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The Role of [bm XOR]-Glu1261 in Activating HOeq Terminal in the Binding Stage of Catalysis in Xanthine Oxidase Enzyme

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Abstract

All molybdo-enzymes except nitrogenase, xanthine oxidase possess structurally similar molybdenum cofactor (Moco) in which molybdenum metal is ligated by a dithiolene side chain to a pyranopterin ring. The pterin cofactor has been proposed to participate in assisting the transfer of electrons from or to the active site containing molybdenum metal. Generally, the role of Moco is to position Mo correctly within the active site, control the redox behavior of the enzymes, allow the enzyme to gain its biological activity, and participate with its pterin ring system in the electron transfer to or from the molybdenum atom. The geometry of the active site in the presence of substrate and binding pocket amino acid (Glu_{1261}) was constructed using GaussView 3.0 software program. All geometry optimizations of the structures were carried out using Gaussian 03 W (version 6.0) program software package. The Mulliken atomic charges and the total energies were computed from the output files of the optimized structures. The percentage compositions of different molecular fragments were generated using AOMIX software package. Oxyanion is responsible for the nucleophilic attack of the deficient carbon atom other than the hydroxyl group itself (OH). Therefore, the most favorable pathway in the initial stage of catalysis is the reaction that occurs after the abstraction of the equatorial acidic proton of the active site. This is because Oxyanion cannot form the stable Structure when it forms a double bond with Molybdenum metal. The amino acid residue, Glu1261 is proposed to play an important role in promoting the nucleophilicity of the HO_{eq} ligand of the active site. That is, Glu_{1261} is in a close proximity to the equatorial hydroxyl group, HOeq capable of abstracting hydrogen from HO_{eq} which causes the equatorial oxygen develops a negative charge (Oxyanion) for the nucleophilic attack on the deficient substrate carbon. Therefore, we can conclude that the role of Glu_{1261} is enhancing the nucleophilicity of HO_{eq} ligand as it acts as a Lewis base to accept a proton from the HO_{eq} ligand as well as stabilizing the tetrahedral Michaelis-Menten type intermediate at the transition state.

Keywords: Oxyanion; Glutamic acid; Hydroxyl group; Xanthine oxidase; Cofactor; Hydroxylation; Substrate carbon

Introduction

Molybdenum with atomic number 42 is found in the 5th row of the periodic table and the 2nd row of the transition metals, below chromium and above Tungsten of the d-block elements [1]. The common ore for Molybdenum is found in the form of Molybdenite (MoS_2) [1-3]. The most common oxidation states of molybdenum lie between +4 to +6, which can form various types of complexes with inorganic or organic ligands. It has been known that molybdenum is an essential micro nutrient for plants, animals and micro-organisms [1,2,4]. Molybdenum is very much abundant in soil of aquatic environment in the form of molybdate (MoO_4^{-2}) anion [1,2] which is the only form of molybdenum available for plants and bacteria [1,2,5]. Molybdenum as a metal is inert in the redox reactions or biological processes, and it requires pyranopterin cofactor to give the active Moco cofactor [1]. In other words, the addition of pyranopterin cofactor to the molybdenum metal promotes its chemical reactivity in the redox reactions [1,5,6]. All molybdo-enzymes except nitrogenase, xanthine oxidase possess structurally similar molybdenum cofactor (Moco) in which molybdenum metal is ligated by a dithiolene side chain to a pyranopterin ring [6-8]. The pterin cofactor has been proposed to participate in assisting the transfer of electrons from or to the active site containing molybdenum metal. Generally, the role of Moco is to position Mo correctly within the active site, control the redox behavior of the enzymes, allow the enzyme to gain its biological activity, and participate with its pterin ring system in the electron transfer to or from the molybdenum atom [7,9,10].

Xanthine oxidoreductase enzymes have been isolated from a wide range of organisms, such as from bacteria [8, 11] to man [1,11] and catalyze the hydroxylation of a wide variety of purine [2,3,12] pyrimidine [6,7,13] pterin [3,4], and aldehyde [1,4,7] substrates. Xanthine oxidoreductase belongs to xanthine oxidase family enzymes, a family that encompasses a wide variety of enzymes that have similar arrangements and composition of redox centers [14-16].

Physiology and biochemistry of xanthine oxidase family enzymes

The history of the xanthine oxidoreductase enzymes is believed to date back to the 19th century, when W. Spitzer (1899) recognized that XOR enzyme catalyzed the oxidation of hypoxanthine to xanthine and then to uric acid [1,14,15]. However, XOR was discovered in 1902 by an Austrian biochemist, Franz Schardinger who described the reaction for the reduction of methyl blue in the presence of aldehyde (formaldehyde) with fresh milk [1,4,5]. The enzyme was initially considered as Schardinger enzyme [4,9]. This XOR enzyme was later isolated from cow's milk, purified, and studied by Malcolm Dixon and SylvaThurlow in 1920s [1,4,17]. The Schradinger enzyme was suggested by VH Booth (1938) to be referred to as xanthine oxidase [1].

In mammalian organs, the highest level of XOR activity is expressed in liver [3,18]. The presence of xanthine oxidoreductase enzymes in the liver can be used as a marker for a hepatic damage, through the XOR

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enzyme circulating in the blood [3]. However, the most observable disease in humans is the deposition of uric acid, known to be responsible for gouty conditions. This condition is more pronounced in the joints, through the deposition of sodium urate crystals [3,6]. In the ischaemia-reperfusion injury hypothesis, during the course of reperfusion, XOR uses dissolved oxygen and hypoxanthine to generate O^{2-} and H_2O_2 [2,3,19]. On the other hand, during the course of ischaemia, the catabolism of cellular ATP to hypoxanthine is believed to create a potential gradient and pumps ions across the membrane. In turn, the dissipation of ions is believed to cause a buildup of calcium concentration that can initiate the proteolytic conversion of the dehydrogenase into oxidase form of the enzyme [6,7,11]. The proteolytic conversion of the enzyme and deposition of xanthine in various tissues may well lead to a multiple organ failure syndrome and inherited XDH deficiency (Xanthinuria) [2,7,12]. Xanthinuria, involving abnormalities of these enzymes, is believed to be caused by the deficiency of XDH [3,10,11], XOR, and AOR [1,5,7], or Moco [2,3,6].

The structures of xanthine oxidoreductase enzymes

According to the X-ray crystallographic data, xanthine oxidoreductase enzymes were obtained from different sources, for example, xanthine oxidase from fresh un-pasteurized mammalian milk (Bos Taurus) [11]. The bmXOR enzyme is a homo-dimer that has a molecular mass of about 300 KDa [1,4,20]. The smallest (S) subunits (20 KDa each, consisting pairs of (Fe_2S_2) clusters are connected by a long segment to the medium (M) subunits (40 KDa each, consisting two FAD cofactors). Similarly, the structure of the OcCu/Mo-CODH is a hetero hexamer that has a molecular mass of about 273 KDa [1,5,7].

Among the components of xanthine oxidase, the flavin and Fe-S center dominate the UV/Visible absorption spectrum of the oxidized Mo^{VI} enzyme and the overall spectral change associated with reduction of the enzyme (at Mo^{IV}). The iron-sulfur contribution to oxidized enzyme includes maxima at 420 nm and 470 nm, while that of the flavin consists of the typical maxima at 360 nm and 450 nm [4,7,21]. The two iron-sulfur clusters exhibit indistinguishable UV/Visible and CD spectral changes on reduction [3,7,11]. They are readily distinguished on the basis of their EPR signals in the reduced state [11,22,23].

Activation of the active Site for nucleophilic reaction

The amino acid residue is located trans to the apical oxo ligand and in a very close proximity to the Mo ion and the HO_{eq} terminal. Because of its unique position, the [XOR]-Glu_{1261} amino acid residue plays an important role in promoting the nucleophilicity of the HO_{eq} ligand at the electron deficient carbon center [1,4,24]. The most important role of Glu_{1261} has been suggested to serve as an active site base in assisting the nucleophilic attack during the initial stage of catalysis for hydroxylation of substrates. The active site amino acid residue (XOR)-Glu_{1261} is located trans to the apical oxo ligand in close proximity to the Mo (VI) and HO_{eq} terminal [1,8,11]. This amino acid residue is capable of abstracting the acidic hydrogen from the HO_{eq} terminal to (XOR)-Glu_{1261}. This role of Glu_{1261} as an active site base is to make a nucleophilic attack on the deficient carbon center of the substrate. The deprotonation of H_{OH} may be suggested to take place before during, or after the nucleophilic reaction (the nucleophilic attack by the equatorial Oxyanion (O^-) on the activated/deficient substrate (Figure 1).

If the deprotonation of the acidic hydrogen takes place before the nucleophilic attack (route i, b), the unstable negatively charged oxygen (O^-) may dissociate to form an unrealistic equatorial oxo ligand ($Mo^{VI}=O_{eq}$) (Figure 1c); that means, the oxo ligand may be formed before the O^- anion interacts with substrate (Figure 1a). On the other hand, if a nucleophilic attack takes place after the deprotonation of H_{OH} (Figure 1f) the positive charge density on the oxygen center (HO^+Mo) may create a high energy barrier that may prevent the formation of the tetrahedral species. However, the concerted formation of O_{Mo}-C_{RH} bond (Figure 1e) is more favorable since the abstraction of an acidic proton by (XOR)-Glu_{1261} is expected to neutralize the charge on the nucleophile (HOMo). The abstraction of an acidic proton by (XOR)-Glu_{1261} is expected to lower the activation barrier for the formation

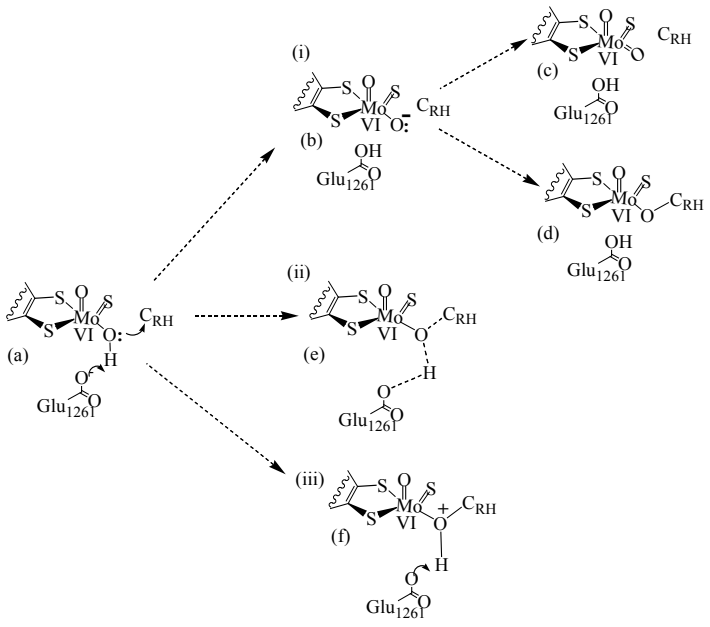


Figure 1: A hypothetical scheme for the de-protonation of $Ö^-_{Mo}$ bound H_{OH} and transfer of H_{OH} from OH_{Mo} to (XOR)-Glu_{1261}. The de-protonation of H_{OH} is proposed to take place before (i-b), during (ii-e), and after (iii-f) the nucleophilic reaction took place.

of a tetrahedral Michaelis-Menten type activated state complexes. A mechanistic implication of lowering the activation energy is in stabilizing the tetrahedral activated state complex and influencing the events taking place such as: the cleavage of C_{RH}-H_{RH} bond, transfer of H_{RH} to S_{Mo}, and allocation of the two electrons on the Mo^{VI} center. Several site-directed mutants of bacterial proteins have been characterized [19,25], including E730A (corresponding to Glu_{1261} in the bovine enzyme), E232A (Glu_{802}), R310M (Arg_{880}), and Q197E (Gln_{767}). Mutation of Glu_{730}/ Glu_{1261} to either Ala or Asp results in complete loss of activity in steady-state assays and the failure of xanthine to reduce the enzyme under anaerobic conditions [19]. The mutation of E730A (Glu_{1261}) enzyme with 100 μM enzyme, indicating that the limiting rate of reduction, kred, has decreased by at least a factor of 10^7 as compared with wild type enzyme: clearly, Glu_{1261} plays a central role in catalysis, as expected for an active base. A similar result is seen with R310M (Arg_{880}) mutant, a lower limit for the effect of the mutant in this case of 10^{6-} fold reduction in kred. From the magnitude of the effect on kred, however, it is evident that the principal role of this residue is not substrate binding, but transition state stabilization, leading to rate acceleration [19].

Given that Glu_{1261} is restrictly conserved, the aldehyde oxidase is likely to operate in principally the same way, and base-assisted catalysis has been demonstrated in the reaction of the bovine xanthine oxidoreductase with an aldehyde substrate [4,19]. Such a role for this Glutamic acid had been explicitly proposed for DgAOR [11,19]. In XOR enzymes, Glu802 and Arg880 have catalytic roles that are specific to action on heterocyclic substrates, through hydrogen bonding and electrostatic interactions with carbonyl groups. Such interactions do not occur between the aldehyde-utilizing enzymes and their substrates since Glu_{802} and Arg_{880} amino acid residues are not conserved in AOR enzymes (Table 1).

Materials and Methods

The structures of interest (such as purine derivatives, the active site, and binding pocket amino acids) were sketched using ChemDraw Ultra 2003, version 8.0 (Cambridge software corporation, Cambridge, MA. USA). These structures were also sketched using GaussView 3.0 (Gaussian, Inc., Pittersburgh, PA. USA) Software package. This software was also used to develop the input geometries, calculate the bond distances, as well as visualize the optimized geometries and frontier orbitals. The input geometries prepared using GaussView 3.0 software program were optimized using Gaussian 03W (2003), version 6.0 (Gaussian, Inc, Pittersburgh PA, USA) software package, on Dell Optiplex780 model computer, 2011 (Dell, Inc; Wilhie Sdh Bhd; Penang, Malaysia). In addition, AOMix 2011/2012, 6.6 (Centre for Catalysis Research and Innovation, University of Ottawa, Ottawa, Canada) software package was used to determine the composition of atomic orbitals.

Structures	Lumo	Homo
A		
B		
C		
D		
E		
F		

Table 1: The frontier orbitals for the structures shown in Figure 2. Molecular orbital and electronic structure visualization was performed from the check point files using Gauss View 3.0 software program.

Materials

The role of glu_{1261} during the initial stage of catalysis: Optimization of the active site in the presence of substrate and Glu_{1261}: The geometry of the active site in the presence of substrate and binding pocket amino acid (Glu_{1261}) was constructed using GaussView 3.0 software program. All geometry optimizations on the structures shown in Figure 2 were carried out using Gaussian 03 W (version 6.0) program software package, as described above.

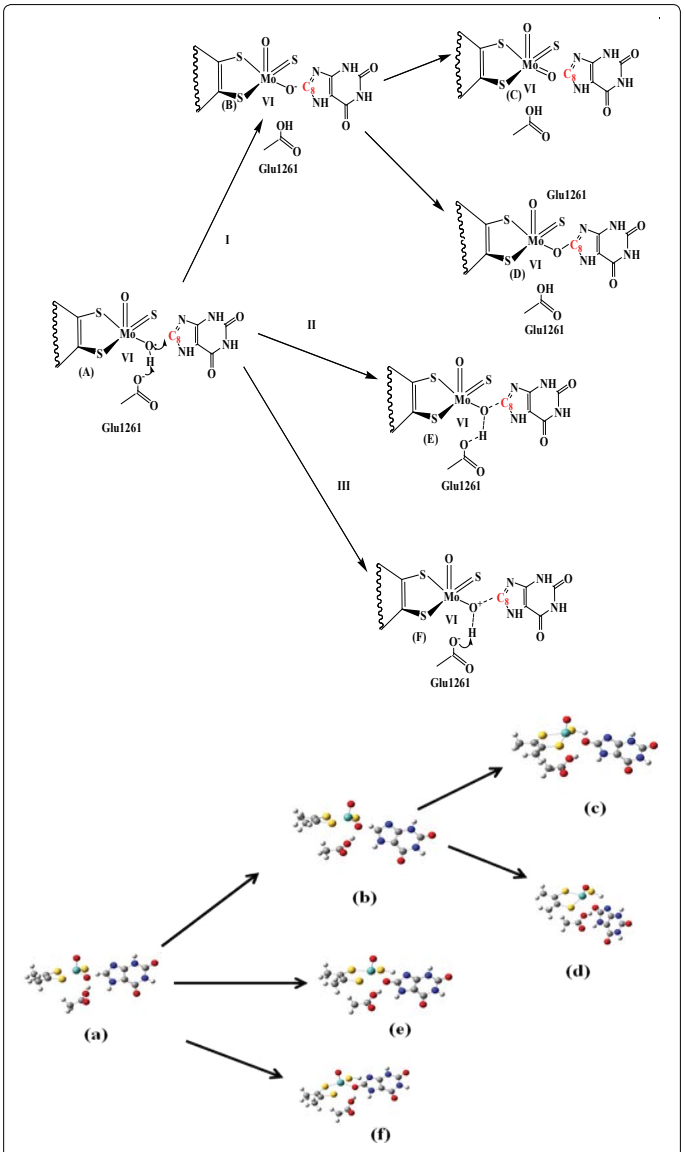


Figure 2: A hypothetical Scheme for the initial stage of catalysis, when H_{OH} was abstracted from OH_{eq} by [*bm*XOR]-Glu_{1261}. The abstraction of H_{OH} is proposed to take place before (I -b), during (II - e), and after (III - f) the nucleophilic reaction took place. The *upper panel* shows the proposed mechanism in the presence of xanthine and Glu_{1261}. The *lower panel* represents, the actual geometries optimized for the structures shown in the *upper panel*. The illustrations (*lower panel*) were prepared with the application of GaussView software program.

The events taking place during the initial stage of catalysis mediated by XOR enzyme in the presence of substrates are the abstraction of acidic hydrogen by Glu_{1261}, nucleophilic attack on the electron deficient carbon, transfer of H_{RH} from C_{RH} to $M_{o=S}$ and transfer of 2e's to Mo^{VI} center.

Characterization of the optimized structures

The Mulliken atomic charges and the total energies were computed from the output files of the optimized structures. The total energies for the optimized geometries were plotted to compare the stabilities of the respective structures. The molecular orbital analyses for the constituent chemical fragments were performed using AOMix software package. The percentage compositions of different molecular fragments were generated using AOMIX software package.

Definition of keywords used in Gaussian job [26,27]: The key word "B3LYP" was used to describe Beck's three-parameter exchange functional combined with the Lee, Yang, and parr's -correlation functional [26,27]. The key word "gen" was used to provide a separate basis set input section and specify an alternate density fitting basis set [26,27]. The key word "#P" was used to describe additional output generated, which included messages at the beginning and end of each link giving assorted machine department information. The key word "opt" was used to describe the geometry optimization to be performed. The key word "pop" was applied to control the outputs of molecular orbitals and orbital energies. Finally, the key word "geom=connectivity" was used to indicate the source of input files.

Results

The role of Glutamic Acid, Glu_{1261} in the initial stage of catalysis (Figure 3-10)

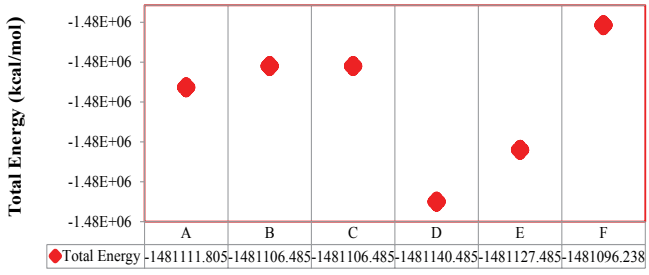


Figure 3: The total energy obtained, from geometry optimization, for the structures shown in Figure 2.

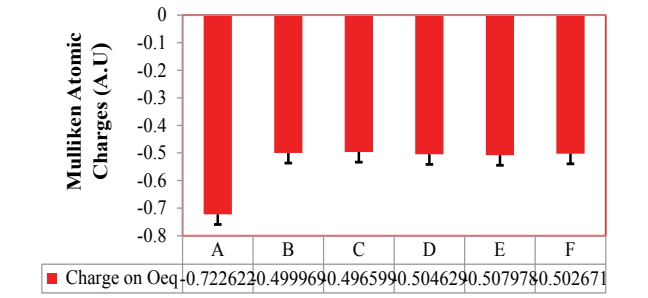


Figure 4: The Mulliken atomic charges on equatorial oxygen terminal atom of the active site. The data was obtained from geometry optimization performed on the structures described in Figure 2.

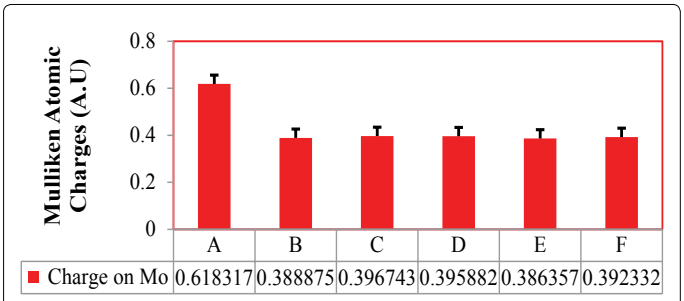


Figure 5: The Mulliken atomic charges on Mo $^{(VI)}$ ion of the active site the data was obtained from geometry optimization performed on the structures described in Figure 2.

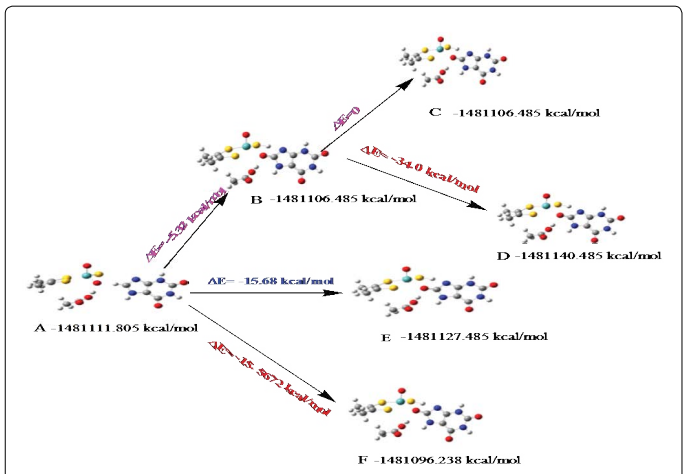


Figure 6: The energy profile obtained from the optimization of the structures shown in Figure 2.

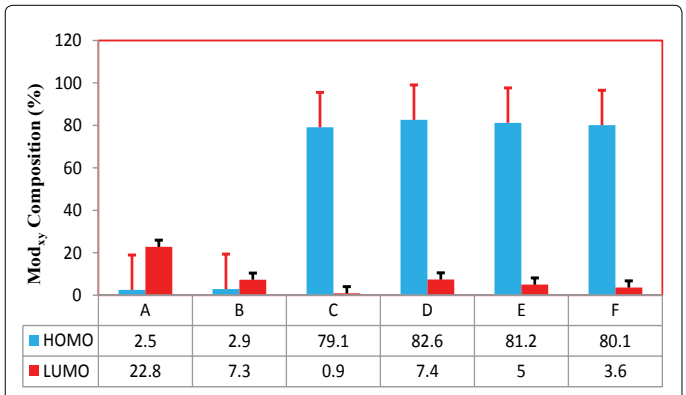
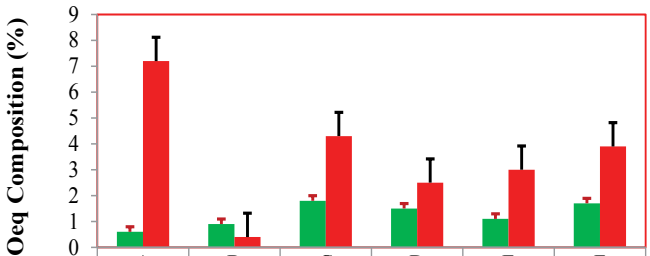


Figure 7: The percent composition for Mod_{xy} in the HOMO and LUMO molecular orbitals, obtained from the structures. The data were developed using AOMix software package.

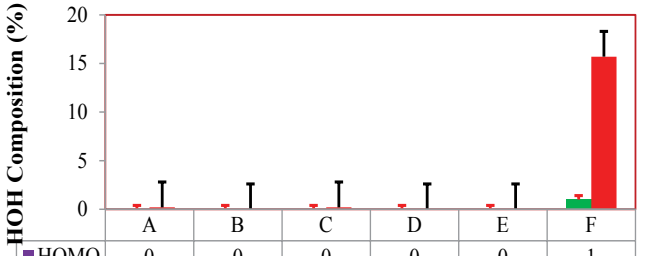
Discussion

All geometry optimizations were performed using Gaussian 03W 6.0 software package by applying a density functional theory (DFT) method to generate several parameters such as total energy, Mulliken atomic charges, orbitals and percent composition. The Mulliken atomic charges and the total energies were compiled from the output files to characterize the stability of the optimized structures. In this section, the role of Glutamic acid will be discussed.



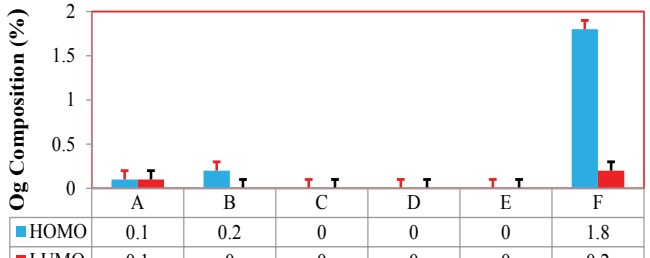
	A	B	C	D	E	F
HOMO	0.6	0.9	1.8	1.5	1.1	1.7
LUMO	7.2	0.4	4.3	2.5	3	3.9

Figure 8: The percent compositions for Oeq, in the HOMO and LUMO molecular orbitals, obtained from the structures. The data were developed using AOMix software package.



	A	B	C	D	E	F
HOMO	0	0	0	0	0	1
LUMO	0.2	0	0.2	0	0	15.7

Figure 9: The percent composition for the proton bound to Oeq, in the HOMO and LUMO molecular orbitals, obtained from the structures shown in Figure 2. The data were developed using AOMix software package.



	A	B	C	D	E	F
HOMO	0.1	0.2	0	0	0	1.8
LUMO	0.1	0	0	0	0	0.2

Figure 10: The composition Glu_{1261}-Og, in the HOMO and LUMO molecular orbitals, obtained from the structures. The data were developed using AOMix software package.

The role of Glu1261 in initial stage of catalysis

Glutamic acid (Glu_{1261}) is thought to act as a general base, deprotonating the Mo-OH of the molybdenum center to facilitate the nucleophilic attack on the substrate. In other words, it was proposed that the role of the Glutamic acid (Glu_{1261}) in activating the equatorial hydroxyl terminal in which the nucleophilic attack is expected to take place after the acidic proton is abstracted from the equatorial hydroxyl terminal. A mutagenic study is important to identify the role of an amino acid residue, in particular, Glu_{1261} in recognition and activating substrates during the initial stage of catalysis [1,3,5]. The active site amino acid residue (Glu_{1261}) could be used as a target of investigation since it is one of the most important active site amino acid residues located in the secondary coordination sphere [3,5,19].

In the initial stage of catalysis as shown in Figure 6, the abstraction of the acidic proton from the equatorial hydroxyl terminal by [bmXOR]-Glu_{1261}, is expected to take place in one of the three routes (such as before the abstraction of the equatorial acidic proton from the equatorial hydroxyl terminal) as shown in Figure 2 which leads to the formation of Structure (B), which further leads to the formation of either Structure (C or D) due to the unstable Oxyanion on O_{eq}, during the abstraction of the equatorial acidic proton from the equatorial hydroxyl terminal which leads to the formation of Structure (E) and after the abstraction of the equatorial acidic proton which leads to the formation of Structure (F). The nucleophilic attack of the equatorial Oxyanion (O^-) on the substrate is proposed to be initiated by the immediate transfer of the acidic hydrogen (HOH) from the HO_{eq} to (XOR)-Glu_{1261}. The deprotonation of H_{OH} may be suggested to take place before, during, or after the nucleophilic reaction (the nucleophilic attack by the equatorial Oxyanion (O^-) on the activated /deficient substrate (Figure 2). Although, the sequence of deprotonation is not well understood the stepwise deprotonation of HOH (Figure 2a) is proposed to take place either before (shown in route I (Structure B)), during (shown in route II (Structure E)) or after the nucleophilic attack (Figure 2 route III (Structure F)). If the deprotonation takes place before the nucleophilic attack (route I (Structure B)), the unstable negatively charged oxygen (O^-) may dissociate to form an unrealistic equatorial oxo ligand (Mo^{VI}=Oeq) (Figure 2 structure (C)). That means, the oxo ligand may be formed before the O^- anion interacts with substrate (Figure 2 (a)). Alternatively, the O^-Mo may undergo a nucleophilic attack on the substrate carbon to form OMo-C_{RH} bond (indicated in Figure 2 (Structure D)) [1,7,11]. In this case, the OMo-C_{RH} bond may be formed before the likely formation of an equatorial oxo ligand (Mo^{VI}=Oeq). On the other hand, if a nucleophilic attack takes place after the deprotonation of H_{OH} (Figure 2 (Structure F)); the positive charge density on the oxygen center (HO_+^{Mo}) may create a high energy barrier that may prevent the formation of the tetrahedral species. However, the concerted formation of OMo-C_{RH} bond (Figure 2 (Structure E)) is more favorable since the abstraction of an acidic proton by (XOR)$^-$$Glu_{1261}$ is expected to neutralize the charge on the nucleophile (HO^{Mo}).

The energy released during the formation of the Structures varies from Structure (A) through Structure (D) (Figure 3). That is, the energy released increases from Structure (A) through Structure (D) during the initial stage of catalysis (from ΔE=-1481111.805 to ΔE=-1481140.485). However, the energy released for (B) and for (C) is almost identical during the formation of the Structures. In addition, the Structure formed during the near attack conformation (A) is energetically unfavorable (ΔE=-1481111.805 Kcal/mol) whereas the Structure (D) is energetically favorable (ΔE=-1481140.485). In other words, the lowest possible minimum amount of energy was attained during the formation of Structure (D). This indicates that Structure (D) is the most stable one. From this, we can suggest that Oxyanion is responsible for the nucleophilic attack of the deficient carbon atom other than the hydroxyl group itself (OH). Therefore, the most favorable pathway in the initial stage of catalysis is the reaction that occurs after the abstraction of the equatorial acidic proton of the active site. This is because Oxyanion cannot form the stable Structure when it forms a double bond with Molybdenum metal. It is evident that the stability of Structure (B) possessing Oxyanion and Structure (C) possessing double bond with Molybdenum is the same (ΔE=-1481106.485 Kcal/mol).

This also confirmed that Oxyanion is unstable to exist and make a nucleophilic attack on the substrate carbon rather than forming a double bond with Molybdenum metal. Thus, the most favorable reaction path way in the initial stage of catalysis occurs after the

abstraction of the equatorial acidic proton of the active site. Therefore, in the most favorable path way, Glu_{1261} is expected to act as a general base which is assumed to be followed by the nucleophilic attack of the Oxyanion on the carbon center of the substrate to yield the Structure. And hence, Glu_{1261} plays a great role in the activation of the active site by abstracting the acidic proton as well as in decreasing the activation barrier for the Structure formation during the initial stage of catalysis.

As shown in Figure 4, the partial charges on the O_{eq} for the Structures shown in Figure 2 (A, B, C, D, E, and F) were given as Δqc=-0.722622, Δqc=-0.499669, Δqc=-0.496599, Δqc=-0.504629, Δqc=-0.507978, and Δqc=-0.502671, respectively. From these partial charges, we can suggest that O_{eq} for Structure (E) is a good nucleophile (Δqc=-0.507978) to attack the deficient substrate carbon more readily than the remaining Structures. This shows that the Oxyanion is more susceptible to attack the deficient substrate carbon during the formation of structure D rather than any other structures. Similarly, the partial charges on the Mo atom for structures (A, B, C, D, E, and F) (Figure 5) were Δqc=0.618317, Δqc=0.388875, Δqc=0.396743, Δqc=0.395882, Δqc=0.386357, and Δqc=0.392332, respectively. The variation of these partial charges on Molybdenum atom indicates that the flow of charges is from the substrate carbon, Cs to the metal center (Mo) of the active site during the initial stage of catalysis. The charge on molybdenum atom at Structure (E) decreases from Δqc=0.618317 to Δqc=0.386357 which indicates that much more charges were flown to the metal center from the substrate carbon of Structure (E). This situation is a direct consequence of the highest accumulation of negative partial charge on the O_{eq} for Structure (E). In addition, the partial charges on the Cs Structures (A, B, C, D, E, and F) (Table 1) were Δqc=0.182387, Δqc=0.480415, Δqc=0.475512, Δqc=0.483265, Δqc=0.487586, and Δqc=0.483945, respectively. The highest positive charge developed on the carbon atom for Structure (E) indicates that the substrate carbon is more susceptible for the nucleophic reaction to take place. This supports the ideas explained earlier in the cases of the partial charges developed on O_{eq} and Mo metal. Therefore, all these suggestions showed that Structure (E) could be the most stable Structure followed by route II (Figure 2E).

As shown in Table 1 the % Contribution of Cs-HOMO and Mo-LUMO for the Structures (A, B, C, D, E, and F) were % Cs-HOMO=0.02 and % Mo-LUMO=22.8; % Cs-HOMO=0.42 and % Mo-LUMO=7.3; % Cs-HOMO=0.46 and % Mo-LUMO=0.90; % Cs-HOMO=0.47 and % Mo-LUMO=7.4; % Cs-HOMO=0.41 and % Mo-LUMO=5.0; and % Cs-HOMO=0.46 and % Mo-LUMO=3.6, respectively. In this case, the % Contributions of Cs-HOMO and Mo-LUMO are highest for Structure (D). This indicates that the interaction between substrate carbon HOMO and the metal center LUMO is strong. Therefore, we can suggest that Structure (D) is considered as the most stable Structure.

Finally, the amino acid residue, Glu_{1261} is proposed to play an important role in promoting the nucleophilicity of the HO_{eq} ligand of the active site. That is, Glu_{1261} is in a close proximity to the equatorial hydroxyl group, HOeq capable of abstracting hydrogen from HO_{eq} which causes the equatorial oxygen develops a negative charge (Oxyanion) for the nucleophilic attack on the deficient substrate carbon. That means, the Oxyanion is responsible for the nucleophilic attack of the deficient carbon atom other than the hydroxyl group itself (OH). Therefore, we can conclude that the role of Glu_{1261} is enhancing the nucleophilicity of HO_{eq} ligand as it acts as a Lewis base to accept a proton from the HO_{eq} ligand as well as stabilizing the tetrahedral Michaelis-Menten type intermediate at the transition state.

Conclusions

In general, we can suggest that Oxyanion is responsible for the nucleophilic attack of the deficient carbon atom other than the hydroxyl group itself (OH). Therefore, the most favorable pathway in the initial stage of catalysis is the reaction that occurs after the abstraction of the equatorial acidic proton of the active site. This is because Oxyanion cannot form the stable Structure when it forms a double bond with Molybdenum metal. In other words, Oxyanion is unstable to exist and make a nucleophilic attack on the substrate carbon rather than forming a double bond with Molybdenum metal. Thus, the most favorable reaction path way in the initial stage of catalysis occurs after the abstraction of the equatorial acidic proton of the active site. Therefore, in the most favorable path way, Glu_{1261} is expected to act as a general base which is assumed to be followed by the nucleophilic attack of the Oxyanion on the carbon center of the substrate to yield the Structure. And hence, Glu_{1261} plays a great role in the activation of the active site by abstracting the acidic proton as well as in decreasing the activation barrier for the Structure formation during the initial stage of catalysis. Furthermore, the flow of partial charges to Molybdenum metal center from the substrate is enhanced by the amino acid residue, Glu_{1261}.

The % Contribution of Cs-HOMO and Mo-LUMO for the respective Structures (A, B, C, D, E, and F) were %Cs-HOMO=0.02 and %Mo-LUMO=22.8; %Cs-HOMO=0.42 and %Mo-LUMO=7.3; %Cs-HOMO=0.46 and %Mo-LUMO=0.90; %Cs-HOMO=0.47 and %Mo-LUMO=7.4; %Cs-HOMO=0.41 and %Mo-LUMO=5.0; and %Cs-HOMO=0.46 and %Mo-LUMO=3.6, respectively. These HOMO-LUMO data were obtained from AOMIX output files. Here, we see that the % Contribution for Cs-HOMO and % contribution for Mo-LUMO vary for each respective Structure. This variation clearly suggests that the amino acid residue plays a great role for the charge transfer from the substrate carbon HOMO to the metal center LUMO. Generally, we can conclude that the flow of partial charges from Cs-HOMO to Mo-LUMO is not possible unless the active site is activated by Glu_{1261}. This clearly shows that the role of Glu_{1261} is activating the active site for the nucleophilic attack to take place. Therefore, this amino acid residue plays a great role in promoting the flow of partial charges from the substrate carbon HOMO orbital to the metal center LUMO orbital.

Finally, the amino acid residue, Glu_{1261} is proposed to play an important role in promoting the nucleophilicity of the HO_{eq} ligand of the active site. That is, Glu_{1261} is in a close proximity to the equatorial hydroxyl group, HO_{eq} capable of abstracting hydrogen from HO_{eq} which causes the equatorial oxygen develops a negative charge (Oxyanion) for the nucleophilic attack on the deficient substrate carbon. That means, the Oxyanion is responsible for the nucleophilic attack of the deficient carbon atom other than the hydroxyl group itself (OH). Therefore, we can conclude that the role of Glu_{1261} is enhancing the nucleophilicity of HO_{eq} ligand as it acts as a Lewis base to accept a proton from the HO_{eq} ligand as well as stabilizing the tetrahedral Michaelis-Menten type intermediate at the transition state.

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