Comparative Analysis of Fold Angles and Aromaticity in Synthetic Models and the Crystal Structure of Nitrate Reductase Enzyme

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Abstract

Many research groups aim to synthesize biomimetic compounds to mimic enzymatic functions. To achieve a complete mimicry, a comprehensive understanding of the structural details at the microscopic level is necessary. In this study, we conducted a structural analysis of synthetic molybdenum-dithiolene complexes and the active site structure of the NIR enzyme. Our analysis focused on the folding of the dithiolene ring and the aromaticity of the five-membered ring.

Keywords: NIR enzyme, Biomimetic compounds, dithiolene unit, DFT.

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Introduction

Nitrate reductase (NIR) is an oxygen transfer enzyme, which catalyses the reduction of nitrate to nitrite and is described by the reaction NO_{3}– + $2e^-$ + $2H^+$ NO_2^- + H_2O. The active site of oxidized NIR enzymes contain two molybdopterins (MPTs) units, an oxo group and an amino acid residue (cysteine (Cys) or aspartic (Asp)). The crystal structure studies conducted in Escherichia coli have revealed two distinct active site structures for NIR, which are resolved at 1.9 Å for (NarGHI) and 2.0 Å for (NarGH) under aerobic conditions. In both crystal structure the dithiolene ligand coordinates with metal by forming a chelate ligand and they differe in conformer of MPT, which exist in tricyclic and bicyclic form [1-5]. Dithiolenes have earned the moniker "non-innocent" ligands because, in their complexes, determining the oxidation state of the metal is not straightforward due to uncertainty about the oxidation state of the ligand(s). For example, in this reaction mechanism like $[Mo(S_2C_2H_2)_2]$ could involve Mo(IV), Mo(V), or Mo(VI), depending on the chosen oxidation state for the ligands. Further, the bicyclic MPT unit in NarGHI is connected to a metal via a highly folded dithiolene unit than the tricylic counterpart. This dithiolene unit's act as an electron transfer conduit from the metal to other prosthetic groups [3, 6-7].

Analysis of the fold angle (Figure 1) and aromaticity using DFT can play an important role in explaining the catalytic property of these enzymes, which would be difficult to explain by experimental methods. Also, comparisons of bond lengths, bond angles and dihedral angles with corresponding three-dimensional crystal structures is necessary to get a better model complex and find out the oxidation state of the metal in the enzyme active site [8, 11-13]. In this direction, we would like to compare the structure, fold angle and aromaticity of the synthetic model along with enzymatic structure.

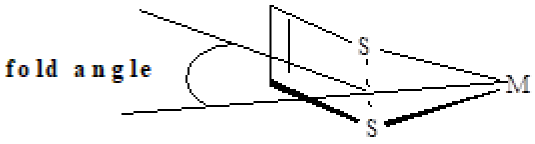

Figure 1: The definition for fold angle

Computational methods

The calculations reported herein were accomplished using the B3LYP [14] density functional approach, as implemented in the Gaussian 09 [15] suite of programs, with the Los Alamos effective core potential on Mo and W (LANL2DZ) [16] and the 6-31G(d) basis set on all other atoms. The structures of all the models which are used in this work are shown in the figure 2. The NICS [17-19] and NMR calculations were performed using the CSGT [20] method for each molecular orbital separately.

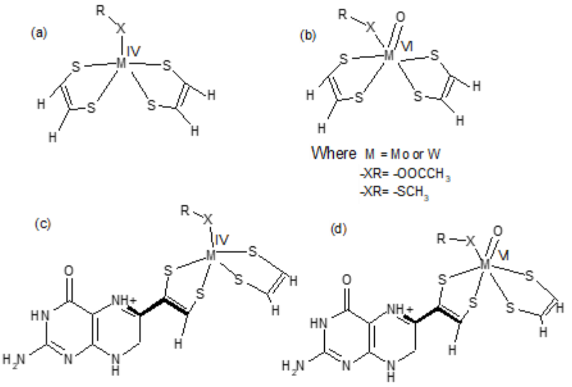

Figure 2: The structures used for the calculations **(a)** $[MXR(S_2C_2H_2)_2]$ **(b)** $[M(XR)(O)(S_2C_2H_2)_2]$ **(c)** $[M(XR)(S_2C_2H_2)MPT]$ **(d)** $[M(XR)(O)(S_2C_2H_2)MPT]$

Results

In order to understand the enzyme crystal structure and to obtain better models for the mechanistic analyses, the various bond length, bond angles and dihedral angles of M^{IV}, M^{V} and M^{VI} model complexes are compared with the NarGHI enzyme active site and the Mo synthetic model complex geometry. The calculated η^2–asp coordinated MVI model complex structural parameters are comparable with the NarGHI active site. Similarly, the η^2–asp coordinated MIV model complex parameters are in good agreement with (η^2–asp) coordinated Mo^{IV} synthetic model. There is small difference observed between the calculated and experimental parameters, which may be due to the inadequacy of the model. To avoid that the bicyclic MPT unit was included in the models (figure 3), and this gave the geometrical parameters of the model in better agreement with the experimental structural parameters.

The fold angle analyses of the dithiolene unit as has been pointed out by Lauher and Hoffmann [6] can be used to understand the oxidation state of the molybdenum or tungsten. The fold angle variation is caused by the occupancy of the metal d–orbital in the equatorial plane with respect to a dithiolene ligand (the orbital is empty (d^0) for the folded case (50°) and filled (d^2) for the more nearly planar case). Here we compare the fold angles for all the models to the corresponding experimental structures and the fold angles in different coordination environment.

The M^{VI} (η^2–Asp) model complex has a higher fold angle than the corresponding $M^{VI}(\eta^1$–Asp)(O) oxo model complex. The calculated fold angles of two dithiolenes for $[M^{VI}(\eta^1$–Asp)(O)$(S_2C_2H_2)MPT]^-$ and $[M^{VI}(\eta^2$–Asp)$(S_2C_2H_2)MPT]$– complexes are 7.4º, 6.4º and 35.6º, 7.9º, which are in good agreement with the crystal structure of NarGH (9.8º, 6.7º) and NarGHI (35.7º,7.3º) respectively. In addition, the $[M^{IV}(\eta^2$–Asp)$(S_2C_2H_2)_2]^-$ model complex fold angle is in agreement with the synthetic model fold angle. The fold angle changes can be explained in terms of the frontier Molecular Orbitals (MOs), these MOs as shown in the figure 4 for the complexes $[M^{VI}(\eta^2$–Asp)$(S_2C_2H_2)_2]$ and $[M^{VI}O(\eta^1$–Asp)$(S_2C_2H_2)_2]$. For the complex $[M^{VI}$ (η^2–Asp)$(S_2C_2H_2)_2]$– the frontier orbitals show that the metal in-plane (M_{ip}) d–orbitals (HOMO–1) is delocalise with the ligand out–of–plane symmetric sulphur orbital. Controversially, in $[M^{VI}O(\eta^1$–Asp)$(S_2C_2H_2)_2]$ the metal d–in plane orbitals are localised by the π–donor oxo group (donates electrons to empty d–orbital), so approximately M–S–C–S metallocycle is planar which minimizes the filled–filled interaction between the ligand out-of-plane orbital and metal in plane orbitals [20-23].

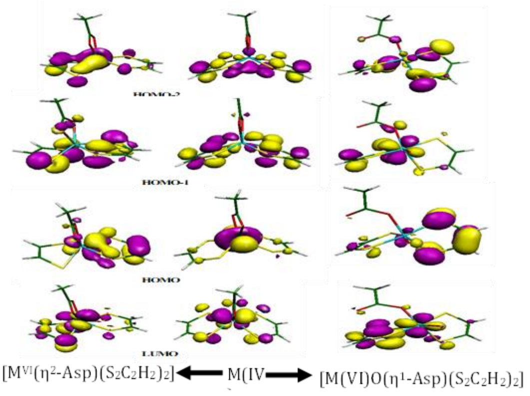

Figure 3: The frontier orbitals diagram for M(IV) and M(VI) model complexes.

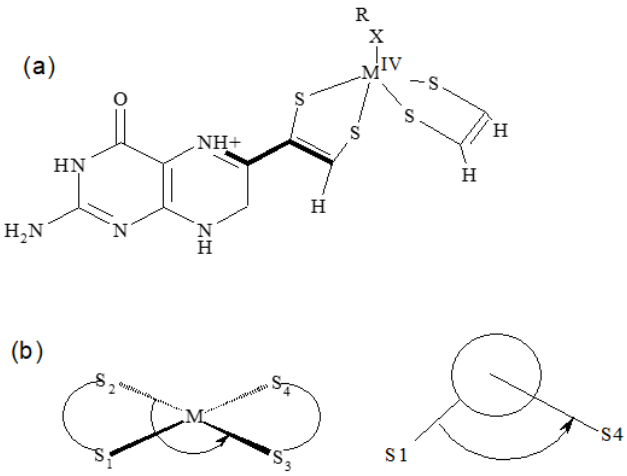

Figure 4: **(a)** The highly conjugated bicyclic tautomer of the pyran ring opened dihyopterin state of a simplified form of MPT (M =Mo or W); **(b)** Description of the orientation of the sulphur atoms at

the metal centre (M=Mo or W).

The above geometric analysis explains the difference between η^2–Asp coordinated synthetic model and NarGHI active site. In NarGHI, the metal is present in the +6 oxidation state and in the synthetic model the metal is in the +4 oxidation state.

The protonation at the pyran oxygen in the tricyclic form of the MPT leads to the bicylcic MPT form. In addition, the ring opening is more feasible in the highly folded dithiolene containing $[M^{VI}(\eta^2\text{–Asp})(S_2C_2H_2)MPT]^-$ since the higher fold angle effects the stereo chemistry of the carbons which is attached to the pyran oxygen, This might be the reason for the bicyclic MPT form in the NarGHI structure.

The calculated fold angles for the optimized structures of the model complexes, $[M(\eta^2\text{-Asp})(S_2C_2H_2)MPT]$ and $[MO(\eta^1\text{-Asp})(S_2C_2H_2)MPT]$ follow the same trend as in the crystal structures of NIR (NarGH and NarGHI). The $[M^{VI}(\eta^2\text{-Asp})(S_2C_2H_2)MPT]$ has a higher fold angle, as in the active site of NarGHI than the model $[MO\eta^1\text{-Asp}(S_2C_2H_2)MPT]$ complex, as in the NarGH active site. In the reduced species (M^{IV}), two electrons are present in the metal d_z^2 non–bonding orbital, which leads to a planar M–S–C–S ring and as minimized the interaction between the out-of-plane ligand orbital and other metal–based orbitals. As a result of this the M(IV) reduced species become non-aromatic, which is shown by the O_π-NICS and O_π-NMR values.

In M^{VI} oxidised form, the model complex can be present in two different coordination environments as reported in the NarGH and NarGHI crystal structures. In the η^2–Asp coordinated complex the oxidized form of the model has a larger fold angle due to the interaction between the empty metal in–plane orbital and ligand pπ orbital. But the corresponding $M(O)(\eta^1\text{-Asp})$ species has a lower fold angle due to the oxo group which changes the coordination environment by localizing the metal orbitals and maintaining the planar M-S-C-S geometry. The conclusion is the d_π-p_π interaction is more feasible in the planar structure with an empty d-orbital than the metal with the d^2 structure as clearly shown by O_π-NICS and O_π-NMR values for the reduced and oxidized forms of the active site model complexes. The calculated structural parameters of the stationary points are in good agreement with the corresponding $[M^{IV}(XC_6H_2\text{-}2,4,6\text{-}Pr^i_3)(S_2C_2H_2)_2]^-$ and $[M^{VI}(O)(XC_6H_2\text{-}2,4,6\text{-}Pr^i_3)(S_2C_2H_2)_2]^-$ (M = Mo or W) synthetic models geometric parameters.

Conclusions

Comparing the structural parameters of synthetic models and enzymatic structures helps elucidate the significance of non-standard residues in natural systems. The presence of a fold angle affects the overlap of orbitals, which plays a crucial role in charge transfer. Aromaticity serves as an index for both synthetic and enzymatic structures, providing insights into the degree of folding. Furthermore, our studies affirm that dithiolene folding determines the formation of bicyclic and tricyclic forms of the MPT unit in nature.

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