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COMMUNICATIONS

Published on Web 09/04/2004

Geometrical Control of the Active Site Electronic Structure of Pyranopterin Enzymes by Metal-Dithiolate Folding: Aldehyde Oxidase

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Pyranopterindithiolate (molybdopterin[1]) is a unique cofactor found in the active sites of more than 50 enzymes containing molybdenum or tungsten.[2] The structure of this cofactor is well-known from recent crystal structures of several enzymes.[3] However, its functional role remains to be established. It is postulated to modulate the redox potential of the metal center and to act as an electron-transfer pathway for the regeneration of the metal center.[4,5] The active sites of the sulfite oxidase (SO) and xanthine oxidase (XO) families of enzymes have one sulfur atom of the pyranopterindithiolate cofactor in an equatorial position and trans to their respective catalytically labile oxygen donor ligands. It has been proposed that this geometry facilitates formal oxygen atom transfer in these enzymes via a kinetic trans effect.[6]

For discrete complexes, the "fold angle" of the five-membered dithiolate chelate ring (Figure 1) depends on the electron configuration of the metal center.[5,7] We have proposed that dynamic variations in protein structure during catalysis can change the fold angle and thereby modulate the charge distribution and reactivity of the active site.[5,8] Such a "dithiolate folding effect" is difficult to establish experimentally because of the lack of atomic resolution protein structures for both oxidized and reduced forms of SO and XO families of enzymes. For XO enzymes, an additional complication is the instability of Mo centers containing both terminal oxo and sulfido groups.[9] The recent report of the high-resolution (1.28 Å) crystal structure of aldehyde oxidase (MOP),[10] a member of the XO family, in the desulfo oxidized state, provides a starting geometry for the electronic structure calculations on the active site presented here. These calculations assess the possible relationships among fold angle changes, catalytic reactivity, and electron-transfer regeneration of *mono* ene-dithiolate active sites.

We have performed geometry optimization and electronic structure calculations at the DFT level of theory[11] on model complexes, **1**−**3** (Figure 2),[12] derived from the X-ray crystal structure of MOP.[10] The calculations indicate that the frontier orbitals (Figures 3 and 4) have significant contributions from both metal in-plane (M_{ip}) and symmetric (S_{π}^{+}) and antisymmetric (S_{π}^{-}) combinations of the dithiolate sulfur out-of-plane *p*-orbitals.[13] This is the first instance where contributions from a dithiolate S_{π}^{+} orbital to the frontier orbitals of a model complex with a geometry like that of an enzyme active site have been demonstrated.

The reaction mechanism of the XO family of enzymes has been studied in detail.[14−16] The proposed mechanism involves a hydride atom transfer to the terminal sulfido group that reduces the Mo(VI) center to Mo(IV).[16−19] Previous theoretical studies of the reductive half-reaction catalyzed by the XO family of enzymes[14,19] provide a framework for the geometrical structure changes, but no orbital correlation was made at that time. The Mo(VI) *aqua* complex (**1**) resembles the resting state of the enzyme that results from the displacement of the product by a water molecule.[16] In the enzyme crystal structure,[3,10,16] the carboxylic group of Glu869 located near the active site has been suggested[16] to be in a position where it is ready to accept a proton from the coordinated water molecule, thus generating an anionic species similar to complex **2**, also observed in the desulfo form in the crystal structure.[10] The calculated fold angles of the model complexes flatten from 42.6° in **1** to 12.3° in

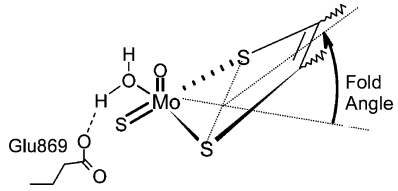

Figure 1. Fold angle definition (along the S···S vector) for the MOP active site.[3]

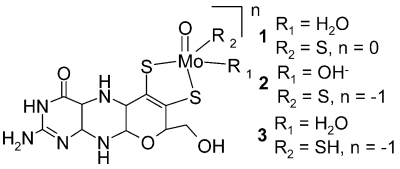

Figure 2. Bioinspired computational models **1**−**3** of the active site of MOP.

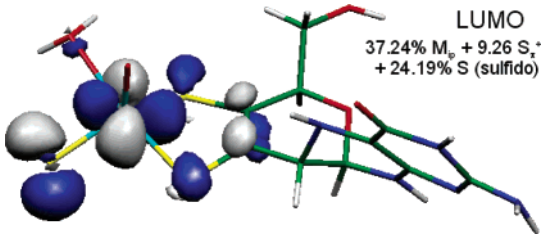

Figure 3. Lowest unoccupied molecular orbital of the oxidized active site of complex **1** (fold angle: 42.6°) that accepts two electrons in the reduction half cycle.

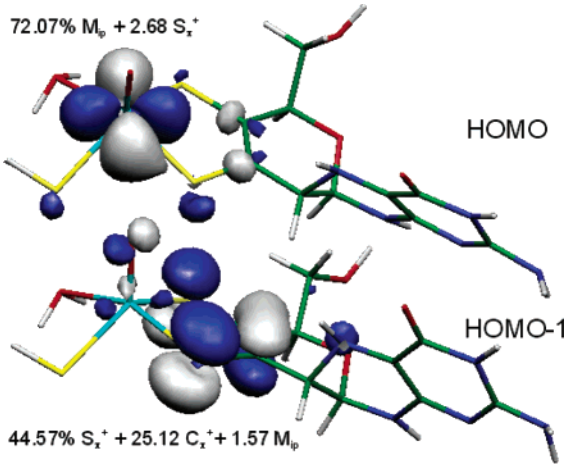

Figure 4. Frontier orbitals (HOMO and HOMO−1) of the reduced active site complex **3** (fold angle: 10.4°).

10.1021/ja046465x CCC: $27.50

2 and 10.4° in **3**. The geometry of complex **2** is very similar[12] to the crystal structure geometry of the active site,[10] which has a fold angle of 16.6°. It is interesting to note that although the formal oxidation state of **2** is Mo(VI), the proton abstraction from **1** leaves an overall negative charge on **2**, and the fold angle reduces to accommodate this change. Geometry-optimized structure **2** has a sulfido−Mo−O(H) angle of 103.9°, and the oxygen lone pair of the hydroxyl group is in an appropriate orientation to attack the carbonyl carbon of the aldehyde substrate.[16] The orbital plot of the lowest unoccupied molecular orbital (LUMO) of compounds **1** (Figure 3) and **2** (Supporting Information) shows that this orbital is antibonding between Mo and the sulfido ligand, as also observed in studies of other model complexes.[20] The subsequent hydride attack on the sulfur atom will weaken the Mo═S double bond by populating the orbital of Figure 3 and thus ease the formation of a sulfhydryl group, a crucial enzymatic step. As a result of the hydride atom transfer, the fold angle in the resultant model complex **3** flattens to 10.4°. In the reduced state (Mo(IV)), complex **3**, the highest occupied molecular orbital (HOMO; Figure 4), is primarily a metal in-plane orbital that is derived from population of the LUMO in **1** during the two-electron cascade associated with incorporation of an oxygen atom into substrate during catalysis.

Notice that in the oxidized active site model (**1**), the metal-dithiolate folding introduces contributions from the dithiolate S_{π}^{+} combination into the redox orbital (LUMO, Figure 3), with concomitant decrease in the metal contribution. The S_{π}^{+} orbital has the right symmetry and energy match to mix with the M_{ip} orbital upon folding, as confirmed experimentally for bent metallocene dithiolate compounds.[5,21] For **1** and **3**, the fold angle defines the orientation of the dithiolate sulfur-pπ orbitals with respect to the M_{ip} redox orbital. Thus, the fold angle can geometrically modulate the electronic structures of the oxidized (**1**) and reduced (**3**) forms, including the M═S bond order in the redox orbital (Figure 3) that becomes stabilized as a S−H σ-bond in reduced **3** (Figure 4).

Figure 4 also shows the second highest occupied molecular orbital (HOMO−1) of **3**, the reduced (Mo(IV)) compound. This orbital is primarily the symmetric combination of the dithiolate sulfur out-of-plane orbitals (S_{π}^{+}), which has an antibonding interaction with the π-orbitals of the carbon−carbon double bond. The constrained unsaturated five-membered chelate ring[7] is essential for raising the energy of the S_{π}^{+} orbitals to have the right symmetry and energy match with metal in-plane orbitals.[5,21] The symmetric combination of the sulfur orbitals (S_{π}^{+}) is more destabilized than the antisymmetric combination (S_{π}^{-}) because of the greater interaction with the C═C π-orbital of appropriate symmetry.[5,21,22]

The calculated structure for the isolated active site of MOP closely resembles that found for the desulfo form of the oxidized protein.[10] The LUMO acceptor orbital of the oxidized form is poised for hydride attack on the terminal sulfido ligand (Figure 3), and the fold angle of the dithiolate ligand decreases upon reduction of the site. These calculations support the proposal that fold angle effects[5] help to buffer the electron density at the metal center.[23] Proton abstraction from the *aqua* ligand of the *oxidized* site (**2**) also flattens the fold angle. This latter result suggests that the dithiolate fold angle can fine-tune the nucleophilicty of the hydroxide ligand that is trans to a dithiolate sulfur atom. Changing the fold angle can also facilitate the attack of hydride on the terminal sulfido ligand by modifying the acceptor properties of the LUMO. Thus, static or dynamic changes in the structure of the protein surrounding the active site can be expected to induce dithiolate fold angle changes that should play an important role in modulating the catalytic reactions of molybdenum and tungsten enzymes.

Acknowledgment. We thank the NIH (Grant GM-37773) for support of this research. H.K.J. gratefully acknowledges a Proctor and Gamble Scholarship.

Supporting Information Available: Geometry-optimized coordinates for **1**−**3**, MO plots of the HOMO and HOMO−1 for **2**, and variation of total energy of **1** with fold angle. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Calculations were performed using the Amsterdam density functional (ADF) theory calculations package (release 2000.02). Further details are provided in ref 5.
(12) In compounds **1**−**3**, the cytosine dinucleotide portion of the native cofactor of MOP has been has been simplified to OH to reduce the computational efforts. On the average, the optimized geometry of **2** differed by less than 2% in bond lengths and 1% in bond angles from the crystal structure geometry (ref 10).
(13) The *z*-axis on Mo points toward the axial oxo ligand, and the *x* axis is the bisector of the angle formed by the dithiolate sulfurs. The Mo in-plane orbital is $d_{x^2-y^2}$; the sulfur out-of-plane orbitals are Sp_z.
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JA046465X