Inorganic Chemistry

Article

pubs.acs.org/IC

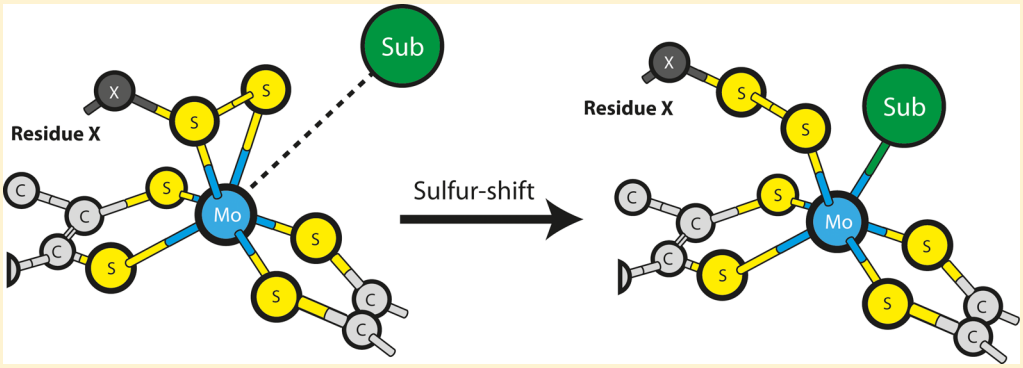
The Sulfur Shift: An Activation Mechanism for Periplasmic Nitrate Reductase and Formate Dehydrogenase

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

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

[‡]REQUIMTE/CQFB, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Quinta da Torre s/n, 2829-516 Caparica, Portugal

Supporting Information



ABSTRACT: A structural rearrangement known as sulfur shift occurs in some Mo-containing enzymes of the DMSO reductase family. This mechanism is characterized by the displacement of a coordinating cysteine thiol (or SeCys in Fdh) from the first to the second shell of the Mo-coordination sphere metal. The hexa-coordinated Mo ion found in the as-isolated state cannot bind directly any exogenous ligand (substrate or inhibitors), while the penta-coordinated ion, attained upon sulfur shift, has a free binding site for direct coordination of the substrate. This rearrangement provides an efficient mechanism to keep a constant coordination number throughout an entire catalytic pathway. This mechanism is very similar to the carboxylate shift observed in Zn-dependent enzymes, and it has been recently detected by experimental means. In the present paper, we calculated the geometries and energies involved in the sulfur-shift mechanism using QM-methods (M06/(6-311++G(3df,2pd),SDD)//B3LYP/(6-31G(d),SDD)). The results indicated that the sulfur-shift mechanism provides an efficient way to enable the metal ion for substrate coordination.

1. INTRODUCTION

Molybdenum (Mo) is a second row transition metal that plays a key role in cellular metabolism catalyzing different redox and non-redox reactions.[1,2] Only two molecular scaffolds that bind Mo and control its redox state and catalytic power are found in proteins: the Fe−Mo and the pyranopterin-based Mo cofactors.[1−3] In the latter case, the Mo ion is coordinated to one or two pyranopterin dithiolene groups from pyranopterin molecules, to oxygen, sulfur, or selenium atoms from residue side chains, and to water. These differences are used to classify these enzymes into three families: xanthine oxidase, sulfite oxidase, or DMSO reductase.[1,4,5]

Enzymes from the DMSO reductase family are the subject of this study. They all present a hexacoordinated Mo ion bound to four sulfur atoms from two dithiolene moieties of two pyranopterin molecules and two more ligands, which are highly variable. The DMSOr family was divided into three subfamilies according to the identity of the fifth and sixth ligands (Figure 1).

In the case of subfamily I, there is no evident substrate binding position since, in contrast to enzymes from subfamilies II and III, there is no solvent molecule coordinating the Mo ion that can act as leaving group. Based on this observation, it has been proposed that in enzymes like Nap and Fdh the substrate transformation is catalyzed at the ligands of the second coordination sphere of the Mo ion rather than on direct coordination with the Mo ion (first sphere mechanism).[7]

The second sphere type of mechanism is supported by the recent X-ray structures of Nap from *Desulfovibrio desulfuricans* ATCC 27774 (2.20 Å, pdb code 2jio)[7] and *Cupriavidus necator* H16 (1.5 Å, pdb code 3ml1),[8] in which the sixth sulfur ligand of the Mo interacts with the sulfur from the cysteine residue and

Received: December 20, 2012
Published: September 25, 2013

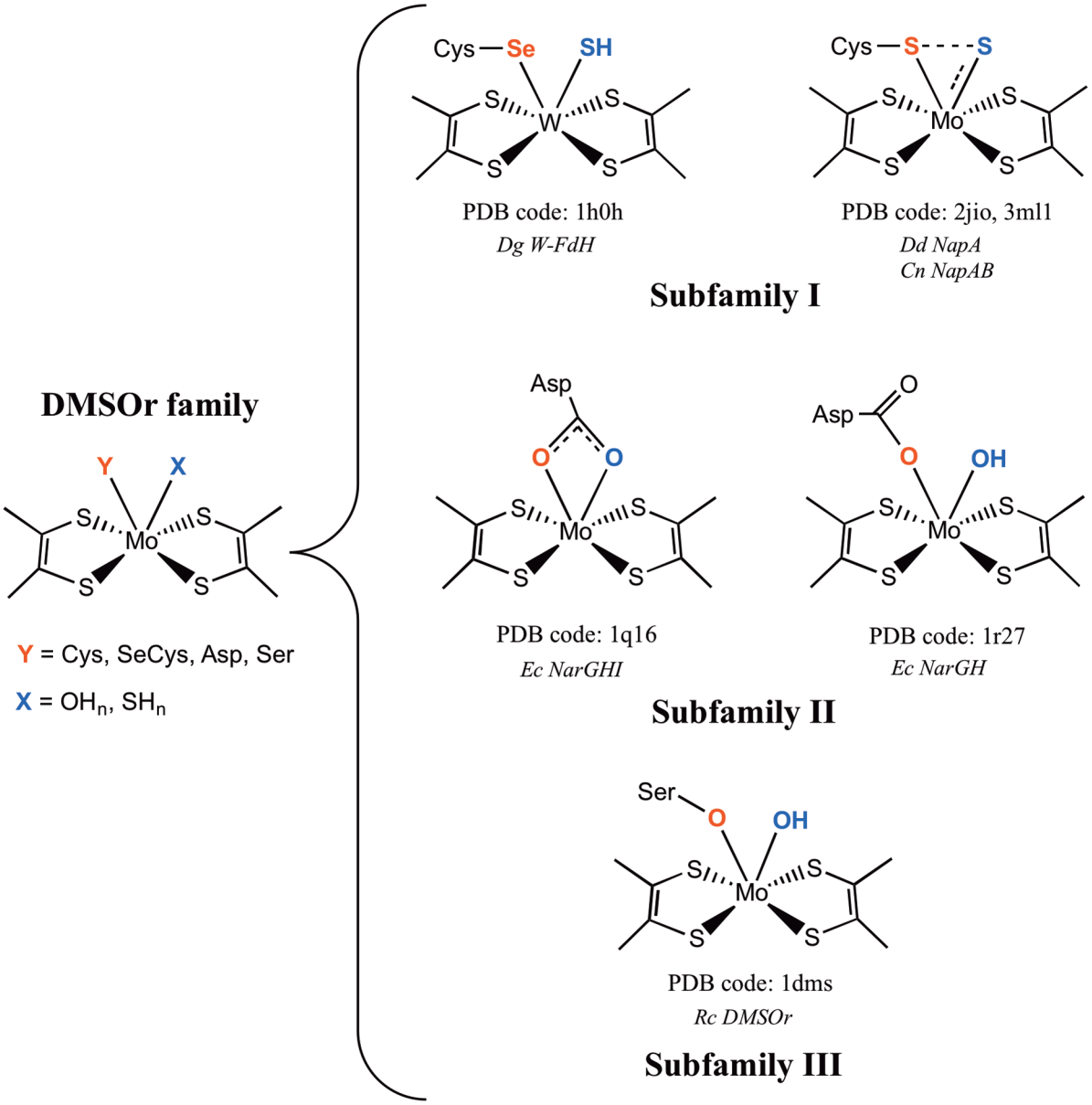


Figure 1. Classification of Mo-pyranopterin dependent enzymes from the DMSO reductase family (adapted from ref 6).

generates a bidentate persulfido ligand that sterically blocks the access of the substrate to the Mo ion. Similar observations are found in the X-ray structures of the Fdh-H from *Escherichia coli* K12[9] and in the W-Fdh from *Desulfovibrio gigas*,[10] though in these cases, the cysteine is replaced by a selenocysteine (SeCys). On the other hand, the first coordination type of mechanism is supported by several experimental and theoretical studies.[11−17] In the case of Fdh, this mechanism gathers more consensus since the X-ray structure of the reduced Fdh-H from *E. coli* K12 shows a pentacoordinated Mo ion, where SeCys is found 7 Å away from the metal.[9]

The viability of the first shell type of mechanism in both Nap and Fdh is dependent upon a new mechanism, called the sulfur shift, in which a cysteine (or SeCys in Fdh) must unbind from the Mo or W to open a site for substrate binding.[15,16] This proposal received strong support from the crystallographic structure of a partially reduced Nap from *Cupriavidus necator* H16 (pdb code 3o5a), which showed that an alternative conformation of the Mo-coordinating cysteine residue is possible upon incubation of the protein with reducing agents.[8]

The sulfur-shift mechanism was first introduced in our previous studies,[15,16] but the energies involved in such conformational rearrangement were relatively high to state this phenomenon as an established concept. Therefore we recalculated the mechanistic steps here using higher levels of theory and with a more realistic model of the enzymes. New results concerning these findings are presented and general conclusions are proposed.

2. COMPUTATIONAL DETAILS

The system used to model each enzyme includes the metallic site and all the residues that participate in the catalytic process or are required to maintain the structure of the active site (Figure 2). The Nap model was based on the PDB structure with the code 2JIO,[7] and the FdH model was based on the PDB structure with the code 1FDO.[18] All the studies were performed with Gaussian09[19] at the M06/6-311++G(3df,2pd)//B3LYP/6-31G(d)[20,21] level for all atoms except Mo,

dx.doi.org/10.1021/ic3028034 | *Inorg. Chem.* 2013, 52, 10766−10772

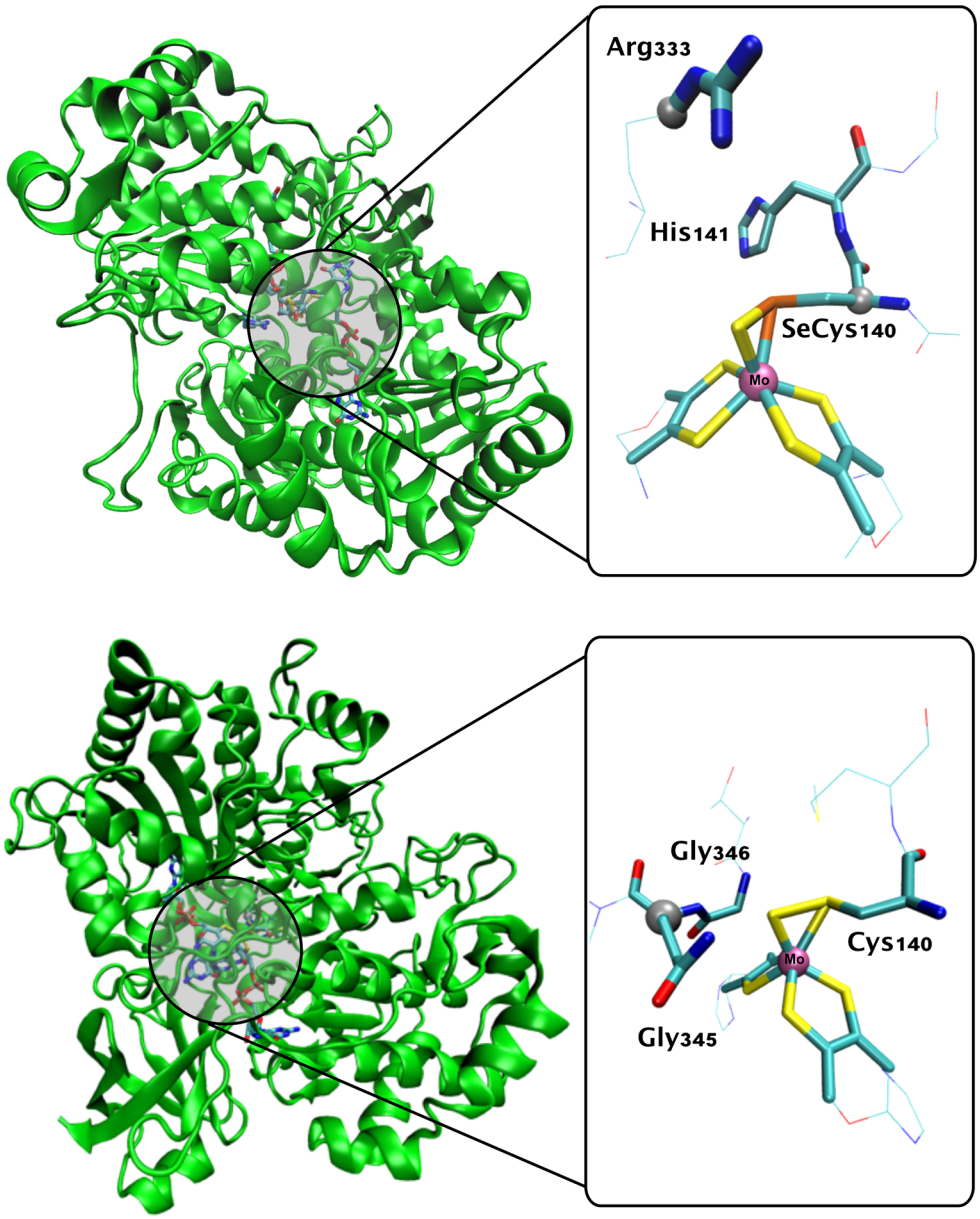


Figure 2. Model systems used to study the sulfur-shift mechanism in Fdh (top) and Nap (bottom). Frozen atoms are represented in ball and stick (including the Mo ion). All the atoms included in model system used to study the sulfur-shift mechanism are displayed in bold sticks. In the structure of the Fdh-H, the oxygen atom that is bound to the molybdenum ion was substituted by a sulfur atom.

which was described with the same functional but with the SDD basis set.[22]

The correct nature of each stationary point was checked by vibrational frequency calculations. Frozen atoms did not lead to additional imaginary frequencies in most of the optimized structures (only a small frequency of 4.5 cm^{-1} was found in the reactants of the model system of FdH), which shows that they are almost free from steric strain. Zero-point corrections and thermal and entropic effects (T = 310.15 K, P = 1 bar) were added to all calculated energies, with the 6-31G(d) basis set. Single point energies included a dielectric continuum model using the integral equation formalism variant (IEFPCM) to account for the long-range interactions of the enzyme. A dielectric constant of 4 was chosen to describe the protein environment of the active site in accordance with previous suggestions.[23−26] Grimme's D3 correction[27] was applied to the functional M06 using the zero damping.

3. RESULTS AND DISCUSSION

We focused on the first step of the catalytic mechanisms of Nap and FdH. Both are members of the DMSOr family and have similar Mo hexa-coordinated shells. In the as-isolated form, the Mo is bound to two pyranopterins, a sulfido, and Cys (Nap) or SeCys (Fdh). Both structures share very similar overall folding and active site arrangements,[4] though key differences in amino acid residues near the active site dictate the very different substrate specificities.[5] Nap catalyzes the reduction of nitrate to nitrite and involves an oxygen abstraction, while Fdh catalyzes the oxidation of formate into carbon dioxide and involves a proton abstraction.

Theoretical calculations[15,16] have shown that the Mo oxidation number, which preserves the coordination shell as seen in the X-ray structures of the two enzymes (2JIO[7] and 1H0H[10]), was Mo(VI). This was also the only oxidation state at which the negatively charged substrates can bind the metal and

dx.doi.org/10.1021/ic3028034 | Inorg. Chem. 2013, 52, 10766−10772

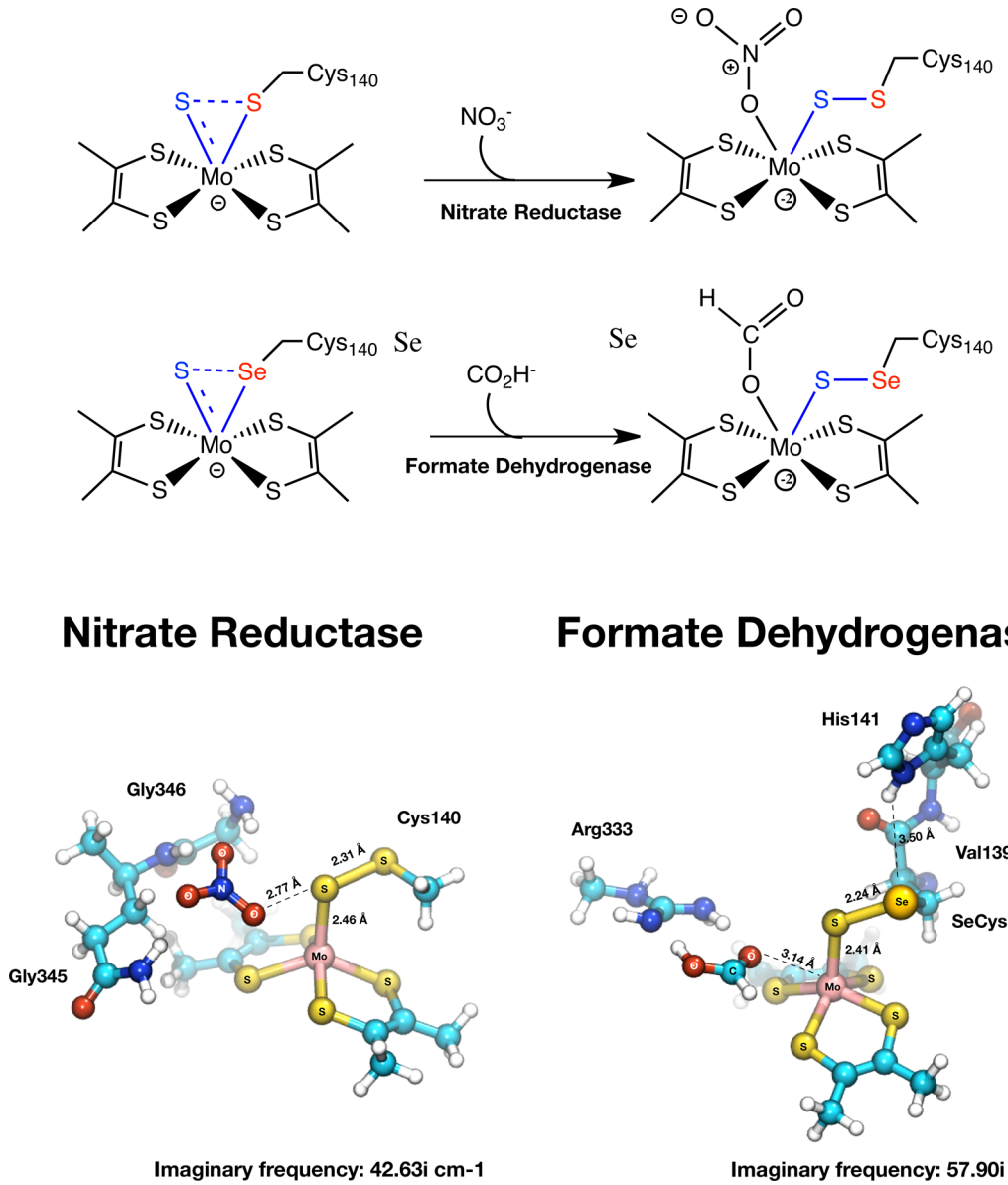


Figure 3. (top) The sulfur-shift mechanism in nitrate reductase (Nap) and in formate dehydrogenase (Fdh). (bottom) Details of the transition state structures for the sulfur shift of Nap and Fdh.

the catalysis proceeds with acceptable kinetics. In such conditions, the sulfido and the residues bound to Mo (Cys140 or SeCys140) interact very closely with each other forming a quasi-covalent bond (2.4 Å in Nap and 2.3 Å in Fdh). Such configuration creates a persulfido ligand that blocks the access of the substrate to Mo, similar to what is found in the X-ray structures of Nap[7](pdb code 2JIO) and Fdh-H (pdb code 1H0H[10]). When the substrate approaches the Mo(VI) ion (4.0 Å in Nap and 3.9 Å in Fdh), it triggers a first-to-second shell movement of the residue bound to the Mo(VI) (Cys140 in Nap and SeCys140 in Fdh) and the simultaneous movement of the sulfido to the position that was previously occupied by the released residue. This process opens a free coordination position at the metal site that is promptly occupied by the substrate, that is, nitrate in Nap and formate in Fdh. The transition states for these concerted transformations are shown in Figure 3. As the substrates further approach the Mo(VI) ion (Mo−O bond length of 3.6 Å in Nap and 3.1 Å in Fdh), the sulfido occupies the position of the residue that moved to the second coordination shell (Mo−S bond length of 2.5 Å in Nap and 2.4 Å in Fdh). The released residue moves further away from the metal (Mo−S_γCys distance of 3.7 Å in Nap and Mo−Se_γCys distance of 3.9 Å in Fdh) but remains in the second coordination shell, connected to the sulfido by a quasi-covalent bond (2.3 Å both in Nap and in Fdh). At the end of this process, the metal site of both enzymes remains hexacoordinated, and the sixth ligand is now an oxygen atom from the substrate (Figure 3).

The energetic profiles of the rearrangement in both enzymes are very favorable, allowing the enzyme to efficiently interchange between both states. In Nap, the reaction has an activation energy of 8.33 kcal/mol and is endergonic in 2.97 kcal/mol. The zero point energy and the thermal and dispersive corrections contributed 1.15 kcal/mol for the activation energy and 1.06 kcal/mol for the reaction free energy. In FdH, the reaction has an activation energy of 6.85 kcal/mol and is exergonic by 4.76 kcal/mol. The zero point energy and the thermal and dispersive corrections contributed −1.09 kcal/mol

dx.doi.org/10.1021/ic3028034 | Inorg. Chem. 2013, 52, 10766−10772

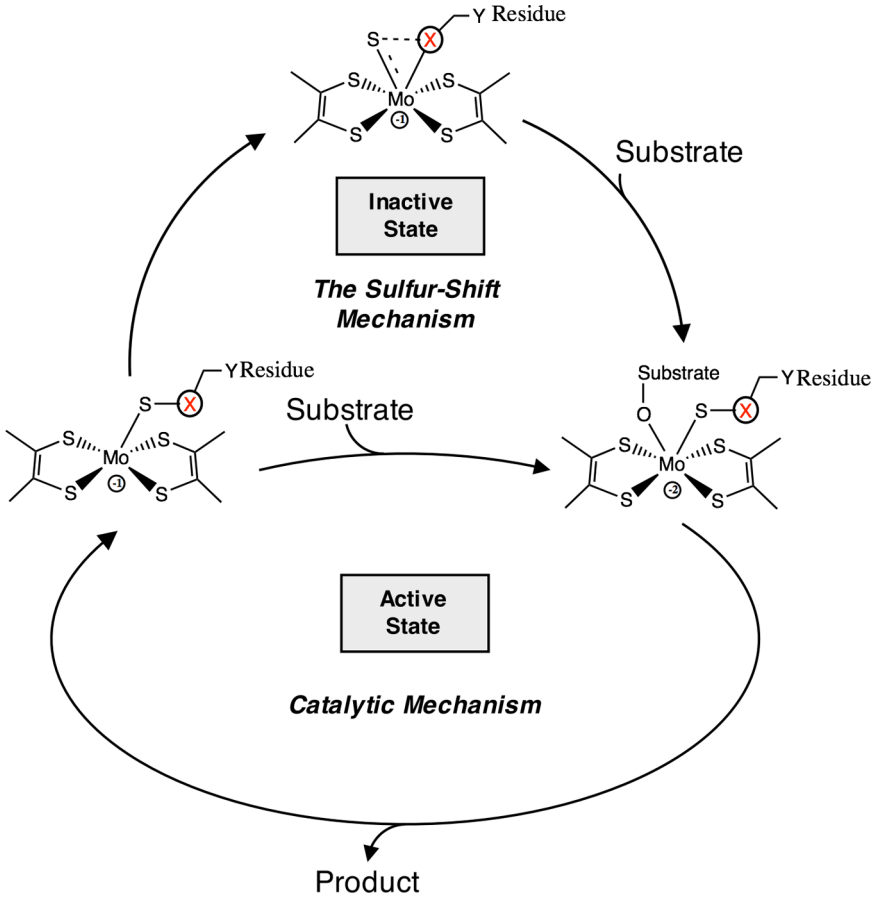


Figure 4. New interpretation of the catalytic mechanism of nitrate reductase (Nap) and formate dehydrogenase (Fdh).

for the activation energy and 0.49 kcal/mol for the reaction free energy.

This mechanism was called "sulfur shift" and can be defined as a metal coordination change involving a first-to-second shell displacement (shift) of the fifth ligand (a sulfur or a selenium atom), opening a free coordination position to bind the substrate without a significant energetic cost. It also allows Nap and Fdh to interchange between two configurations: the inactive in which the S−S_γCys ligand blocks the access of the substrate to Mo(VI) and the active in which the substrate binds directly to Mo(VI).

These results suggest a new interpretation of the catalytic mechanisms of Nap and Fdh, in which the full mechanism can be divided in two main parts: the first comprises the sulfur shift that controls the activation/inactivation of the enzyme, while the second deals with the redox transformation of the substrates (Figure 4).

In this new interpretation, the cycle begins with the metal site in the inactive conformation. This is observed in the oxidized X-ray structures of Nap and Fdh.[7,9] When the substrate approaches the cofactor, the sulfur-shift mechanism is triggered, and the substrate binds Mo(VI). Once the chemical transformations of the respective substrates are complete and the product dissociates, two situations can occur: (1) no further substrate is available or (2) substrate is still available. In the first case, the reverse of the sulfur-shift mechanism takes place and the enzyme returns to the inactive state, with the persulfido ligand blocking the access of other molecules to the Mo(VI) ion. This effect is supported by earlier computational results.[15] In Fdh, the reverse reaction of the sulfur shift requires an activation energy of 12.9 kcal/mol, and the reaction requires 6.9 kcal/mol, which is fast at physiological temperature. This means that the inactive state is attained until the substrate is again available in the active site. In the second case, if the substrate is available after completing a catalytic cycle, then catalysis continues without returning to the inactive Mo configuration. With the new substrate bound to the Mo ion right after the product is release, the catalytic efficiency of the full process is increased.

These reaction pathways fit very well within the experimental observations[13,18,28,29] based on which the direct binding of the substrate with Mo has always been proposed. Additionally, these results also go in line with recent theoretical findings regarding FdH, in which it was found that the oxidation of formate into carbon dioxide requires an unbound selenocysteine.[30,31] Hoffman[32] and Xi[33] observed similar conclusions in Nap. The theoretical studies performed by these authors have shown that a favorable pathway for nitrate reduction involves the initial dissociation of the coordinating cysteine from the Mo ion, similarly to what is found in FdH. The only thing that these studies did not reveal is how the active site residue (Cys in Nap and SeCys in FdH) dissociates from the metal ion at the same time that the substrate binds to the metal ion. This answer is now provided by the sulfur-shift mechanism that is presented in this paper.

The new results are also of particular interest in the light of recent results, where partially reduced crystals of Nap from *Cupriavidus necator* H16 revealed two alternate conformations of the cysteine residue bound to the Mo ion,[8] resembling what is proposed with the sulfur-shift mechanism.

dx.doi.org/10.1021/ic3028034 | *Inorg. Chem.* 2013, 52, 10766−10772

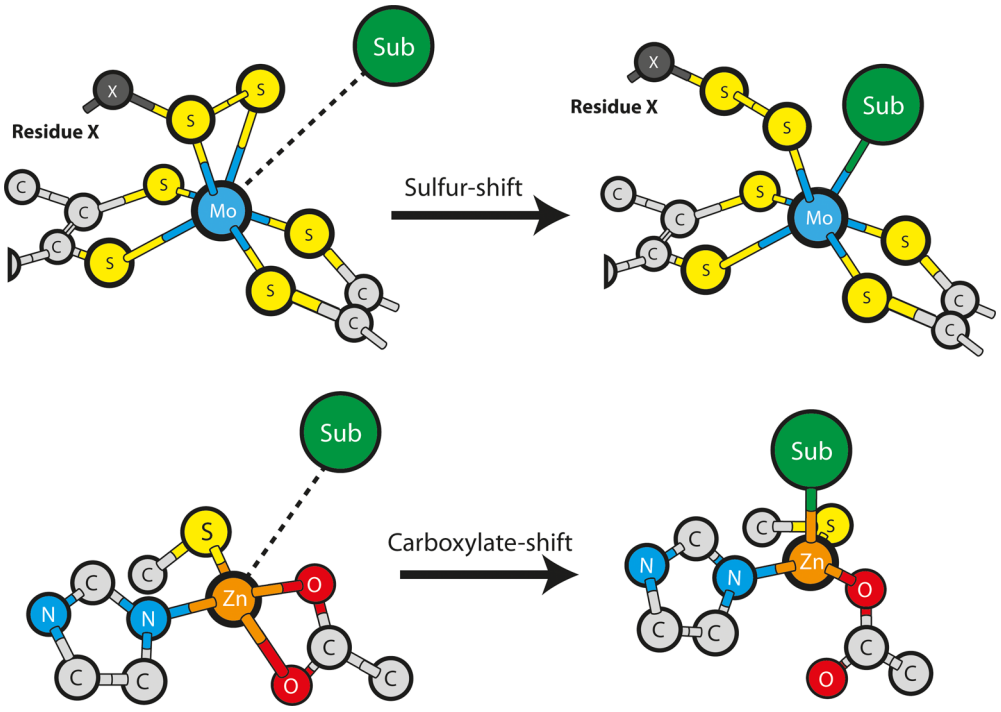


Figure 5. Schematic representation of the sulfur-shift (top) and the carboxylate-shift mechanisms (bottom).

4. CONCLUSIONS

The results obtained highlight a new mechanism that was named sulfur-shift (Figure 5). This mechanism was found to occur in prokaryotic mononuclear Mo-containing enzymes of the DMSOr family, which present cysteine (or SeCys) and an inorganic sulfur atom as fifth and sixth ligands to the metal. These ligands allow the enzyme to exchange between inactive and active forms, in which substrate binding to the metal ion is either blocked or allowed. This rearrangement provides an efficient mechanism to lower the activation energy for ligand exit or entrance and maintains Mo hexacoordinated through the entire catalytic cycle. All observations are well supported by recent experimental evidence that emphasizes its existence.[8] These studies also evidence another role for the pyranopterin cofactors in the catalytic process: they promote the interaction between the metallic site and the substrates that otherwise would be repulsive since both of them are negatively charged.

The sulfur-shift mechanism is very similar to the well-known carboxylate-shift mechanism that is found in other enzymes such as farnesyltransferase,[34,35] ribonucleotide reductase,[36−38] and methane monooxygenase,[38,39] as well as in a number of model compounds containing Fe,[40,41] Mn,[42−44] and Zn[35,45] (Figure 5). Both mechanisms present an efficient way for ligand exit or entrance and maintain a constant coordination number on the metal ion through the catalytic cycle. The main difference between these mechanisms is that the sulfur-shift mechanism involves the change in the coordination of a sulfur atom from cysteine or SeCys (moving from the first to the second shell), while the carboxylate shift involves a monodentate/bidentate exchange of a carboxylate group within the first shell. The energetic profiles of both mechanisms are very similar with low activation barriers and almost thermoneutral reactions, allowing the enzymes to rapidly interchange between the two forms.

The functional similarity between these mechanisms becomes even clearer if we look at the active site of respiratory nitrate reductase (Nar) from *E. coli* K12, where we can find a carboxylate shift assisting the binding of an oxygen atom of an OH_n molecule (Figure 1).[46,47] These results also suggest that this type of mechanism should be more common in nature than we might suspect.[48] In fact, they might be part of a common activation mechanism of the enzymes to control the access of nonsubstrate molecules to metal cofactors.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Chemical structures in xyz format. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: nscerque@fc.up.pt.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

N.S.C. and P.J.G. thank program Ciência 2007 and 2008 of Fundação para a Ciência e a Tecnologia (FCT) for funding. This work has been supported by FCT through the projects EXCL/QEQ-COM/0394/2012, PTDC/QUI-QUI/102760/2008 and PTDC/QUI/67052/2006 and Grant No. PEst-C/EQB/LA0006/2011.

■ REFERENCES

(1) Hille, R. *Chem. Rev.* **1996**, *96*, 2757.
(2) Hille, R. *J. Biol. Inorg. Chem.* **1997**, *2*, 804.
(3) Schwarz, G.; Mendel, R. R.; Ribbe, M. W. *Nature* **2009**, *460*, 839.
(4) Moura, J. J. G.; Brondino, C. D.; Trincao, J.; Romao, M. J. *J. Biol. Inorg. Chem.* **2004**, *9*, 791.
(5) Romao, M. J. *Dalton Trans.* **2009**, 4053−4068.
(6) Gonzalez, P. J.; Rivas, M. G.; Mota, C. S.; Brondino, C. D.; Moura, I.; Moura, J. J. G. *Coord. Chem. Rev.* **2013**, *257*, 315.

(7) Najmudin, S.; Gonzalez, P. J.; Trincao, J.; Coelho, C.; Mukhopadhyay, A.; Cerqueira, N. M. F. S. A.; Romao, C. C.; Moura, I.; Moura, J. J. G.; Brondino, C. D.; Romao, M. J. *J. Biol. Inorg. Chem.* **2008**, *13*, 737.
(8) Coelho, C.; Gonzalez, P. J.; Moura, J. J. G.; Moura, I.; Trincao, J.; Romao, M. J. *J. Mol. Biol.* **2011**, *408*, 932.
(9) Raaijmakers, H. C. A.; Romao, M. J. *J. Biol. Inorg. Chem.* **2006**, *11*, 849.
(10) Raaijmakers, H.; Macieira, S.; Dias, J. M.; Teixeira, S.; Bursakov, S.; Huber, R.; Moura, J. J. G.; Moura, I.; Romao, M. J. *Structure* **2002**, *10*, 1261.
(11) Butler, C. S.; Charnock, J. M.; Bennett, B.; Sears, H. J.; Reilly, A. J.; Ferguson, S. J.; Garner, C. D.; Lowe, D. J.; Thomson, A. J.; Berks, B. C.; Richardson, D. J. *Biochemistry* **1999**, *38*, 9000.
(12) George, G. N.; Colangelo, C. M.; Dong, J.; Scott, R. A.; Khangulov, S. V.; Gladyshev, V. N.; Stadtman, T. C. *J. Am. Chem. Soc.* **1998**, *120*, 1267.
(13) Khangulov, S. V.; Gladyshev, V. N.; Dismukes, G. C.; Stadtman, T. C. *Biochemistry* **1998**, *37*, 3518.
(14) Leopoldini, M.; Russo, N.; Toscano, M.; Dulak, M.; Wesolowski, T. A. *Chem.—Eur. J.* **2006**, *12*, 2532.
(15) Cerqueira, N. M. F. S. A.; Gonzalez, P. J.; Brondino, C. D.; Romao, M. J.; Romao, C. C.; Moura, I.; Moura, J. J. G. *J. Comput. Chem.* **2009**, *30*, 2466.
(16) Mota, C. S.; Rivas, M. G.; Brondino, C. D.; Moura, I.; Moura, J. J. G.; Gonzalez, P. J.; Cerqueira, N. M. F. S. A. *J. Biol. Inorg. Chem.* **2011**, *16*, 1255.
(17) Metz, S.; Thiel, W. *Coord. Chem. Rev.* **2011**, *255*, 1085.
(18) Boyington, J. C.; Gladyshev, V. N.; Khangulov, S. V.; Stadtman, T. C.; Sun, P. D. *Science* **1997**, *275*, 1305.
(19) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09*; Gaussian, Inc.: Wallingford, CT, 2009.
(20) Zhao, Y.; Truhlar, D. G. *Theor. Chem. Acc.* **2008**, *120*, 215.
(21) Zhao, Y.; Truhlar, D. G. *Acc. Chem. Res.* **2008**, *41*, 157.
(22) Andrae, D.; Haussermann, U.; Dolg, M.; Stoll, H.; Preuss, H. *Theor. Chim. Acta* **1990**, *77*, 123.
(23) Cerqueira, N. M. F. S. A.; Fernandes, P. A.; Ramos, M. J. *J. Chem. Theory Comput.* **2011**, *7*, 1356.
(24) Cerqueira, N. M. F. S. A.; Fernandes, P. A.; Eriksson, L. A.; Ramos, M. J. *Biophys. J.* **2006**, *90*, 2109.
(25) Ramos, M. J.; Fernandes, P. A. *Acc. Chem. Res.* **2008**, *41*, 689.
(26) Oliveira, E. F.; Cerqueira, N. M. F. S. A.; Fernandes, P. A.; Ramos, M. J. *J. Am. Chem. Soc.* **2011**, *133*, 15496.
(27) Grimme, S.; Antony, J.; Ehrlich, S.; Krieg, H. *J. Chem. Phys.* **2010**, *132*, 154104.
(28) Rivas, M. G.; Gonzalez, P. J.; Brondino, C. D.; Moura, J. J. G.; Moura, I. *J. Inorg. Biochem.* **2007**, *101*, 1617.
(29) Jepson, B. J. N.; Mohan, S.; Clarke, T. A.; Gates, A. J.; Cole, J. A.; Butler, C. S.; Butt, J. N.; Hemmings, A. M.; Richardson, D. J. *J. Biol. Chem.* **2007**, *282*, 6425.
(30) Leopoldini, M.; Chiodo, S. G.; Toscano, M.; Russo, N. *Chem.—Eur. J.* **2008**, *14*, 8674.
(31) Tiberti, M.; Papaleo, E.; Russo, N.; De Gioia, L.; Zampella, G. *Inorg. Chem.* **2012**, *51*, 8331.
(32) Hofmann, M. *J. Biol. Inorg. Chem.* **2009**, *14*, 1023.
(33) Xie, H. J.; Cao, Z. X. *Organometallics* **2010**, *29*, 436.
(34) Duchackova, L.; Schroder, D.; Roithova, J. *Inorg. Chem.* **2011**, *50*, 3153.
(35) Sousa, S. F.; Fernandes, P. A.; Ramos, M. J. *J. Am. Chem. Soc.* **2007**, *129*, 1378.
(36) Assarsson, M.; Andersson, M. E.; Hogbom, M.; Persson, B. O.; Sahlin, M.; Barra, A. L.; Sjoberg, B. M.; Nordlund, P.; Graslund, A. *J. Biol. Chem.* **2001**, *276*, 26852.
(37) Hogbom, M.; Andersson, M. E.; Nordlund, P. *J. Biol. Inorg. Chem.* **2001**, *6*, 315.
(38) Torrent, M.; Musaev, D. G.; Basch, H.; Morokuma, K. *J. Comput. Chem.* **2002**, *23*, 59.
(39) Gherman, B. F.; Baik, M. H.; Lippard, S. J.; Friesner, R. A. *J. Am. Chem. Soc.* **2004**, *126*, 2978.
(40) Kuzelka, J.; Spingler, B.; Lippard, S. J. *Inorg. Chim. Acta* **2002**, *337*, 212.
(41) Lemercier, G.; Mulliez, E.; Brouca-Cabarrecq, C.; Dahan, F.; Tuchagues, J. P. *Inorg. Chem.* **2004**, *43*, 2105.
(42) Rardin, R. L.; Bino, A.; Liu, S. C.; Lippard, S. J. *Abstr. Pap. Am. Chem. Soc.* **1990**, *199*, 506.
(43) Pursche, D.; Triller, M. U.; Reddig, N.; Rompel, A.; Krebs, B. *Z. Anorg. Allg. Chem.* **2003**, *629*, 24.
(44) Baffert, C.; Collomb, M. N.; Deronzier, A.; Kjaergaard-Knudsen, S.; Latour, J. M.; Lund, K. H.; McKenzie, C. J.; Mortensen, M.; Nielsen, L.; Thorup, N. *Dalton Trans.* **2003**, 1765.
(45) Demsar, A.; Kosmrlj, J.; Petricek, S. *J. Am. Chem. Soc.* **2002**, *124*, 3951.
(46) Jormakka, M.; Richardson, D.; Byrne, B.; Iwata, S. *Structure* **2004**, *12*, 95.
(47) Bertero, M. G.; Rothery, R. A.; Palak, M.; Hou, C.; Lim, D.; Blasco, F.; Weiner, J. H.; Strynadka, N. C. J. *Nat. Struct. Biol.* **2003**, *10*, 681.
(48) Moura, I.; Pauleta, S. R.; Moura, J. J. G. *J. Biol. Inorg. Chem.* **2008**, *13*, 1185.