MODIFIED-METHANOL DEHYDROGENASE ENZYMATIC CATALYSTS FOR FUEL CELL APPLICATIONS

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Abstract

The role of $Ca^{2+}$and Ba^{2+} ions in the active site of Methanol Dehydrogenase (MDH) enzymes is studied to tailor modified-MDH enzymes with the best performance as catalysts for the fuel (methanol) oxidation reaction. Portions (models) of the MDH active site considering both Ca^{2+} and Ba^{2+} ions are investigated to come up with a model that accurately represents the complete active site of the enzyme. Density Functional Theory (DFT) is used for this study and information about the ground state configurations of active site models is obtained. Calculated structural parameters are compared with corresponding published experimental information, and models representing the Ca^{2+}- and Ba^{2+}-MDH active sites are suggested. The response of Ca^{2+}- and Ba^{2+}- MDH active site models upon the presence of methanol is also investigated using DFT calculations. Geometrical and electronic configurations and total and binding energies of the complexes are reported. Finally, the immobilization of MDH enzyme on functionalized electrodes is investigated using molecular simulations. Molecular Dynamics simulations are carried out to study transport properties such as diffusion coefficients associated to the MDH immobilization process on functionalized electrodes for bio fuel cell applications.

Introduction

Methanol Dehydrogenase (MDH) is a quinoprotein that oxidizes methanol, a comparatively cheap fuel for operation of electronic devices, and other primary alcohols to their corresponding aldehydes. The crystal structure of bacterial MDH from *Methylobacterium extorquens* [1, 2] and from *Methylophilus W3A1* [3-5] has been characterized and it has been determined that the enzyme active center contains a Ca^{2+} ion.

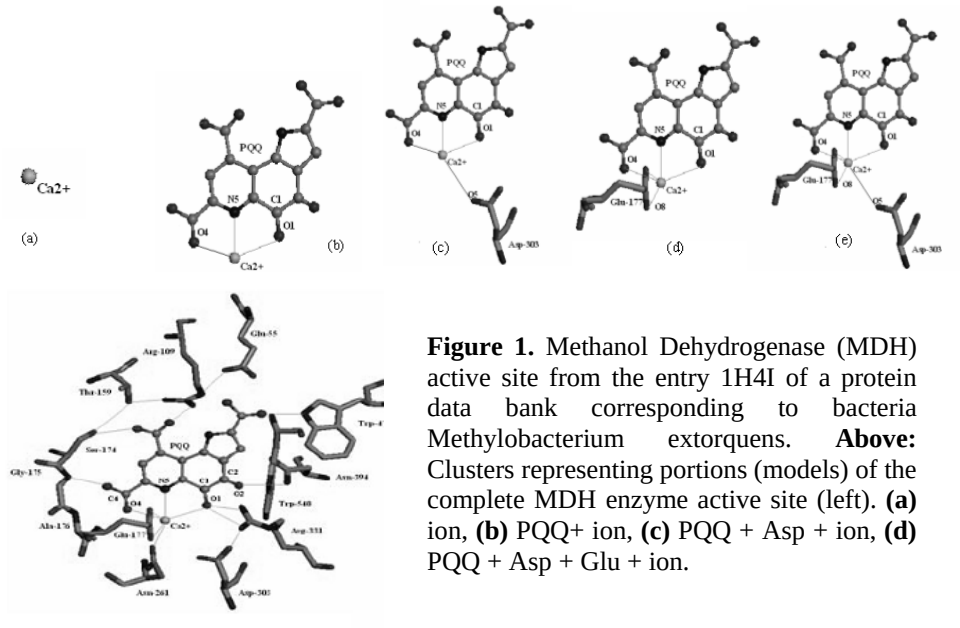

Figure 1. Methanol Dehydrogenase (MDH) active site from the entry 1H4I of a protein data bank corresponding to bacteria Methylobacterium extorquens. **Above:** Clusters representing portions (models) of the complete MDH enzyme active site (left). **(a)** ion, **(b)** PQQ+ ion, **(c)** PQQ + Asp + ion, **(d)** PQQ + Asp + Glu + ion.

The role of Ca^{2+} is not clearly

understood.[6] It has been suggested that apart from holding PQQ in place, Ca^{2+} might have an important role in the methanol oxidation reaction. It was proposed that Ca^{2+} acts as a Lewis acid contributing to the mechanism by its interaction with O1 (Figure 1).[7] Experimental studies have been conducted in order to elucidate the function of Ca^{2+} ion. Some authors [8] have used Ca^{2+}-free MDH enzymes to obtain enzymes containing Sr^{2+} and Ba^{2+} in their active sites. Their experimental results have shown that there are no major differences between these enzymes in the interactions between PQQ and the metal ions in the active site. However, even though the Ba^{2+}-modified enzyme has a relative low affinity for methanol, its activation energy for the oxidation reaction is half that of the normal Ca^{2+}-containing MDH enzyme.[8] This result was not expected since the replacement with Ba^{2+}, a weaker Lewis acid, should decrease the activity of the enzyme, and therefore increase its activation energy.[8] This means that Ba^{2+} has the potential to activate PQQ, but apparently subtle differences in the active site of PQQ-containing enzymes determine the way and the extent to which the activation is expressed. Itoh et. al [9] have used spectroscopic methods and performed semi-empirical molecular orbital calculations to characterize and study the alkaline metal ion binding to the PQQ coenzyme. Their results suggested that the binding of Ca^{2+} to PQQ is much stronger than that of Sr^{2+} and Ba^{2+} and was attributed to the size of Ca^{2+}, which best fit in the binding pocket of PQQ in the enzyme.[9] Coordination of the larger metal ions caused a distortion of the PQQ molecule making the binding of these ions smaller than that of Ca^{2+}.

Methodology

Applied quantum chemical methods [10, 11] such as *Ab Initio* methods [12] and DFT [13, 14] have been successfully used for getting information about the geometric and energetics of atomic and molecular systems, activation energies, reaction paths and mechanisms.[15] DFT incorporates electron correlation (Coulomb correlation),[11] which is neglected in the simplest *Ab Initio* methods such as Hartree-Fock, at a similar computational cost.[11] DFT provides a relatively efficient tool with which to compute the ground state energy in realistic models of clusters and bulk materials. Data collected from DFT calculations can be further combined to get binding and adsorption energies of the complexes, activation energies, potential energy surfaces and transition states.[16-20] In this work, both *Ab initio* and DFT calculations were carried out using the program Gaussian'03.[21] *Ab Initio* calculations were performed at the Hartree Fock (HF) theory level in combination with the Los Alamos National Laboratory basis set (LANL2dz) for the effective core potentials of double-ζ type.[10] DFT calculations were performed using the hybrid Becke 3 Perdew-Wang 91 (B3PW91) method and the 6-311+g** basis set for all atoms except the Ba^{2+} ion for which LANL2dz is used.[10]

Molecular Dynamics (MD) simulations are carried out in order to investigate the immobilization of MDH enzyme on functionalized electrodes. MD simulations are conducted under a weak coupling heat bath system with constant temperatures using the Berendsen thermostat.[22] The relaxation time is selected to be small enough (1 fs) so that thermodynamic equilibrium is reached and the simulation system approaches the canonical ensemble (NVT), at constant number of atoms N, volume V and temperature T, without periodic boundary conditions.[23] The COMPASS forcefield is used in these studies and the simulations are run for 500ps.

Results

MDH Active Site Models

Ground state electronic configurations are obtained for the MDH models represented in Figure 1 (*a-d*). Calculated structural parameters are compared with corresponding published experimental information.

The geometry of all the active site models shown in Figure 1 are in very good agreement with the configurations observed experimentally showing that Ca^{2+} in the active site of MDH is bonded to the C1 quinone oxygen (O1), the oxygen of the C4 carboxylate (O4), and the N5 atom of the pyrrolo-quinoline quinine (PQQ) molecule. Also Ca^{2+}-N5, Ca^{2+}-O1 and Ca^{2+}-O4 bond lengths for the models shown in figure 1 (b), (c), (d) & (e) are in good comparison with experimental values. In Figure 1(e), the distance from Ca^{2+} and O8 of the GLU is 2.50Å and Ca^{2+}-O5 of ASP is 2.38Å. X-ray crystallographic information from Williams et.al. gives a value of 2.38Å for the Ca^{2+}-O8 and from Xia et.al. (at 1.94Å from *Methylophilus W3A1*) the Ca^{2+}-O5 is found to be 3.60Å. The distance between O5 of Asp and O1 of PQQ as shown in figure 1(c) is found to be 3.84Å and from figure 1(e) it is 3.35Å compared to the experimental information from Xia et.al. (4.10Å).

Using crystallographic methods for the Ca^{2+}-containing MDH enzyme, Williams et al. reported the Ca^{2+}-N5, Ca^{2+}-O1, and Ca^{2+}-O4 bond lengths of 2.32, 2.25 Å, and 2.44 Å respectively (Figure 1). Looking at the corresponding bond lengths in the case of Ba^{2+}-containing MDH active site models, we find an increase of 0.3-0.6 Å with respect to Ca^{2+}-containing MDH active site models. The increase in the bond lengths for Ba^{2+}-containing MDH active site models is due to the ionic size of the element, i.e., Ba ionic radius (1.34 Å) is larger than that for Ca (0.99 Å). There is a general prediction that changing the active site components (ions or residues) in an enzyme would cause distortion effects and in some cases loss of activity also. But here, the geometry of the Ba^{2+}-containing MDH active site models are in very good agreement with the configurations observed experimentally for Ca^{2+}-containing MDH active site models, indicating that no significant distortion is introduced by the replacement of Ca^{2+} by Ba^{2+} in the MDH active site. From the above comparisons, we can hypothesize that MDH active site can also be well represented with the presence of Ba^{2+} (Figure 2).

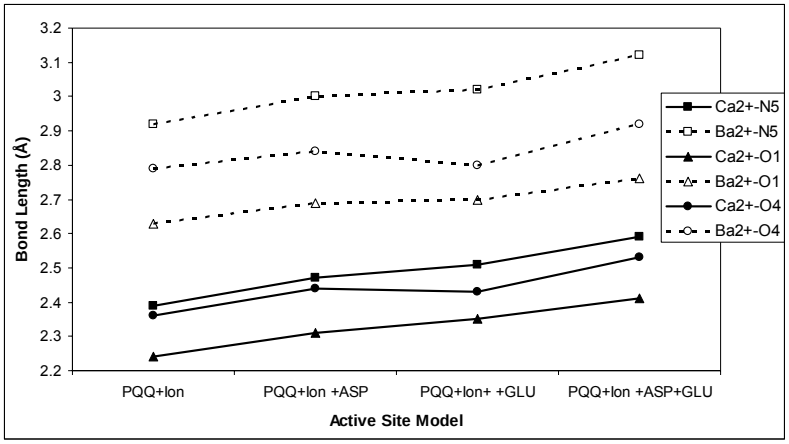

Figure 2. Comparison of bond lengths obtained from DFT (B3PW91/6-311+g** and LANL2dz for Ba^{2+}) calculations for both $Ca^{2+}$and Ba^{2+} MDH active site models.

Binding of Methanol to the Ca^{2+}, Ba^{2+}-containing Active site models

The binding of methanol to various active site models involving both Ca^{2+}, Ba^{2+} are investigated, but of particular importance is the PQQ + ion + methanol case shown in Figure 3. It is observed that the orientation of methanol with respect to the Ion-PQQ complexes is different

depending on the ion under consideration. The distance between O1 of PQQ and H7 of methanol when Ca^{2+} is present is 4.47 Å, but it is 2.45 Å when Ba^{2+} is present in the active site of MDH. This result may be of great importance for the dissociation of methanol during its oxidation by MDH.

B.E (methanol) = E (active site model + methanol) - E (active site model) – E (methanol) (1)

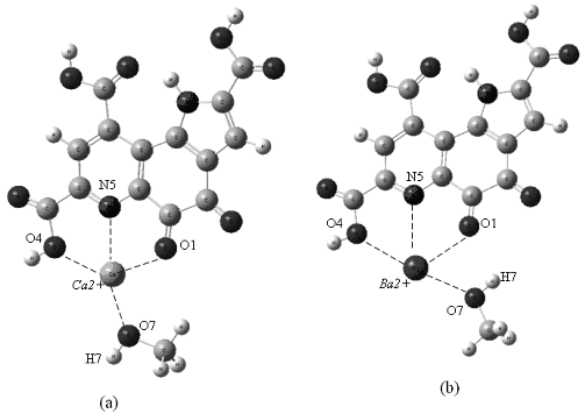

Figure 3. Ground state geometries for (a) PQQ + Ca^{2+} + methanol (b) PQQ + Ba^{2+} + methanol.

The binding energy of methanol (equation 1) to the PQQ + Ion + ASP + GLU active site model is smaller than the other two cases (PQQ + Ion + GLU and PQQ + Ion + ASP), indicating that the oxidation of methanol might be easier as the size of the MDH active site model increases (Figure 4), thus highlighting the importance of the presence of additional amino acids in the complete MDH active site. The model consisting of PQQ, Ca^{2+}, and ASP has the highest binding energy (more negative) compared to the other models. Thus, it seems that the role of ASP is to increase the methanol binding energy to the MDH active site, indicating that methanol oxidation could not be easier in this case.

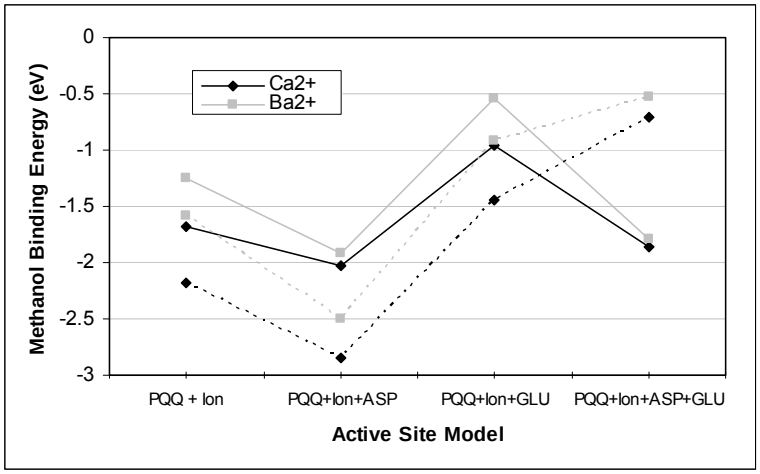

Figure 4. Comparison of binding energies of methanol for the modeled Ca^{2+}-, Ba^{2+}-MDH active sites. Solid (DFT), dotted (HF) calculations

Ca^{2+}-containing Active Site Model: Molecular Dynamics Simulations

The active site of MDH enzyme used to carry out MD simulations consisted of its complete active site surrounded by a number of amino acid chains in order to obtain a more realistic model of the complete MDH enzyme. MD simulations considering this MDH model (Figure 5 left) in the presence of a methanol molecule and including explicit water effects suggest that the nature of the ion modifies the binding and orientation of methanol with respect to the active site of the enzyme, facilitating the

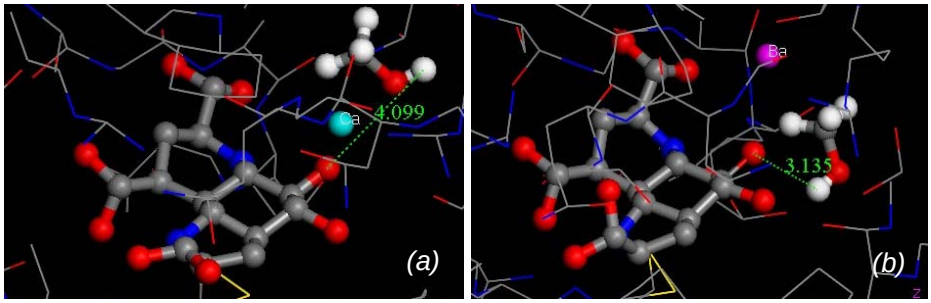

Figure 5. (left) MDH active site model used in MD simulations. **(right)** The Binding of Methanol in **(a)** Ca-MDH and **(b)** Ba-Modified MDH Enzymes. Distances shown in Angstroms.

methanol oxidation reaction as the hydrogen-oxygen distance highlighted in Figure 5 (a and b) decreases. These results are in complete agreement with our DFT calculations where is observed that the orientation of methanol with respect to the Ion-PQQ complexes is different depending on the ion under consideration (Figure 3).

MDH Immobilization

In order to establish appropriate electron transfer from the enzyme active site to the electrode, Tris-(methoxy) carboxyl ethyl silane (TMCES) is used as mediator, which offers electrostatic, H-bond, and hydrophilic interaction with charged amino acid residues of the enzyme molecules. After the MD simulations were complete, the enzyme-TMCES complex was found to be attached to the substrate. Thus, the most favorable MDH model orientation when immobilized on graphite (Figure 6) electrode is found.

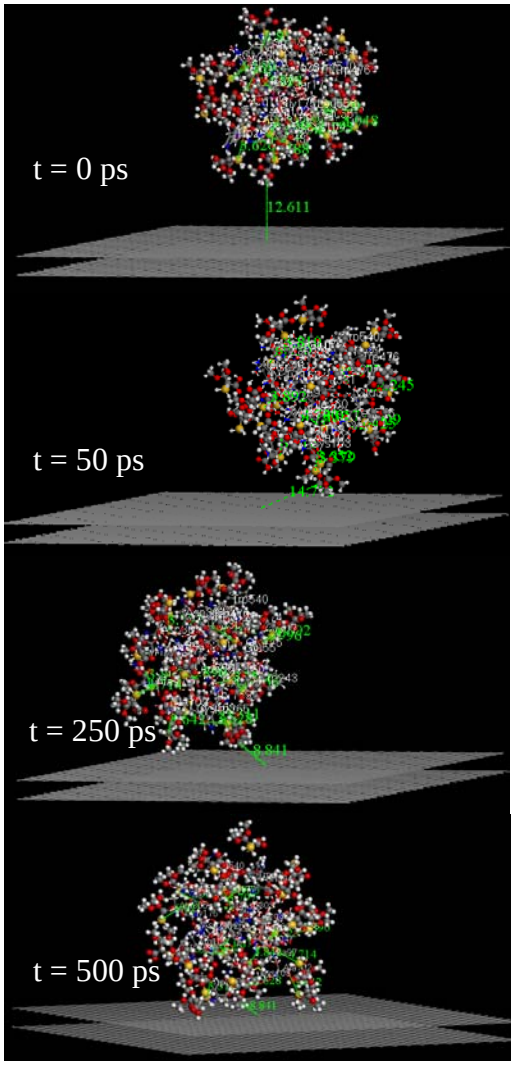

Figure 6: Dynamics of the immobilization of MDH model on graphite substrate at 298K.

Ca^{2+} Diffusion Coefficient Calculation

The ion diffusion coefficient resulting from the immobilization process is calculated according to equation (2). The diffusion coefficient (*D*) is related to the mean square displacement $\prec \Delta x^2(t) \succ$, which is given by Einstein's relation (equation 2) as equal to 2*bDt* where *b* is the dimensionality associated with the diffusion process.

$$D = \ell t_{t\to\infty} \frac{\prec \Delta x^2(t) \succ}{2bt} \qquad (2)$$

The Einstein relation can thus be used to calculate the diffusion coefficient from an equilibrium simulation, by plotting the mean square displacement as a function of time and then attempting to obtain the limiting behavior as $t \to \infty$. So, for sufficiently long trajectory files and taking *b*=3 (3-D motion), the calculated Ca^{2+} diffusion coefficient at 298K is calculated to be $(2.5\pm0.4)10^{-9}$ cm^2/s.

Conclusions

First, Ca^{2+} and Ba^{2+}-containing MDH active site models were investigated in order to obtain an accurate representation of the actual active site using a small portion of it (PQQ, ion, ASP, GLU). Calculated bond lengths were compared to published x-ray crystallographic information to validate our findings. Second, the binding energy of the metal ion (Ca^{2+} or Ba^{2+}) to the rest of the active site model was calculated in order to elucidate how important (weak vs. strong) the interactions within selected active site models are. Finally, the dynamics of a MDH

enzyme model was studied and its immobilization on graphite electrodes was modeled by functionalizing it with TMCES. A transport property such as the Ca^{2+} diffusion coefficient during the immobilization process was calculated to be $(2.5\pm0.4)10^{-9}$ cm^2/s.

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