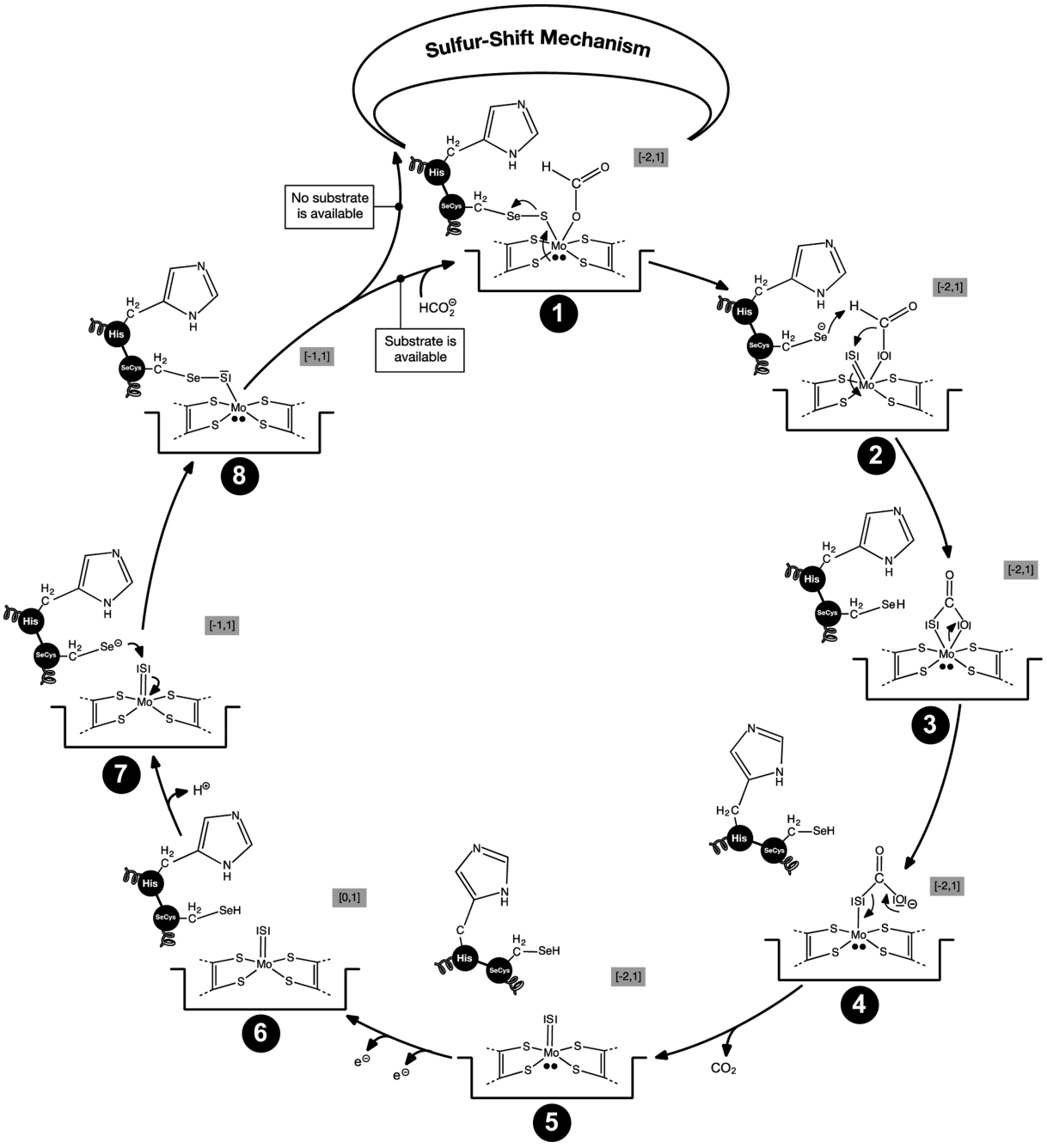

Figure 5. Proposed catalytic mechanism for formate dehydrogenase.

the sulfur-shift mechanism involves the change in the coordination of a sulfur or selenium atom from Cys or SeCys, while the carboxylate shift involves a monodentate/bidentate exchange of a carboxylate group within the first shell.

The Catalytic Mechanism of the Periplasmic Nitrate Reductase (Nap)

Nap catalyzes the reduction of nitrate to nitrite, in which an oxygen atom from the substrate is transferred to the Mo ion, and later released as a water molecule. The reaction requires two electrons, which are provided by external reducing species, and two protons that are obtained from the solvent either directly or indirectly mediated by residues from the enzyme catalytic pocket. Figure 4 summarizes the currently accepted catalytic mechanism for the conversion of nitrate into nitrite catalyzed by Nap.[17]

After the sulfur-shift has been completed, a free coordination position becomes available at the metal center of nitrate reductase that is promptly occupied by nitrate. Taking into account the presence of nitrate, the total charge and spin multiplicity of the system becomes −2 and 1. The energy profile of this reaction is very favorable and at the end of the reaction step, nitrate is coordinated to the Mo ion through one of its oxygen atoms (Figure 4: 1). The negative charge that is obligatorily clustered during the substrate-binding step is spread in both tricyclic pyranopterin molecules. Therefore, the role of both pyranopterin fractions of the PGD molecules is very important for the catalytic process, as they cushion the

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excess of electron density, facilitating the interaction between two negatively charged molecules.

Next, the reduction of nitrate into nitrite is the rate-limiting step of the full reaction[17] (Figure 4: 1 → 2). In this process, the N−O bond (coordinated to the Mo ion) is cleaved, resulting in the formation of nitrite. The O atom remains attached to the Mo ion through a double bond. The persulfide moiety formed by the gamma sulfur from Cys140 and the sulfido ligand during the sulfur-shift, remains coordinated.

From the binding of nitrate until the formation of nitrite, the total charge and spin multiplicity of the total system remains constant (i.e., −2 and 1, respectively) and no electrons were traded from external reducing species to the metal cofactor. This might result counterintuitive as a Mo(VI) formal oxidation state was assumed initially. However, QM calculations indicates that the electrons needed to reduce nitrate to nitrite originate from the reduction of the Mo ion (from Mo(VI) to Mo(IV)) during the sulfur-shift event. This redox interplay between the sulfur-ligands and the Mo ion was reported by Stiefel and co-workers,[23,24] and was discussed when the sixth ligand to the Mo ion was identified as a sulfur atom in the *Dd* Nap X-ray structure.[1]

Once the nitrite molecule leaves the catalytic pocket, the formation of a water molecule and the reduction of the active site by an external reducing species complete the enzymatic turnover. Theoretical calculations have shown that the metal cofactor with a charge −1 and spin multiplicity of 1 is a poor nucleophile and cannot facilitate the protonation of the oxo group at the Mo ion.[17] Moreover, the water molecule only dissociates from the Mo ion after the reduction of the metal cofactor, a process that occurs in two sequential steps of one electron. After the transfer of one electron from the external redox partner of the enzyme (Figure 4: 2 → 3), the system acquires an odd number of electrons, having a total charge of −2 and spin multiplicity of 2. Experimentally, in *Dd* Nap, it was observed that a shortage of electron donors favors the presence of paramagnetic species that can be detected by EPR spectroscopy.[25,26] Analysis of the EPR signal suggested the nature of this intermediate, resulting from two protons of a water molecule bound either to the sulfido group or directly coordinated to the molybdenum atom. Theoretical calculations favor the existence of the latter intermediate and should correspond to state 5 of Figure 4. The detection of this intermediate, after electron donor shortage, is favored by the fact that the proton transfer should be faster (Figure 4: 3 → 4 and 4 → 5) than the electron transfer from the electron donors (Figure 4: 2 → 3 and 5 → 6).

When the active site receives the second electron, the water molecule is released from the Mo ion and leaves the active site pocket. As mentioned before, the enzyme can then follow two different pathways. If no further substrate is available at the catalytic pocket, the sulfur-shift mechanism is reversed and the Mo ion arrives at the hexacoordinated distorted-trigonal prismatic geometry (Figures 1 and 4: 6 → D → A). However, if nitrate is available, it can promptly occupy the free coordination position at the Mo ion, and the catalytic process continues without the need to pass through the structure observed by X-ray crystallography (Figure 4: 6 → 1). This should turn the reduction process more efficient.

The Catalytic Mechanism of Formate Dehydrogenase (Fdh)

Fdhs catalyzes the oxidation of the formate anion to CO_2 in a redox reaction that involves the transfer of a H atom and two electrons from the substrate to the enzyme.[27] X-ray crystallographic structures are available for Fdh-H in two oxidation states.[3] In the oxidized state, the Mo site comprises a hexacoordinated Mo in a distorted trigonal prismatic geometry. The Mo first coordination sphere is very similar to the one described for Nap, but with a Se from a SeCys residue instead of a S from a Cys residue.[3] On the other hand, the crystallographic structure of the reduced Fdh-H showed a pentacoordinated Mo ion in a distorted square-pyramidal geometry, in which the four sulfur atoms from the dithiolene moieties occupy the vertices of the equatorial plane, and the Se from the SeCys is in the axial position.[3] Later, the crystallographic data of the Fdh-H reduced form was reinterpreted and it was found that the SeCys ligand was 7 Å away from the metal site, not coordinated to the Mo ion, and that the axial position was occupied by a sulfido ligand.[2]

Computational studies have shown that the initial part of the mechanism of Fdh is very similar to that of Nap, as depicted in Figure 5.[18,20,28] As for Nap, the mechanism starts with the Mo ion hexacoordinated and, when substrate is available at the active site, the sulfur (selenium)-shift mechanism is triggered and opens a free coordination position at the metal ion that is promptly occupied by one of the oxygen atoms of formate (Figure 5: 1). At this stage the total net charge of the metal cofactor is −2, and the spin multiplicity 1. Similarly to what was observed for Nap, the two pyranopterin molecules have a key role spreading the negative charge that is generated during the binding of the formate.

The following reactions involve the oxidation of formate.[18] The first stage involves the cleavage of the bond that connects SeCys to the inorganic sulfur (Figure 5: 1 → 2). This cleavage is possible thanks to the side chain of a histidine (His141), which forms a hydrogen bond with the resulting selenol anion. This interaction is crucial to stabilize the selenol anion that is responsible to perform the proton abstraction from formate (Figure 5: 2 → 3). Once this reaction is complete, the SeCys moves away from the active site, resembling what has been observed in the reinterpretation of the X-ray data of reduced Fdh-H[2]. At the end of these two steps, the resulting carbon dioxide molecule does not dissociate from the active site, and remains bound to the Mo ion, that retains a hexacoordination number (Figure 5: 3).

The next steps of the catalytic cycle involve the release of carbon dioxide, the oxidation of the active site and the enzymatic turnover. The carbon dioxide release occurs in two sequential steps (Figure 5, steps 3 → 4 and 4 → 5). First, the Mo−O bond is broken and only afterward the bond between the carbon and the sulfido ligand is *cleaved*. After these two steps, carbon dioxide is released and the inorganic sulfur remains covalently bound to the Mo ion by a double bond, resembling what is observed in the reinterpreted X-ray structure of reduced Fdh-H[2]. As observed in Nap, from the binding of the formate to the Mo ion until the release of the carbon dioxide, the total charge and spin of the system remains constant, and the transfer of electrons to an external electron acceptor is not required. According to the computational studies, this only occurs after the formation of intermediate 5. Only then, the sequential electron transfer to the external oxidant species occurs, which leads to the oxidation of the metal cofactor (Figure 5: 5 → 6). In the next steps, the proton bound to the SeCys side chain is transferred to the solvent or to another residue in the catalytic pocket. This event forces a conformational rearrangement of the loop where SeCys is

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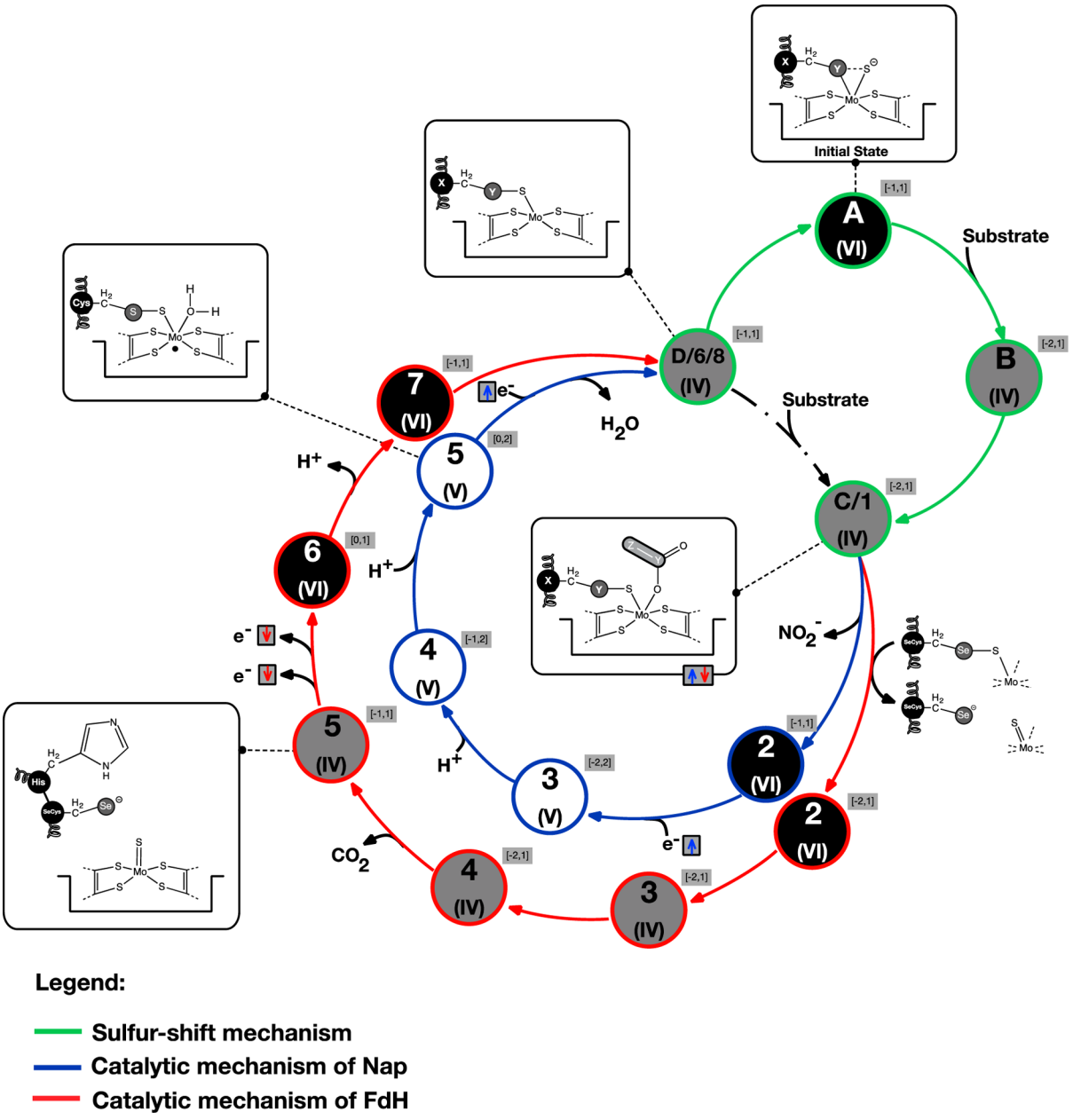

Figure 6. Changes in the oxidation states of the Mo ion during the catalytic mechanisms of Nap (inner circle) and Fdh (outer circle). The numbers and letters represent the reaction intermediates of the catalytic mechanisms shown in Figures 3–5. X stands for Cys or SeCys and Y for S or Se depending on the enzyme that is analyzed.

located, to occupy a position nearby the Mo ion (Figure 5: 6 → 7). Once this is accomplished, the SeCys forms a covalent bond with the sulfido ligand that is coordinated to the Mo ion (Figure 5: 7 → 8).

Similar to what was observed for Nap, two pathways can be followed from this point. If no substrate is available at the active site, the sulfur-shift mechanism is reversed and the metal site adopts the original resting conformation (Figures 1 and 5: 8 → D → A). If more formate is available, it can occupy the free coordinating position at the Mo ion and the catalytic process continues (Figure 5: 8 → 1).

A key difference between the catalytic mechanisms of Nap and FdH is the fact that only Mo is used to reduce nitrate but in Fdhs both Mo and W are catalytically competent to oxidize formate to carbon dioxide. Presently, it is not fully established why both Mo and W are capable of catalyzing the oxidation of formate to CO_2 in Fdh, while Nap containing W in the active site are inactive. Theoretical calculations have shown that no significant changes on the energy profile of the catalytic mechanism are observed when the Mo is exchanged by W in the model used for the calculations.[18] Nevertheless, in line with the computational studies, our experimental results showed that the K_m and k_{cat} values of the molybdenum and tungsten-containing Fdh enzymes isolated from *Desulfovibrio* sp. are of the same order of magnitude.[18]

The differences in the reactivity of enzymes having W and Mo in their actives sites should be more related with the reduction potentials of the Metal(VI/V) and Metal(V/IV) redox couples. Schulzke and Doring have shown that molybdenum is able to provide more stable conditions for redox processes with a smaller change of its reduction potential upon temperature change and a smaller entropy gain or loss

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upon oxidation or reduction.[29] This means that the enzymes containing Mo instead of W should be more efficient on the electron transfer which will turn the enzymes more efficient on the catalysis.

OXIDATION STATE OF THE Mo ION DURING THE CATALYTIC CYCLES OF Nap AND Fdh

The formal oxidation state of the Mo ion through the catalytic cycles of Nap and Fdh is a subject of controversy. Figure 6 depicts how the formal oxidation state of Mo would change during the catalytic cycle of both Nap and Fdh. These proposals are based on our previous theoretical results and the available experimental data.

There is a general consensus that the initial oxidation state of the Mo ion in the X-ray structures of the oxidized-forms of both Nap and Fdh is VI (Figure 6: A). This is also supported by several computational studies which showed that only with this oxidation state (where the charge and spin multiplicity of the system are −1 and 1) we can reproduce the coordination number and geometry around the Mo ion as observed in the available X-ray structures.[3]

As described above, the first stage of the catalytic mechanism of both enzymes involves the sulfur-shift mechanism, induced by substrate approach and binding. When the Mo−S_{Cys} (or Mo−Se_{Cys} for Fdh) bond is broken, the metal ion receives the two electrons of the sulfur/selenium, gaining electron density (i.e., becoming reduced). The two S atoms (or S and Se for Fdh) establish a covalent single bond and are therefore oxidized. The oxidation state of the dithiolene S atoms remains unaltered throughout the whole catalytic cycle. Furthermore, the total charge of the system (−2) does not change in this process (Figure 6: B→ C). These results are in agreement with early observations of Stiefel and co-workers[23,24] and show that the sulfur shift-mechanism involves internal redox processes at the active site, and this resembles the chemical nature of the thiol−disulfide exchange reaction, which is ubiquitous in the chemistry of living systems.

In Nap, the reduction of nitrate and the concomitant release of nitrite implies the oxidation of the metal cofactor, from Mo(IV) to Mo(VI). According to the computational studies, the two electrons needed to complete the nitrate reduction are not necessarily transferred from the electron donor until the final stages of the catalytic cycle. The transfer of two electrons from the external electron donor and of two protons (from the solvent or residues of the catalytic pocket) are only mandatory to protonate the oxo-group (after nitrite release) and to form the water molecule that is released later on and from which result enzyme species ready to bind the next substrate molecule. DFT calculations showed that the formation of the water molecule involves the participation of Mo(V).[17] However, it only dissociates spontaneously from the metal when the second electron is transferred to the metal and forms Mo(VI).

In FdH, the formate binds to the metal site in the presence of Mo(IV). Subsequently, the covalent bond between SeCys and the sulfido is cleaved, and since the Se atom grabs the electron density to form the selenol anion, the oxidation of the Mo (from Mo(IV) to Mo(VI)) by the sulfido ligand takes place. After the proton transfer from the substrate to the selenol anion of SeCys, the metal cofactor is again reduced from Mo(VI) to Mo(IV). In all of these reactions the substrate remains covalently bound to the Mo ion. The release of carbon dioxide only occurs after the proton transfer from formate to SeCys. This is accomplished in four sequential steps, during which several internal reduction and oxidation processes occur within the metal cofactor but do not alter the overall oxidation state of the Mo(IV) ion.

After the release of CO_2, the metal keeps the (IV) oxidation state. It is then oxidized to Mo(VI) through the transfer of two electrons to the external electron acceptor. Subsequently, SeCys looses a proton to the solvent or to an active site residue and binds to the sulfido. S and Se are oxidized to form a covalent bond and Mo(VI) receives two electrons from the broken Mo−S bond, becoming reduced to Mo(IV). After this point and similar to what is observed in Nap, the enzyme is ready for a new turnover (Figure 6: 8 → 1) or returns to the initial state (Figure 6: 8 → A).

Both mechanisms have in common that once the sulfur-shift has been triggered, the release of the product of the reaction (nitrite in Nap and CO2 in Fdh) requires the presence of Mo(IV), suggesting that these reactions (Figure 6: 1 → 2 in both pathways) require species rich in electrons. Another point in common is that both reactions occur without the transfer of electrons from the external reducing or oxidizing species, but through internal redox reactions. This proposal has generated a lot of speculation in the last years, since it was expected that the reduction of nitrate into nitrite and the oxidation of formate to CO2 should be concomitant with the transfer of two electrons from the external electron donor or acceptor, respectively.[30] However, the sulfur shift mechanism, which is another redox reaction (dithiolate to disulfide and vice versa), is the redox pair that reduces nitrate and oxidizes formate.

CONCLUSIONS

It is remarkable how nature has been able to construct enzymes that, despite sharing many similarities, have simple but key differences that have tuned them for completely different functions. Nap and Fdh from the DMSOr family are examples of this. The structures of these enzymes and their active sites are almost identical in terms of coordination number, geometry, and nature of the ligands. Their substrates are also similar. Both are polyatomic anions sharing similar charge and stereochemistry. They also share a common activation mechanism, called the sulfur-shift. In addition, in both cases, the formation of the reaction product occurs before to the transfer of electrons from/to the electron donor/acceptor. In spite of these similarities, Fdhs do not catalyze (at least efficiently) the nitrate reduction or Naps the oxidation of formate. The structural reasons behind these facts rely on the change of two key amino acid residues on the active site of both enzymes: Cys/Met residues present in Naps are replaced by SeCys/His residues in Fdhs. The role of Cys in Nap and SeCys in FdH is apparently similar, as it is evidenced by the sulfur-shift mechanism or by the recent studies performed by Schrapers et al.[31] In this study, it was found that *Rodobacter capsulatus* Fdh has a Cys in the place of SeCys and no subtle differences are observed in the catalytic reaction. Similar observations were also observed in previous theoretical calculations where it was found that formate can bind the Nap active site and trigger the sulfur-shift mechanism in the same way as the natural substrate.[16] However, the reaction becomes stalled once formate coordinates to Mo because Nap has a Met in the position of His in Fdh. In contrast to the latter, the Met residue cannot establish a H-bond to stabilize the potential cys-thiolate (instead of selenol) that leads to the electron density rearrangement that reoxidize the Mo ion to the

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VI oxidation state. The formation of the thiolate anion and Mo(VI) intermediate would be mandatory to catalyze the proton transfer and the formation of carbon dioxide. The nature of this residue is therefore one of the key factors that controls the reactions catalyzed by the two enzymes.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: nscerque@fc.up.pt.
*E-mail: jose.moura@dq.fct.unl.pt.

Present Address

§P.J.G.: Departamento de Física, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, S3000ZAA Santa Fe, Argentina.

Notes

The authors declare no competing financial interest.

Biographies

Nuno M. F. S. A. Cerqueira hails from Braga, Portugal, and obtained his degree in Chemistry from University do Minho (Portugal). Afterward, he received his Ph.D. from Porto University under the direction of Prof. Maria João Ramos. Following postdoctoral studies with Prof. José Moura at Faculdade de Ciências e Tecnologia at Universidade Nova de Lisboa, he became a research scientist (Ciência 2007) at Faculdade de Ciências of Porto University (Porto, Portugal). Since 2014, he is a research scientist (IF-FCT) at UCIBIO@REQUINTE. His main research interest is to understand reaction mechanisms at the molecular level and controlling factors for the reactivity and selectivity of important chemical transformations, using computational means.

Pablo J. González was born in Santa Fe (Argentina). He received his B.S. in Biotechnology from Universidad Nacional del Litoral (Argentina) and then performed his Ph.D. in Biophysical Chemistry under the supervision of Prof. José J.G. Moura at the Faculdade de Ciências e Tecnologia of Universidade Nova de Lisboa (FCT-UNL, Portugal). He had postdoctoral training in biophysical techniques applied to the study of metalloenzymes before joining as a research scientist to REQUIMTE at FCT-UNL. At present, he is a professor and research scientist (CONICET) at the Facultad de Bioquímica y Ciencias Biológicas of Universidad Nacional del Litoral (Argentina).

Pedro A. Fernandes was born in Setúbal, Portugal. He obtained his degree in Chemistry at the University of Porto, Portugal. Afterward he has obtained his Ph.D. (1999) in Molecular Dynamics Simulations of Liquid Interfaces and Ion Transfer, under the supervision of Prof. José Ferreira Gomes, at the same University. He joined the University of Porto as an Assistant Professor in 1999 and the research group of Prof. Maria João Ramos in 2000. He is now Associate Professor, dedicated to the field of computational biochemistry, in the areas of protein structure and dynamics, enzymatic catalysis and inhibition, and drug design.

José J. G. Moura has a degree in Chemical Engineering and a Ph.D. in Chemistry and is Professor of Chemistry at Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa. Main field of research is Bioinorganic Chemistry and the role of Metals in Biology. President of Chemistry Department and President of Scientific Council at FCT-UNL, Research Specialist University of Minnesota, Adjunct Professor University of Georgia, Athens, Portuguese Delegate to NATO, COST, and INTAS, member of Scientific Panel in the Calouste Gulbenkian Foundation and FCT-MCTES, and of several scientific editorial boards. In 2006, he was elected Member of Academia das Ciências de Lisboa, and in 2010 elected President of the Society of Biological Inorganic Chemistry. Director of FCT-UNL Campus Library since 1996.

Maria João Ramos obtained her first degree in Chemistry at the University of Porto, Portugal, and her Ph.D. in muon research at The University of Glasgow, U.K., and performed postdoctoral research in molecular modeling at the University of Oxford, U.K. Back in Portugal since 1991, she is now a Professor at the University of Porto, the leader of a group working in computational biochemistry with three main lines of research: computational enzymatic catalysis, protein structure and dynamics, and drug design. She was nominated Doctor Honoris Causa by the University of Stockholm and is now Vice-Rector of the University of Porto.

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