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The effect of protonation of a substrate,on a catalytic mechanism of Nicotinate-dehydrogenase

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Abstract: The active site of Nicotinate Dehydrogenase is somewhat similar to xanthine oxidoreductase (XOR) except the equatorial sulfido group in Xanthine oxidase is replaced by sellenido group in Nicotinate Dehydrogenase. In addition, the two enzymes were reported to vary in the binding pocket amino acid residue composition. Modeling the substrate binding step using acetaldehyde as a substrate and active site terminal containing selenium in the place of sulfido terminal is mechanistically important. In order to probe the oxidation of acetaldehyde by molybdenium hydroxylases, Density functional theory with B3LYP level theory has been applied. Initially, a linear transit calculation is applied to obtain transition state structures. The exact positions of transition state structure was proved by maximum energy, inflection point in Mulliken charge profile and single negative frequency. Protonation of the carbonyl oxygen of acetaldehyde in the complexes resulted in stabilization of the transition state by around 420 kcal. But it retarded the way of dissociation of the transition state structure towards product bound complex, which was confirmed by decreased electron density and negative Mulliken charge on $H_{acetaldehyde}$ along its waytowards the terminals of the active sites, low extent Mo-O_{eq} elongation profile, low extent of O_{eq}-$C_{acetaldehyde}$ shortening and higher frequency.

Keywords: Density functional calculations, Nicotinate-Dehydrogenas, acetaldehyde, Single point energy, mulliken charge and single negative frequency.

1. INTRODUCTION

Even though there is a thorough study on catalytic mechanism of Xanthine-oxidoreductase, which is a typical member of molybdoenzyme family, we cannot usually find such study on the mechanisms of Nicotinate dehydrogenase. We can see a very close similarity between the two enzymes. The evidences presented in different experimental approaches including EPR studies proves that, in the crystal structure of NDH selenium indeed replaces sulfur as a molybdenum ligand as Mo=Se. Besides Most of the amino acid residues present in NDH active sites are also present in *b*XOR.Xanthine oxidoreductase (XOR) is a molybdenum-containing enzyme that is the prototypical member of the molybdenum hydroxylase family of proteins[1].

As a result of their close similarity between XO and NDH, mainly in the primary and secondary coordination sphere of the reductive half reaction, the proposed mechanisms for XOR can be used as a starting clue inorder to know the effect of protonation of model substrate on its catalytic property.

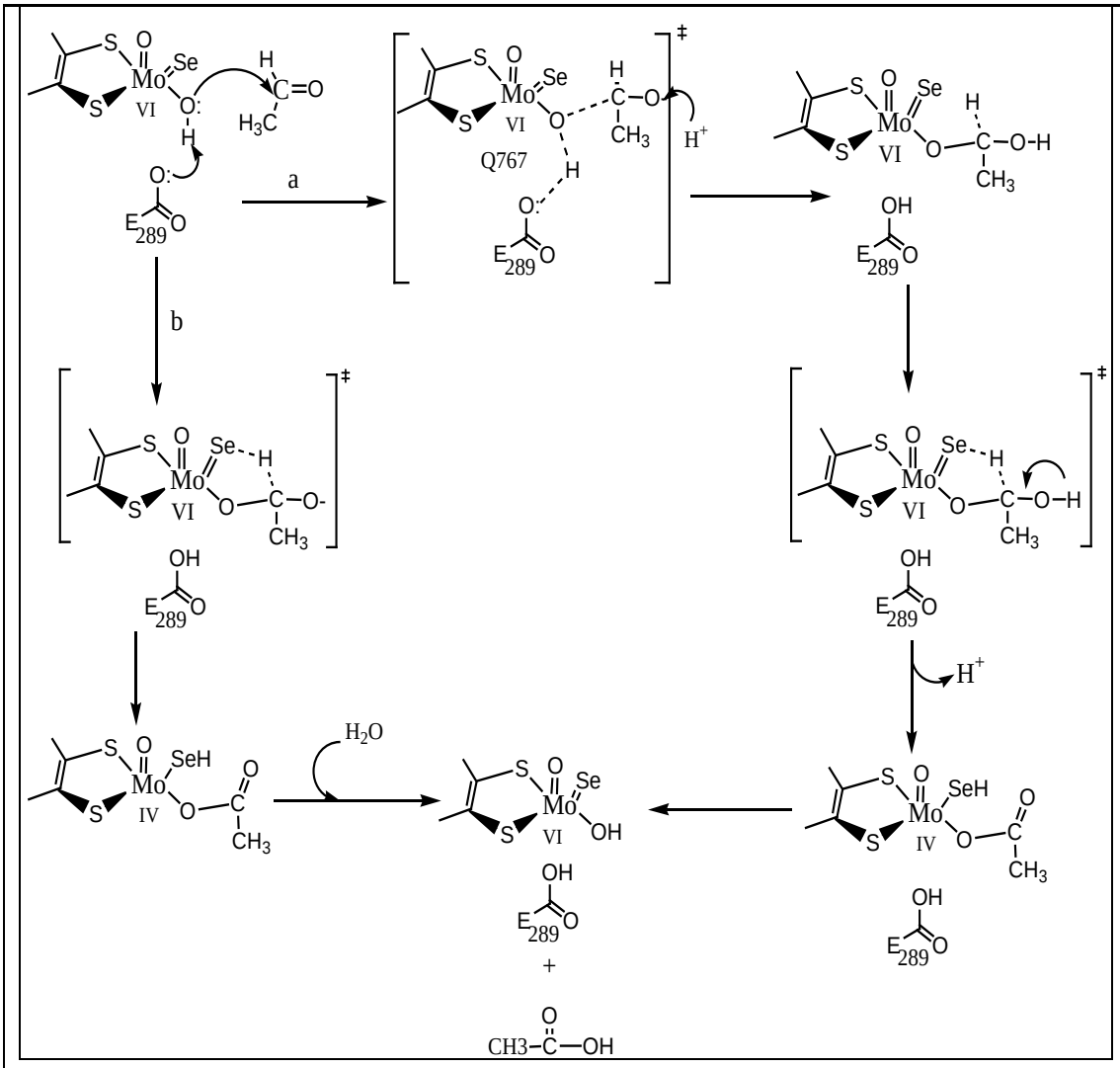
Nicotinate (niacin, vitamin B3) is an important constituent of all living cells in the form ofNAD(P). Organisms contain NAD(P) concentrations of 0.1-1 mM which upon cell-death supply nicotinate as a nitrogen, carbon and energy source to dedicated nicotinate-utilizing microorganisms. Regardless of organism and environment catabolism of nicotinate starts with hydroxylation to 6-hydroxynicotinate by the well-characterized molybdopterin enzyme nicotinate dehydrogenase.

In aerobic organisms 6-hydroxynicotinate is oxidatively decarboxylated to 2, 5-dihydroxypyridine or subjected to a second hydroxylation yielding 2, 6-dihydroxynicotinate. Under microaerobic or anaerobic conditions the ferredoxin-dependent reduction to 1, 4, 5, 6-tetrahydro-6-oxonicotinate (THON) is observed. The latter pathway is followed by the anaerobic soil bacterium *Eubacterium barkeri*, which belongs to the Clostridia. *E. barkeri* ferments nicotinate to propionate, acetate, CO_2, NH4+ and ATP[2].

Current understanding of the catalytic mechanism of the enzyme is based on these studies, which are supplemented by model compounds and model reactions[3]. Considering these circumstances, the oxidation mechanism of NDH has been investigated theoretically, using acetaldehyde as a substrate.

Protonation reaction is a process of taking proton by nucleophile species from its surrounding. Since there is negative charge accumulation on the oxyl group of the substrate, starting from where the substrate carbon is being attacked by equatorial oxygen; it may prefer to undergo protonation reaction as shown below in **Scheme 1.1.**

As shown in the Scheme, the protonation of the substrate-active site complex is proposed to takes place at the first transition state presumed to occur before the formation of Michael-Menten type complex. On the other hand deprotonation of the complex is expected to takes place at the second transition state before the formation of product bound complex. Protonation of acetaldehyde in complexes of each active site have been done in order to know its effect.



Scheme 1.1: Postulated base-assisted hydride transfer mechanism for the oxidation reaction by NDH. In this mechanism the protonation reaction of carbonyl oxygen of acetaldehyde is proposed[3-10].

2. MATERIALS AND METHODS

2.1. Materials: All computational calculations were performed using Gaussian 03W (version 6.0) program software package (Gaussian, Inc., Wallingford, CT, USA). Molecular orbital and electronic structure visualization were performed using Gauss View 3.0 (Gaussian, Inc., Pittsburgh, PA, USA). The software was also used to compute the bond distances from the output files of the optimized structures. The pictorial views, for the frontier (HOMO and LUMO) orbitals, were generated from the checkpoint files using this software. The structures were initially developed using ChemDraw ultra 6.0

(Cambridge soft wares, Cambridge, MA, USA). All data were analyzed using Microsoft office excel 2003(Microsoft, Inc., Redmond, Washington, USA).

2.2. Methods

2.2.1. General computation procedure: Electronic structure calculations have been performed to generate Mulliken atomic charges (ΔqMo, ΔqS, etc.), total electronic energies, wave-function descriptions, bonding descriptions, and bond orders. The parameters were generated from the geometry optimization, single point energy, frequency, and linear transit calculations. All computation calculations were performed using Gaussian 03W using density functional theory (DFT) method of the B3LYP correlation functional formalism (DFT-B3LYP).

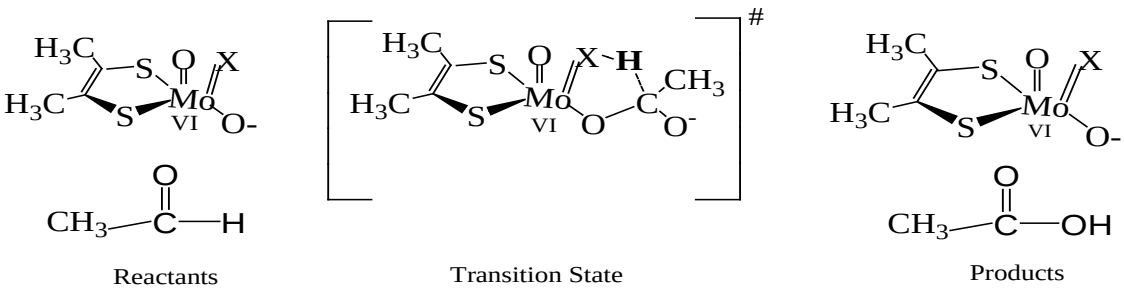


Figure 2.2.1: The reactants (acetaldehyde and active sites separately), TS and product (acetic acid and regenerated active sites separately) structures that was modeled for energy comparision. Where X: Se.

3. RESULTS AND DISCUSSION

The reductive half reaction active sites with sellenido terminal truncated structure, was modeled using GaussView software. Single point Energy, Mullikens charge and bond descriptions of the complex were among the very important tools obtained from the transition state structure, to study the properties of the active site. These were obtained by linear transit calculations were made to show the transfer of substrate bound to the active sites with terminals Se. Electron density and frequency were additional information that were obtained.

Though transition state has a fleeting existence, studies upon its formation and decay reveals crucial events taking place during catalytic activity of the catalyst on the substrate. Thus, the transition state complexes of the active sites were modeled with sellenido terminals. The energy for the transition state of unprotonated substrate bound active site of the complex was -2336399.53 kcal at Se-H bond distance of 2.11861 angstrom. The transition state energy for the respected protonated TS was -2336824.55 kcal at Se-H bond distance of 1.7171 angstrom (Fig. 3.1.). Thus from the energy profile of unprotonated and protonated transition states of the complex, it can be seen that in protonation state, the TS structure was further stabilized by energy of 425.02 kcal.

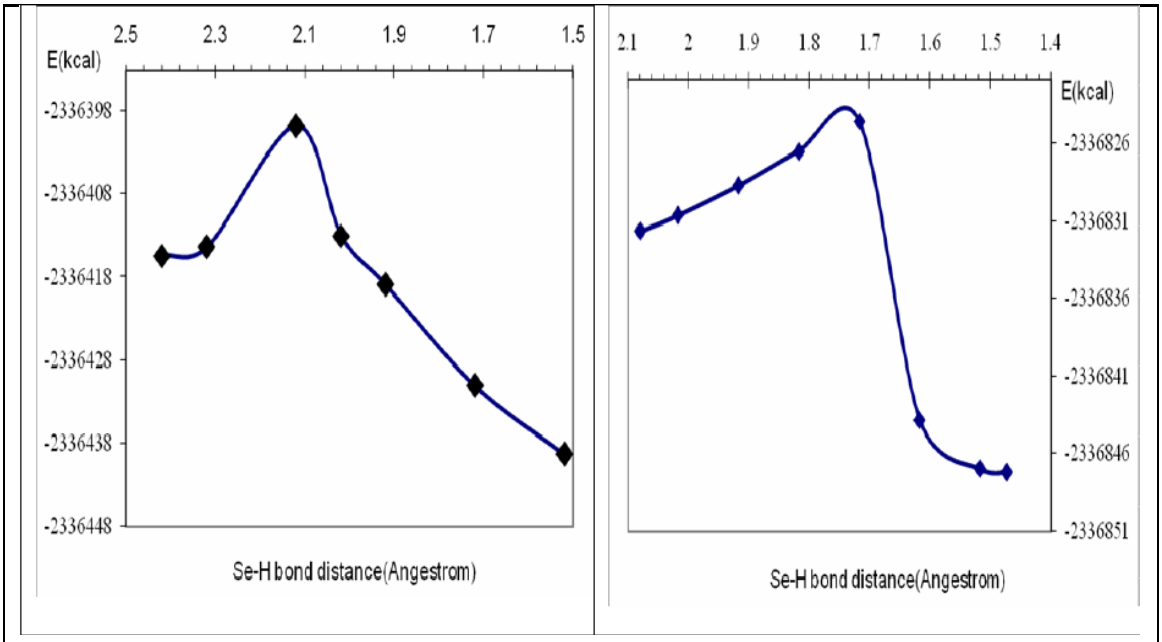


Figure 3.1: Total energy profile from linear transit calculation which have been carried out by stepping up the $H_{acetaldehyde}$-Se bond distance (A^{O}), at constant intervals. Once a series of structures are optimized, the total energies (kcal/mol) from each optimization steps were plotted against respective H-Se bond distances in protonated (right) and unprotonated (left) substrate-active site complex.

The highest barrier, on the unprotonated complex is proposed to be due to the charge delocalization on the carbonyl carbons of the aldehydes.

The positions of the transition state structures were also located from inflections point shown in the Mulliken's charge profile on selected groups in the complex. The Mulliken charges were obtained from the output files of the optimized structures of each atom of the complexes. The change in Mulliken atomic charges of groups around the active site ($C_{acetaldehyde}$, Mo, O_{eq}, $H_{acetaldehyde}$, $(S_{pterin})_{\beta}$, $(S_{pterin})_{\alpha}$, O_{app} and X_{Mo}) as $H_{acetaldehyde}$ migrated from the $C_{acetaldehyde}$ of acetaldehyde (interaction site of acetaldehyde) to the X_{Mo} (X-H) terminals. For active sites with sellenido terminal, the Mulliken charge profile on $(S_{pterin})_{\beta}$, $(S_{pterin})_{\alpha}$, Mo, C_{CRH}, H_{RH}, O_{eq}, O_{app} and Se_{Mo} of the active site has shown inflection at Se-H bond distance of 2.11861 and 1.7171 angstrom in its unprotonated and protonated complexes respectively, which are the approximate positions for the respective transition state structures (**Figure 3.2.**).

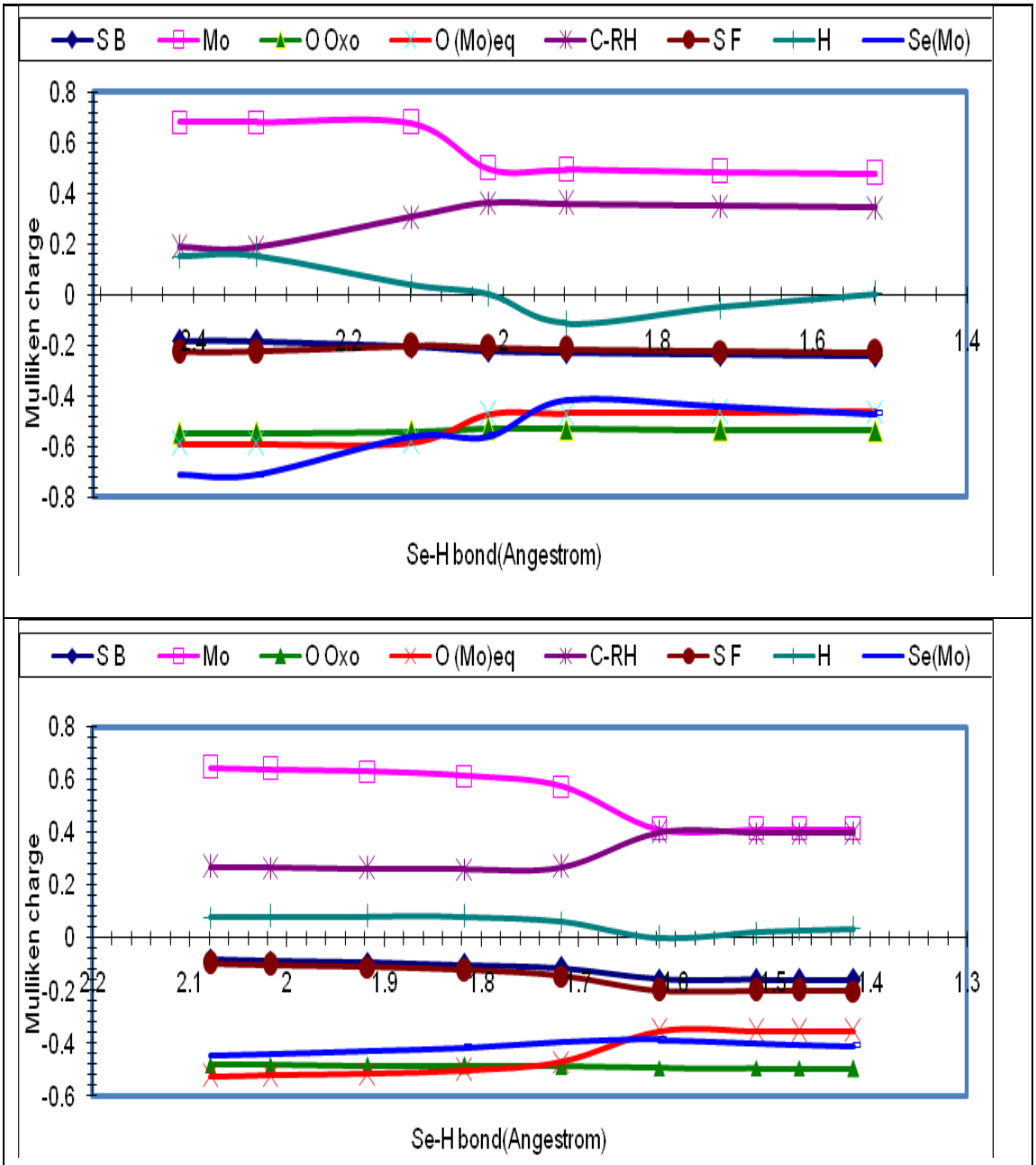


Figure 3.2: Mulliken atomic charge profile obtained from linear transit calculation. It is computed for S B, Mo, Oxo, O_{eq}, $C_{acetaldehyde}$, S F, $H_{acetaldehyde}$ and Se_{Mo} during the migration of $H_{acetaldehyde}$ from the substrate to the Se_{Mo} (Se-H) terminal (A^{O}) in the unprotonated (upper pannel) and protonated (lower panel) substrate-active active site complex. Where; S B, Mo, Oxo, O_{eq}, $C_{RH(acetaldehyde)}$, S F, $H_{acetaldehyde}$ and Se_{Mo} are sulfur back to the terminal, molybdenum, apical oxo, equatorial oxygen, carbon of acetaldehyde, transversal sulfur to the terminal, transferring substrate hydrogen and sellenido terminal respectively.

The increase in Mulliken charge on H was much larger for unprotonated, 0.004363 to 0.149153, than protonated complex, 0.034432 to 0.077708 (the range is from reactant bound to product bound complex). From coulombs law of force between charged objects, the bond energy directly proportional with the developing charge upon the two and is inversely related to the distance between them. Thus we can see greater charge on $H_{acetaldehyde}$ in unprotonated complex than protonated which indicates more favored. The transfer of $H_{acetaldehyde}$ to selenium was proven from the decrease in positive Mulliken charge on molybdenum, while there is increase in positive Mulliken charge on $C_{acetaldehyde}$ of the substrate.

During the initial movement of $H_{acetaldehyde}$ transfer towards the terminals (X), Mo-O_{eq} bond becomes smaller but after the formation of the transition state, it became longer and longer and then broke eventually. The extent of Mo-Oeq bond elongation, in unprotonated sellenido terminated transition state, extends by 7.605% to 1.76197 A^o while protonated transition state complex by 2.636 %, to 2.02802 A^o (Fig 3.3). The protonated complex seem to require significantly lesser relative deformation energy to reach the transition state, and the energy barrier towards/at the transition state was thus lesser for the protonated complex. But when we observe the Mo-Oeq bond elongation after the transition state was formed for protonated complex, its percentage elongation is very small by 2.568%, while that of unprotonated is very large by 7.067%.

The extent of shortening for O_{eq}- $C_{acetaldehyde}$ bond in unprotonated complex was by 17.49 while for that of protonated complex by 6.32%. So that unprotonated complex was faster in formation of product bound complex through breaking ultimately, as shown in Fig. 3.3. The extent of Mo-Sebond elongation in unprotonated substrate–active site at transition state extends by 0.9826 %, to 2.38512 A^o while protonated substrate–active site by 2.613 %, to 2.42649 A^o. The protonated substrate–active site thus requires a significantly larger relative deformation in the overall processes of substrate bound transformation to product bound complex.

According to Hammond Postulate, the transition state for an exothermic reaction occurs early along the reaction pathway so that it resembles the reactants. The bond distances for the transition state of all active site sellenido terminal, could reveal shorter $C_{acetaldehyde}$ - $H_{acetaldehyde}$ bond and longer Se_{Mo}-$H_{acetaldehyde}$ bonds, which are according to Hammond Postulate. This is proposed to indicate a substrate type transition state structure rather than a product type. Thus upon protonation the transition state is being stabilized so that it favors reactant bound complex rather than product bound. Thus protonation is less favored and retards the product formation.

The exact position of the transition state structures with highest energy were further proven by single imaginary negative frequency. This is to distinguish the minimum characterized by all other positive frequencies. According to Arrhenius equation activation energy and frequency factor are directly proportional. The frequencies for the unprotonated and protonated acetaldehyde bound active site, are -721.35 and -1018.10 respectively. The protonated complex has higher frequency than the respective unprotonated complex, so that they have bigger activation energy than the respective unprotonated complexes which indicates they are less favored. Generally protonation by itself actually used to stabilize the transition state but it retards the way of the TS towards product bound complex. It is

because the high electron density on oxo of acetaldehyde can't return back to $C_{acetaldehyde}$, so that it can't let $H_{acetaldehyde}$ move towards Se_{Mo} terminal (i.e. it is going to be more energy consuming).

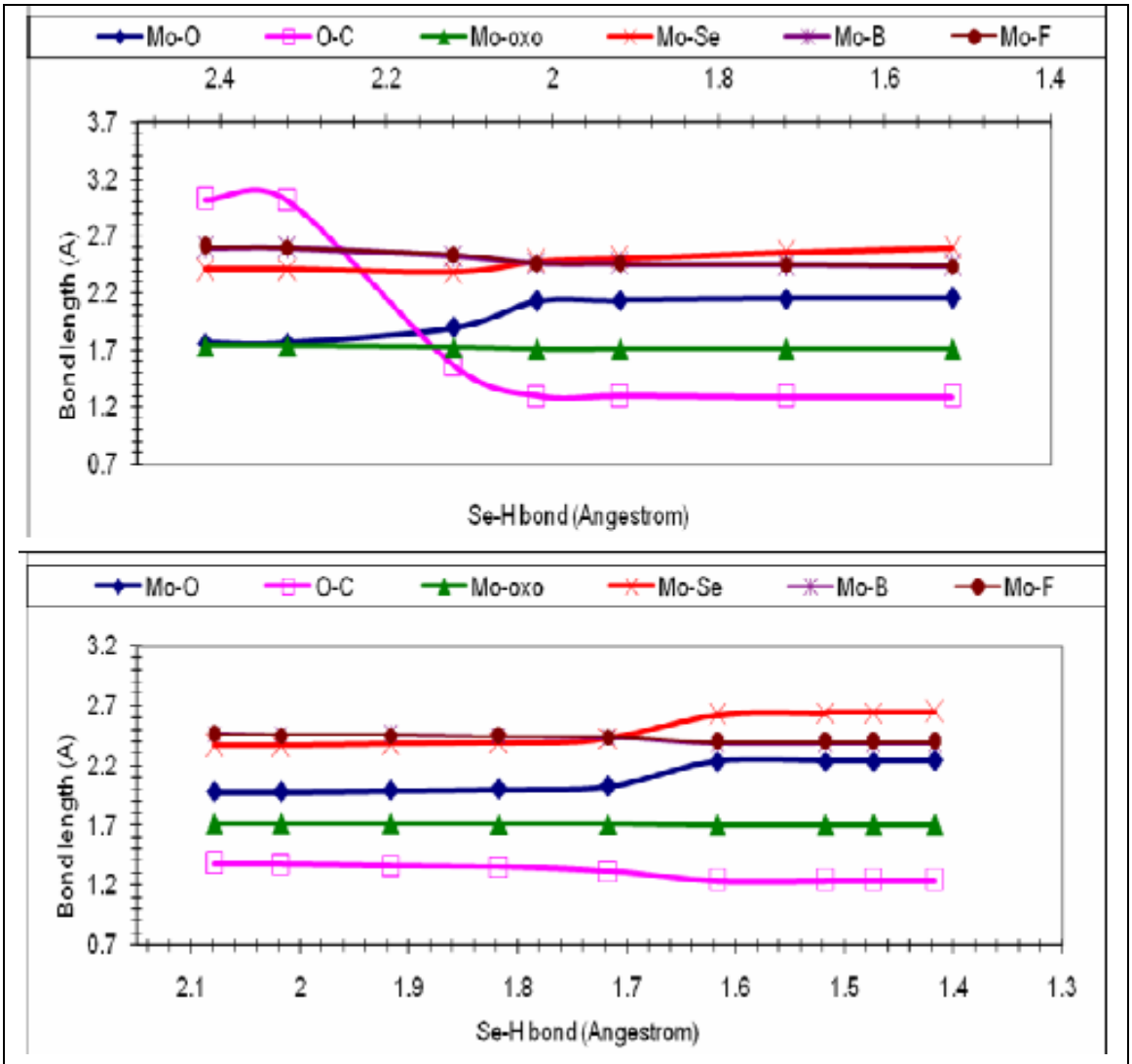


Figure 3.3: Bond length Profile obtained from linear transit calculation of Mo-Oeq, Oeq-C_{RH}, O_{ap}-Mo and Mo-Se_{Mo} bond distance (A^0) verses Se-H bond distance (A^0), in the unprotonated (upper panel) and protonated (lower panel) substrate-active site complex. The bonds associated with these groups are supposed to shown to either weaken or strengthen.

4. CONCLUSION

Protonation of carbonyl oxygen of acetaldehyde in complex of the active sites with sellenido terminal, resulted in stabilization of the transition state. But it retarded the way of dissociation of the transition state structure towards product bound complex. This was confirmed by decreased in electron density

and negative Mulliken charge on $H_{acetaldehyde}$, low extent Mo-O_{eq} bond elongation profile, low extent of O_{eq}-$C_{acetaldehyde}$ bond shortening and higher frequency. The retardation of transition state dissociation was due to high electron density on oxo of acetaldehyde couldn't return back to $C_{acetaldehyde}$, so that it didn't let $H_{acetaldehyde}$ move towards X_{Mo} terminals.

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