Unraveling the Way Acetaldehyde is Formed from Acetylene: A Study Based on DFT

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ABSTRACT: Acetylene hydratase (AH) of Pelobacter acetylenicus is a tungsten (W)-containing iron–sulfur enzyme that catalyzes the transformation of acetylene to acetaldehyde, the exact true reaction mechanism of which is still in question. Scientists utilized different computational approaches to understand the reaction mechanism of acetylene hydration. Some identified it as a multistep (4–16) process that starts with the displacement of a water molecule present at the active site of AH with acetylene. However, some said that there is no need to displace water with acetylene at the active site of AH. As the reaction mechanism for the conversion of acetylene to acetaldehyde is still controversial and needs to be investigated further, DFT studies were performed on the model complexes derived from the native protein X-ray crystal structure of AH. Based on the computational results, here we are proposing the nucleophilic reaction mechanism where the water (Wat1424) molecule is coordinated to the W center and Asp13 is assumed to be in an anionic form. The Wat1424 molecule is activated by W and then donates one of its protons to the anionic Asp13, forming the W-bound hydroxide and protonated Asp13. The W-bound hydroxide then attacks the C1 atom of acetylene together with the transfer of a proton from Asp13 to its C2 atom, resulting in the formation of a vinyl alcohol intermediate complex. The energy barrier associated with this step is 14.4 kcal/mol. The final, rate-limiting, step corresponds to the tautomerization of the vinyl alcohol intermediate to acetaldehyde via intermolecular assistance of two water molecules, associated with an energy barrier of 18.9 kcal/mol. Also, the influence of the metal on the hydration of acetylene is studied when W is replaced with Mo.

INTRODUCTION

Acetylene hydratase (AH) of Pelobacter acetylenicus is a tungsten (W)-containing iron–sulfur enzyme. It catalyzes a nonredox reaction, the hydration of acetylene to acetaldehyde, as part of an anaerobic degradation pathway of unsaturated hydrocarbons.1

\[ \text{C}_2\text{H}_2 + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{CHO} \]

The protein X-ray crystal structure of AH2 from P. acetylenicus reveals a mononuclear W center at the active site and in a nearby iron–sulfur [4Fe-4S] cluster. At the active site, W is coordinated by two metallopterin guanine dinucleotide (MGD) cofactors, a sulfur atom of cysteine, and an oxygen species, which was assigned to be a water molecule because of its distance (2.04 Å) from the W center. The location of the [4Fe-4S] cluster is not far from the W center2 (Figure 1).

The protein X-ray crystal structure2 also shows that there are at least 16 well-defined water molecules in a vestibule directly adjacent to the active site, and these molecules may help in the catalytic activity, the hydration of acetylene to acetaldehyde. Due to these 16 water molecules, the water molecule coordinated with the tungsten atom at the active site, and the nearby protonated Asp13, the reaction mechanism of acetylene hydration through acetylene hydratase is still obscure. Different scientists presented multiple reaction mechanisms, for example, Seifert et al.3 suggested an electrophilic addition mechanism for the hydration of acetylene. As the bound water molecule gains a partial positive charge through protonated Asp13, it directly attacks the triple bond of acetylene as an electrophile. Density functional theory (DFT) calculations on small models of AH by Antony and Bayse4 show that the displacement of a water molecule at the active site by an acetylene molecule is an exothermic reaction. As the bound water molecule gains a partial positive charge through protonated Asp13, it directly attacks the triple bond of acetylene as an electrophile. Density functional theory (DFT) calculations on small models of AH by Antony and Bayse5 show that the displacement of a water molecule at the active site by an acetylene molecule is an exothermic reaction. Based on this result, they suggest the nucleophilic attack of a water molecule at the \( \eta_2 \)-acetylene bound to W to form vinyl alcohol assisted by protonated Asp13. Vincent et al.6 computed high energy barriers (higher than 40 kcal/mol) for both the mechanisms suggested by Seifert using DFT methods and therefore ruled out both. Instead, they speculated a 16-step reaction mechanism, which started with the displacement of
the water molecule by acetylene bound to the metal center in an \( \eta^2 \) fashion. The reaction proceeds through intermediate vinylidene (\( W = C = CH_2 \)) and carbene (\( W = C(OH)CH_3 \)) complexes. However, the energy barriers for the formation of these intermediates are also quite high (28 and 34 kcal/mol, respectively). 4

Subsequently, Himo et al. 5 performed quantum chemical calculations on considerably larger models of the active site of AH derived from the protein X-ray crystal structure and proposed a nine-step reaction mechanism. This mechanism starts with the displacement of the \( W^{\text{IV}} \)-bound water molecule with \( \eta^2 \)-acetylene. The water molecule, activated by the ionized Asp13, performs a nucleophilic attack on acetylene, resulting in the formation of a vinyl anion intermediate, which is stabilized by metal coordination. The protonated Asp13 then acts as an acid and donates a proton to the vinyl anion, generating a vinyl alcohol intermediate. This is the rate-limiting step (energy barrier of 23 kcal/mol). The final two steps involve the tautomerization of vinyl alcohol to acetaldehyde with the help of Asp13 and the W metal center. 5 Keeping in view Himo’s works, Najasian et al. 6 performed computational analysis on bioinspired model complexes that mimic the active site of acetylene with Group 6 transition metals and substituents attached at the dithiolate part of the molybdopterin cofactor to assess their effect on the catalytic cycle of acetylene hydration. Hydration of acetylene to

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**Figure 1.** Cofactors and the active site of AH. (A) Tungsten atom (blue) is coordinated by the dithiolene groups of both MGD cofactors and the side chain of Cys141. A water molecule completes the slightly distorted octahedral geometry. This water is also hydrogen-bonded to Asp13, a residue adjacent to the [4Fe:4S] cluster ligand Cys12. (B) Binding pocket positions an acetylene molecule directly above the water molecule and Asp13. (C) Bond distances of 2.04 Å to W and 2.41 Å to the OH atom of Asp13 indicate a highly activated water molecule positioned right below a binding pocket for acetylene. (Pictures are taken from ref 2 cited in this article; Copyright [2009] [Proc. Natl. Acad. Sci. USA. PMID: 17360611; PMCID: PMC1805521]).

**Figure 2.** Schematic description of the mechanism of acetylene hydration at acetylene hydratase, where A = electrophilic pathway, B = nucleophilic pathway, ES = educt−substrate complex, TS = transition state, EP1 = alcoholic product, and EP2 = tautomerized (to aldehyde) product.
acetaldehyde with the help of a series of zinc catalysts was also studied through quantum chemical calculations by Li et al.\textsuperscript{7} The reaction mechanisms for the hydration of acetylene, the binding of $C_2H_2$, and the results presented to date are still ambiguous. Therefore, more investigations are needed to understand the structural information on the binding of $C_2H_2$ with tungsten present at the active site of acetylene hydratase as well as the reaction mechanism for acetaldehyde formation to present a decisive answer. Also, the effect of replacement of tungsten with molybdenum needs to be investigated. In this research, we considered the initial proposal of Seifert et al.\textsuperscript{2} and investigated the two mechanisms proposed for catalysis, which do not take place through an organometallic intermediate (Figure 2) where DFT calculations were performed on small and large model complexes of the AH active site designed based on the protein X-ray crystal structure of AH.\textsuperscript{2}

## RESULTS AND DISCUSSION

Acetylene hydratase (AH) is a unique tungsten-containing enzyme as it does not appear to catalyze a redox reaction. The protein X-ray crystal structure of AH$^{+}$ provides important clues on the catalytic mechanism and the role of the W center. For catalysis, the enzyme is activated by the reduction of W center from W$^{I}$ to W$^{V}$, as only W$^{V}$ participates in the catalysis of acetylene hydration, and the [4Fe-4S] cluster facilitates this step. The reactive species in AH is either a hydrox or a water molecule coordinated to the W center. Extensive research has been carried out on the hydration of acetylene in the last decade. However, the results presented to date are still not conclusive and, therefore, under investigation till date. Considering the nature of the oxo-ligand attached to the W, we have computed two pathways (as suggested by Seifert et al.\textsuperscript{2}): an electrophilic pathway, where the coordinated water molecule would act as an electrophile, and a nucleophilic pathway, where the hydroxide would act as a nucleophile (Figure 2).

In the electrophilic pathway, Asp13 was considered to be protonated, due to the presence of water molecules as a solvent. The W-bound water molecule (Wat1862) is activated by the nearby protonated Asp13 residue and a second water molecule (Wat1424). The transition state involves the electrophilic attack of the activated Wat1862 molecule on the triple bond of acetylene with the simultaneous transfer of protons among Asp13, Wat1862, Wat1424, and acetylene. The proton from the Asp13 residue (−COOH) is transferred to the Wat1862 molecule, and one proton of the Wat1862 molecule is transferred to the α carbon atom (Cα or C1) of acetylene. From Wat1424, one proton is transferred to Asp13, while its electron-donating part (−OH) is transferred to the second carbon atom (Cβ or C2) of acetylene. This proton shuttle results in the formation of vinyl alcohol, which subsequently may tautomerize to aldehyde (Figures 2A and 3).

In the nucleophilic pathway, Asp13 was considered to be in the anionic form. The W center, which acts as a Lewis acid, activates water molecule Wat1862 and generates a W-bound hydroxide and protonated Asp13. The transition state involves the nucleophilic attack of this W-bound hydroxide at the alpha carbon atom (Cα or C1) of acetylene together with the simultaneous transfer of a proton from Asp13 to the second acetylene carbon atom (Cβ or C2), resulting in the formation of vinyl alcohol, which subsequently may tautomerize to aldehyde (Figures 2B and 4).

The density functional theory (DFT) computations were, first, performed on the small model complexes (3 Å) derived from the protein X-ray crystal structure of AH.\textsuperscript{2} The computational results for the small model complexes show that the nucleophilic pathway (Figure 5) is energetically more favorable than the electrophilic pathway (Figure 6) as the energy barrier for the electrophilic pathway transition state SE-TS (28.5 kcal/mol in the continuum) is higher than the energy barrier for the nucleophilic pathway transition state SN-TS (17.0 kcal/mol). To have a more global appraisal, geometry optimizations and energy barrier calculations were also performed in the gas phase; however, the continuum results were used here for the discussion (relative computed energy values for the geometry optimization and single-point energies are presented in Tables 1–4). It is highlighted that all of the relative computed energy values for geometry optimization and single-point energy values are consistent with the continuum
results. The formation of vinyl alcohol product SE-EP1 is \( \sim 7 \) kcal/mol lower in energy than that of SN-EP1. The final step may involve the tautomerization of the vinyl alcohol intermediate to acetaldehyde. The formation of acetaldehyde product SN-EP2 is \( \sim 4 \) kcal/mol lower in energy than the formation of SE-EP2 (Table 1).

The tautomerization of the vinyl alcohol intermediate to acetaldehyde also needs an energy barrier. Suenobou et al. computed the energy barrier for the tautomerization of vinyl alcohol to acetaldehyde with and without the assistance of a water molecule. They suggested that when the reaction is catalyzed by a water molecule, the energy barrier decreases from 55.8 to 29.6 kcal/mol in the gas phase. Lledo’s et al. suggested that the intervention of a chain of two water molecules further reduces the potential energy barrier to 21.8 kcal/mol. The importance of water molecules for the conversion of atmospheric VA into AA was also imposed by Peeters et al. debating that the lifetime of VA decreases as it is taken up by aqueous aerosol or cloud droplets and converting it into AA with a fast speed.

Keeping the above in view, the energy barrier for the tautomerization of vinyl alcohol (VA) to acetaldehyde (AA) was also computed in this research (without the educt complex) without the assistance of a water molecule and with the assistance of one and two water molecules (Table 2, Figures 7 and 9). The computed energy barrier for the intramolecular conversion of VA into AA was 58.8 kcal/mol in the polarizable continuum. This energy barrier decreased to 30.7 kcal/mol when the reaction was catalyzed by a water molecule. As the energy barrier dropped dramatically with the

**Table 1. Computed Energies [kcal/mol] Relative to the Educt–Substrate Complex for Stationary Points Relevant in the Hydration of Acetylene by Small Model Complexes of AH**

| Electrophić pathway, SE | Nucleophić pathway, SN |
|------------------------|------------------------|
| ES                     | 0.0                    | 0.0 | //BP86<sup>b</sup> |
| ES                     | 0.0                    | 0.0 | SDD<sup>c</sup>   |
| ES                     | 0.0                    | 0.0 | COSMO<sup>d</sup> |
| TS                     | 30.1                   | 12.9 | //BP86<sup>b</sup> |
| TS                     | 30.4                   | 15.7 | SDD<sup>c</sup>   |
| TS                     | 28.5                   | 17.0 | COSMO<sup>d</sup> |
| EP1                    | –27.9                  | –27.1 | //BP86<sup>b</sup> |
| EP1                    | –33.6                  | –31.3 | SDD<sup>c</sup>   |
| EP1                    | –35.6                  | –30.3 | COSMO<sup>d</sup> |
| EP2                    | –43.5                  | –52.2 | //BP86<sup>b</sup> |
| EP2                    | –46.6                  | –50.2 | SDD<sup>c</sup>   |
| EP2                    | –46.6                  | –50.2 | COSMO<sup>d</sup> |

<sup>a</sup>Here, ES = educt–substrate complex, TS = transition state complex, EP1 = alcohol product complex, and EP2 = tautomerized product complex.<br> <sup>b</sup>B3LYP/Lanl2DZ(p).<br> <sup>c</sup>B3LYP/SDDp//B3LYP/Lanl2DZ (p).<br> <sup>d</sup>COSMO-B3LYP/SDDp//B3LYP/Lanl2DZ(p) (see Computational Details).

**Table 2. Computed Energy Barriers (kcal/mol) for the Tautomerization of Vinyl Alcohol to Acetaldehyde**

| VA | TS | AA |
|----|----|----|
| 1A | 0.0 | 62.6 | –9.6 | //B3LYP<sup>b</sup> |
| 2A | 0.0 | 26.4 | –7.3 | //B3LYP<sup>b</sup> |
| 3A | 0.0 | 13.3 | –5.3 | //B3LYP<sup>b</sup> |
| 4A | 0.0 | 20.3 | –11.0 | //B3LYP<sup>b</sup> |
| 5A | 0.0 | 18.3 | –8.6 | //BP86<sup>e</sup> |
| 6A | 0.0 | 28.5 | –9.7 | //BP86<sup>e</sup> |
| 7A | 0.0 | 18.9 | –13.0 | //BP86<sup>e</sup> |

<sup>a</sup>Here, 1A = intramolecular reaction, 2A = single water molecule-catalyzed reaction, 3A = additional water molecules, VA = vinyl alcohol, TS = transition state, and AA = acetaldehyde.<br> <sup>b</sup>B3LYP/Lanl2DZ(p).<br> <sup>c</sup>B3LYP/SDDp//B3LYP/Lanl2DZ (p).<br> <sup>d</sup>COSMO-B3LYP/SDDp//B3LYP/Lanl2DZ(p).<br> <sup>e</sup>COSMO-B3LYP/SDDp//BP86/Lanl2DZ(p).<br> <sup>f</sup>COSMO-B3LYP/SDDp//BP86/Lanl2DZ(p) (see Computational Details).


Table 3. Computed Energy Barriers [kcal/mol] Relative to the Educt–Substrate Complex for Hydration of Acetylene by the Large Model (Water-Containing (6 Å)) Complexes of AH$^a$

|          | electrophilic pathway, LE | nucleophilic pathway, LN |
|----------|--------------------------|--------------------------|
| ES       | 0.0                      | 0.0                      |
| TS       | 31.7                     | 21.5                     |
| EP1      | −20.3                    | −39.4                    |
| EP2      | −36.1                    | −47.1                    |
| EP3      | −36.5                    | −38.3                    |

$^a$Here, ES = educt–substrate complex, TS = transition state complex, and EP1 = alcohol product complex. $^b$B3LYP/Lanl2DZ(p). $^c$B3LYP/SDDp//B3LYP/Lanl2DZ(p). $^d$COSMO-B3LYP/SDDp//B3LYP/Lanl2DZ(p) (see Computational Details).

Table 4. Computed Energy Barriers [kcal/mol] Relative to the Educt–Substrate Complex for Hydration of Acetylene by the Large Model (Water-Containing (8Å)) Complexes of AH$^a$

|          | electrophilic pathway, XE | nucleophilic pathway, XN | nucleophilic pathway, Mo |
|----------|--------------------------|--------------------------|--------------------------|
| ES       | 0.0                      | 0.0                      | 0.0                      |
| TS       | 26.5                     | 14.3                     | 14.3                     |
| EP1      | −36.1                    | −47.1                    | −50.7                    |
| EP2      | −32.9                    | −61.4                    | −63.7                    |
| EP3      | −36.5                    | −51.4                    | −55.5                    |

$^a$Here, ES = educt–substrate complex, TS = transition state complex, EP1 = alcohol product complex, EP2 = tautomerized product complex, and EP3 = product complex, where acetaldehyde is replaced by the surrounding water molecule but acetaldehyde is also present in the structure. $^b$B3LYP/Lanl2DZ(p). $^c$B3LYP/SDDp//B3LYP/Lanl2DZ(p). $^d$COSMO-B3LYP/SDDp//B3LYP/Lanl2DZ(p) (see Computational Details).

assistance of a water molecule, we then computed the tautomerization of vinyl alcohol to acetaldehyde in the presence of two water molecules, which gave an energy barrier of 20.7 kcal/mol in the continuum (Table 2 and Figure 9). The tautomerization process was also analyzed using three and four water molecules; however, no significant difference was observed. Detailed mechanisms are presented in the Supporting Information, and only transition states for the tautomerization of vinyl alcohol to acetaldehyde are presented in Figure 7.

Considering the importance of water molecules in the tautomerization of VA to AA, the same was computed in the educt complex, WN, which was generated by the introduction of two water molecules in the optimized small model SN-EP1 complex geometry (Figure 8). The computed energy barrier for this conversion of VA into AA in WN-TPS was 18.9 kcal/mol in the polarizable continuum (Table 2). Now, when we compared this energy barrier of WN-TPS with the energy barrier for alcohol formation in the electrophilic (SE-TPS = 28.5 kcal/mol) and nucleophilic (SN-TPS = 17.0 kcal/mol) pathways, the tautomerization of VA to AA seems to be the rate-limiting step in the nucleophilic pathway.

To identify the most probable reaction mechanism, large model complexes (6Å) are analyzed considering some of the surrounding amino acid residues (Trp179, Trp293, and Trp472) as well as the water molecules (Wat1209, Wat1212, Wat1424, and Wat1432), which are in the proximity of the W metal center, connected through hydrogen bonding, and may take part in the reaction (Figures 10 and 11). The computational results for the large model complexes also favor the nucleophilic pathway for the formation of vinyl alcohol as the energy barrier for LN-TPS is 15.1 kcal/mol, whereas for the electrophilic pathway, it is 29.4 kcal/mol (Table 3). The formation of vinyl alcohol complexes LE-EP1 and LN-EP1 is exothermic for both pathways; however, LN-EP1 is ~4 kcal/mol lower in energy than LN-EP1.

Another important factor observed here is that the large model complexes when computed without the water molecules Wat1209, Wat1212, Wat1424, and Wat1432, the energy barrier for the vinyl alcohol formation from acetylene was ~20 kcal/mol higher than that with the inclusion of water molecules (Table 3). Therefore, we can conclude that the nucleophilic reaction mechanism for hydration of acetylene is a preferable pathway and the water molecules adjacent to the active site, present in the second shell, are very important for the hydration of acetylene to acetaldehyde.

Now to confirm or validate the above-mentioned finding, we have increased the size of our model complexes and considered all of the amino acid residues and water molecules present within the 8 Å distance from the metal at the active site (Figure 12).

The computational results for the large model complexes (8 Å) also favor the nucleophilic pathway for vinyl alcohol formation. The energy barrier for the nucleophilic pathway transition state complex XN-TPS (14.4 kcal/mol) is ~9 kcal/mol lower than the energy barrier for the electrophilic pathway transition state complex XE-TPS (23.1 kcal/mol) (Figures 13 and 14). The relative energies for the acetaldehyde product complexes XN-EP2 and XE-EP2 are also exothermic, but XN-EP2 is ~16 kcal/mol lower in energy than XE-EP2. In the nucleophilic pathway, the W center is regenerated by the replacement of acetaldehyde with one of the surrounding water molecules. The reaction energy for this step (XN-EP3) is also

Figure 7. Optimized transition state geometries for the intramolecular (1A-TS) and intermolecular (2A-TS and 3A-TS) tautomerization of vinyl alcohol to acetaldehyde.
exothermic, $-7.2$ kcal/mol in the polarizable continuum and $-1.1$ kcal/mol in the gas phase, relative to the XN-EP2 complex (Table 4). As the results produced by the small model and large model complexes (6 and 8 Å) are comparable, the results are validated, and it is proposed that the nucleophilic reaction pathway is the most probable reaction mechanism for the hydration of acetylene.

When these results are compared with the rate-limiting steps of the previously reported pathways, it has been observed that the reported energy barrier of 14.4 kcal/mol for the nucleophilic reaction pathway is the most probable reaction mechanism for the hydration of acetylene.

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or C\textsubscript{1} atom of acetylene together with the transfer of a proton from Asp\textsubscript{13} to its C\textsubscript{\beta} or C\textsubscript{2} atom, resulting in the formation of a vinyl alcohol intermediate complex. The energy barrier for this step is 14.4 kcal/mol in the polarizable continuum. The final step corresponds to the tautomerization of a vinyl alcohol intermediate to the acetaldehyde via intermolecular assistance of two water molecules. The energy barrier for this step is 18.9 kcal/mol in the polarizable continuum, which is calculated only for the small model complexes, SN. The reason is that when we compare the energy barriers calculated for vinyl alcohol formation from acetylene between the small model (SN-TS) and large model (XN-TS) complexes, the difference is negligible (~3 kcal/mol). Therefore, the energy barrier for tautomerization in the large model (XN) was not calculated. However, it is highlighted that tautomerization of vinyl alcohol to acetaldehyde should be the rate-limiting step in the nucleophilic pathway (see Tables 2 and 4).

Therefore, this research concludes that the nucleophilic reaction pathway presented here is more favorable for the hydration of acetylene. It is not only lower in energy relative to the already reported\textsuperscript{3-7} ones but it also involves a simple two-step mechanism compared to the others for which up to sixteen-step reaction pathways have been reported. Another important point highlighted here is that the water molecules, which are not directly coordinated with the metal at the active site but present in the second shell around the active site, play the most important role in the hydration of acetylene. Also, the effect of metal, when W is replaced with Mo, on the hydration of acetylene is inconclusive as no difference between computed energies/energy barriers is observed. In future, QM/MM studies can also be performed for the reaction pathway analysis of acetylene hydration as studied for uricase-catalyzed oxidation and hydration of uric acid,\textsuperscript{14} water wires catalyzing long-range proton pumping reactions,\textsuperscript{15} or reaction pathways for proteasome inhibition.\textsuperscript{16}

\section*{METHODOLOGY}

\textbf{Computational Details. Small Model Complexes.} All of the small model geometries (~44 atoms) were fully optimized using Gaussian 03 with the density functional BP86\textsuperscript{17-19} and the LANL2DZ basis set.\textsuperscript{20-23} The self-consistent field (SCF)\textsuperscript{24} method was used with the IntRep option for the SCF procedure to account for integral symmetry and NoVaracc for full integral accuracy. Whenever there was an SCF convergence problem, the QC option was used, which involves linear searches when far from convergence. The starting geometries for transition state searches were generated by shortening and lengthening of forming and breaking bonds, respectively. Frequency calculations were performed to confirm that no imaginary frequency exists for the minimized geometries, whereas one negative frequency must exist for the transition state. Single-point energies were computed with the B3LYP\textsuperscript{25} functional and the Stuttgart–Dresden effective core potential basis set (SDD)\textsuperscript{26,27} augmented by polarization functions for all atoms except W and H (\(\zeta = 0.600, 1.154, 0.864,\) and 0.421 for C, O, N, and S, respectively).\textsuperscript{28} In addition, self-consistent reaction field (SCRF) computations were performed on the optimized geometries by a conductor-like polarizable continuum method (CPCM).\textsuperscript{29,30}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure11}
\caption{Optimized geometries for the large model (6 Å) complexes involved in the nucleophilic pathway of acetylene hydration by AH.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure12}
\caption{Chemical structure of the large active site model (water-containing) complexes for the electrophilic pathway derived from the protein X-ray crystal structure of AH.\textsuperscript{2}}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure13}
\caption{Optimized geometries for the large model (8 Å) complexes involved in the electrophilic pathway of acetylene hydration by AH.}
\end{figure}
**Large Model Complexes.** All of the large model geometries (~144 atoms for 6 Å and ~244 atoms in 8 Å model geometries) were optimized using Gaussian 09 with the hybrid density functional B3LYP\(^{25}\) and the LANL2DZ basis set\(^{26,27}\) augmented by polarization function on sulfur atoms (\(\zeta = 0.600, 1.154, 0.864, \) and 0.421 for C, O, N, and S, respectively).\(^{38}\) Self-consistent reaction field (SCRF)\(^{24}\) computations were performed on the optimized geometries by the conductor-like polarizable continuum method (CPCM)\(^{25,30}\) as implemented in Gaussian 09. To make it consistent with the results of small model complexes, the default Gaussian 03 procedure and parameters were used. The molecular cavity was specified using a minimum radius (RMin) of 0.5 Å and an overlap index (OFac) of 0.8.\(^{31}\)

**Active Site Models.** Active site model complexes were designed based on the protein X-ray crystal structure of *P. acetylenicus* (PDB-ID: 2E7Z).\(^7\)

**Small Model Complexes (3 Å).** Small active site models were considered to identify the most probable reaction mechanism. These models include the W metal center coordinated with two molybdopterin ligands (MGD), a metal-bound water (Wat1862) molecule, a cysteinate (Cys141) ligand, and an additional aspartate (Asp13) residue.

The water molecule (Wat1424), nearby Asp13, and Wat1862 were also considered in the case of the electrophilic reaction mechanism (Figure 2A). Cys141 was truncated to a H3C-group, Asp13 to acetate (CH3COO-), and MGD to 2,3-dithiolato-2-butene (ene-dithiolate). Hydrogen atoms were added manually. The beta (\(\beta\)) carbon atom of acetate and methyl carbon atoms of the ene-dithiolato ligands were kept fixed to their crystal structure positions during the calculations to mimic the steric constraints of the protein matrix (Figure S1 of the Supporting Information).

**Large Model Complexes with Water Molecules (8 Å).** From the protein crystal structure,\(^2\) it was deduced that Asp13 forms hydrogen bonds with the oxygen species attached to W as well as to the peptide bond of Cys12 and the side chain of Trp179. Therefore, Trp179, Trp293, and Trp472 were included for the large models to account for the effect of second shell ligands on the energy profile and reaction mechanism. Hydrogen atoms were added manually. During the optimizations, alpha (\(\alpha\)) carbon atoms and nitrogen atoms attached to the beta (\(\beta\)) carbon atoms of Asp13, Trp179, Trp293, and Trp472 were kept fixed to their crystal structure positions to mimic the steric constraints by the protein matrix. Nitrogen attached to the beta (\(\beta\)) carbon atom of Cys141 was also kept fixed. The MGD ligands were truncated to pyran rings, and the oxygen atoms of these pyran rings were kept fixed (Figure S2 of the Supporting Information).

**Large Model Complexes with Water Molecules (8 Å).** The protein X-ray crystal structure\(^2\) shows that there are at least 16 well-defined water molecules in a vestibule directly adjacent to the active site, and these molecules may help in the catalytic activity, the hydration of acetylene to acetaldehyde. Thus, Wat1209, Wat1212, and Wat1432 water molecules were considered in the large active site models. Ala137, Met138, Ile113, Ile142, and Phe611 were also considered to keep the water molecules at their locations as they form hydrogen bonds with these water molecules. Hydrogen atoms were added manually. Alpha (\(\alpha\)) carbon atoms and nitrogen atoms attached to the beta (\(\beta\)) carbon atoms of Asp13, Ile113, Trp179, Trp472, Phe611; the beta (\(\beta\)) carbon atom of Ala137; the nitrogen...
...atom attached to the $\beta$ carbon atom of Cys141; $\alpha$ carbon of Ile142; $\alpha$ and $\beta$ carbon atoms of Trp293; C4 of Met138; and the oxygen atoms of the pyran rings of dithiolenes were kept fixed to their crystal structure positions during optimizations to mimic the steric constraints by the protein matrix (Figure S3 of the Supporting Information).

For all of the active site model complexes, first, the hydrogen atoms were optimized, applying two negative charges for the nucleophilic pathway (assuming W at the +IV oxidation state and Asp13 in the deprotonated form) while one negative charge for the electrophilic pathway (assuming W at the +IV oxidation state and Asp13 in the protonated form), keeping all of the heavy atoms fixed at their positions. The resulting geometries served as starting geometries when generating input geometries for the study of the mechanism for acetylene hydratase. The starting geometries of educt–substrate complexes (ES) and the alcohol product complexes (EP1) for geometry optimizations were generated from the optimized transition state (TS) geometries.

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