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Enzyme-substrate interaction based approach for screening electroactive microorganisms for Microbial Fuel Cell applications

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Alcohol dehydrogenase (ADH) of *A. aceti* is one of the potential enzyme for biological fuel cell applications. The modeled structure of ADH of *A. aceti* and its validation has been reported. The docking investigations have been made to assess the catalytic activity of the modeled ADH. The experimental results show that the modeled ADH of *A. aceti* has lowest binding affinity to its substrate when compared with the ADH structures of few other microorganisms. It indicates that the ADH of *A. aceti* has higher catalytic activity and better electrogenic activity. This study documents a computational procedure for screening electroactive microorganisms for biological fuel cell applications.

Keywords: Alcohol dehydrogenase, Enzymes, Ethanol, Homology Modeling

Microbial fuel cells (MFC) are bioelectrochemical devices that can be used for the production of electricity from organic substrates using microorganisms[1-4]. *Acetobacter aceti* is one of the promising microorganisms for Microbial Fuel Cell applications[5]. *A.aceti* is *a* gram-negative acetic acid bacterium and has high potential for the oxidation of ethanol and lactate[6]. They are one of the highly potent model organisms for electrochemical investigations. These bacteria possess alcohol dehydrogenase and methanol dehydrogenase enzymes[7]. The ethanol oxidase system of *A. aceti* consists of ADH, ubiquinone, and cytochrome[8]. ADH acts as the primary dehydrogenase in the respiratory chain of the microorganisms. These bacteria have the excellent ability to perform the electrocatalysis of ethanol to acetic acid by the subsequent activities of alcohol dehydrogenase and aldehyde dehydrogenases which are located on the periplasmic side of the membrane[9]. The use of these bacteria for bioelectricity generation in microbial fuel cells with different substrates is well explored[10-14]. The present work is attempted to model the structure of ADH of *A. aceti* and investigate the catalytic activity based on its binding affinity. Homology modeling is an interesting tool to build the three dimensional model of the protein[15,16]. Modeling of ADH of *A. aceti* aids to identify its structure and understand its biological function.

Electrochemically active bacteria are the key players of MFCs. The use of suitable microorganism will greatly aid in enhancing the power output of MFC. Currently electrochemical techniques such as cyclic voltammetry, amperometry, impedance analysis, polarisation techniques are used to screen the electrochemical activity of the microorganisms[4,9]. These techniques are laborious and time consuming. Hence, an attempt was made to develop a new computational technique for screening electroactive microorganisms for MFC applications. The computational screening techniques offer several advantages over the conventional electrochemical techniques[17-20]. The catalytic activity of the modeled ADH of *A. aceti* is compared with the catalytic activity of ADH structures of *Pseudomonas putida* HK5, *Saccharomyces cerevisiae* and *Schewanella denitrificans* OS-217. The binding affinity of the substrate to the enzyme can be used as a tool for screening electroactive bacteria for MFC applications.

Experimental Section

Homology Modeling

The amino acid sequence of ADH from *A. aceti* was retrieved from Swissprot protein sequence database for this experiment (accession Number: Q335W6)[21]. The retrieved ADH from *A. aceti* consists of 162 amino acids. The template protein sequence closely related to the target protein sequence was identified and used as template for the modeling experiment. The PSI-BLAST search was used to identify the homologous protein from the Brookhaven Protein Data Bank (PDB)[22,23]. The target protein sequence was searched in PSI-BLAST. The best template for ADH protein from *A. aceti* was identified based on the e-value and sequence identity. The Clustal W was used to align the template and the target sequences.[24] Further, the target ADH of *A. aceti* and its template was aligned using Clustal W. Homology modeling was carried out for the ADH

protein with the particular template using Modeller 9.11. The newly built structures were ranked based on the internal scoring function. The newly constructed model with the least internal scores were identified and utilized for model validation.

Validation of the modeled structure

The overall stereochemical quality of the newly modeled ADH was analyzed after building the 3D structure of the protein. The PROCHECK program was used to analyze the Ramachandran plot[25,26]. Further, the modelled 3D structure was validated using the ERRAT[27]. The ProSA was utilized to compare the stereochemical quality of the template and the newly modeled protein. The root mean square deviation (RMSD) was obtained by superimposing the modeled protein with the template structure using Chimera. The active site of the modeled protein was identified using q site finder.

Docking experiment

Docking studies were performed with the modeled protein structure and ethyl alcohol as the substrate to find the binding energy. The ethyl alcohol was chosen as the ligand and its structure was drawn with the Chem sketch. The ethyl alcohol and modeled ADH protein from *A. aceti* were processed by adding hydrogen atom with the MGL tools. The ethyl alcohol ligands was minimized by computing gasteiger charges before storing in the pdbqt format for the docking studies with the Autodock vina 4.2. The active binding region for docking was found. The entire ligand binding region of the protein was covered within the GRID. The Autodock tools were used to select the active region based on the amino acid sequence obtained with the q site finder. The dimensions of the Grid are 40 along all the three directions. The AutoDockVina was used for improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading[28]. Docking study was performed with the Autodock Vina with AMBER force field and Monte Carlo simulated annealing. The newly modeled protein was kept as rigid and the ligand molecules are kept flexible throughout the docking process. The binding studies are made with ethyl alcohol as substrate and alcohol dehydrogenase structure of *Pseudomonas putida*, *Saccharomyces cerevisiae* and *Schewanella denitrificans* OS-217. The ADH enzyme structures of *Pseudomonas putida* HK5, *Saccharomyces cerevisiae* and *Schewanella denitrificans* OS-217 were obtained from the PDB database from the ids 1KV9, 1PIW and 3rf7 respectively.

Results and Discussion

Structure of a Quinohemoprotein ADH from *Pseudomonas putida* HK5 at 1.9A resolution was selected as template for modeling new ADH structure of *A. aceti*. The selected templates had the maximum similarity and e-value of 46% and 2e-21 respectively.

The ADH of *A. aceti* and the *P. putida* template sequences were aligned with the Clustal W. The result of alignment was shown in the supplementary information. Modeling of the ADH of *A. aceti* with the Modeler 9.11 yielded five new models. The dope scores of five models of ADH of *A. aceti* were shown in the Table 1.

The third model had the minimum dope score of -7177.48633 and is considered as the best model for the ADH of *A. aceti*.

Figure 1 represents the newly modeled structure of ADH, its superimposition with the template structure, Ramachandran plot, and its active site.

Homology modeling resulted in a new high quality structural model for ADH of *A. aceti* and the model with the least dope score is shown in Fig. 1-A. The modeled structure was subjected to validations to confirm the good quality of the model. Further, the newly constructed three dimensional model of protein subunit of the ADH protein of *A. aceti* was validated by superimposing it with the template structure and by calculating the RMSD value. Superimposition resulted in a very low RMSD value of 0.278 indicating the good quality of modeled structure. The superimposed template and target structure is shown in the Fig. 1-B. Finally, the good quality of the modeled 3D structure of ADH is confirmed by the using PROCHECK by analyzing the Ramachandran plot. The percentages of phi and psi angles that occur in the allowed and disallowed regions were found for each of the predicted model. Ramachandran plots of modeled 3D structure of ADH protein of *A. aceti* are

Table 1 — Dope score of experiment models.

S.No.	Best model	DOPE score
1	TvLDH.B99990001.pdb	-6990.16113
2	TvLDH.B99990002.pdb	-6976.76270
3	TvLDH.B99990003.pdb	-7177.48633
4	TvLDH.B99990004.pdb	-7096.23389
5	TvLDH.B99990005.pdb	-6902.71631

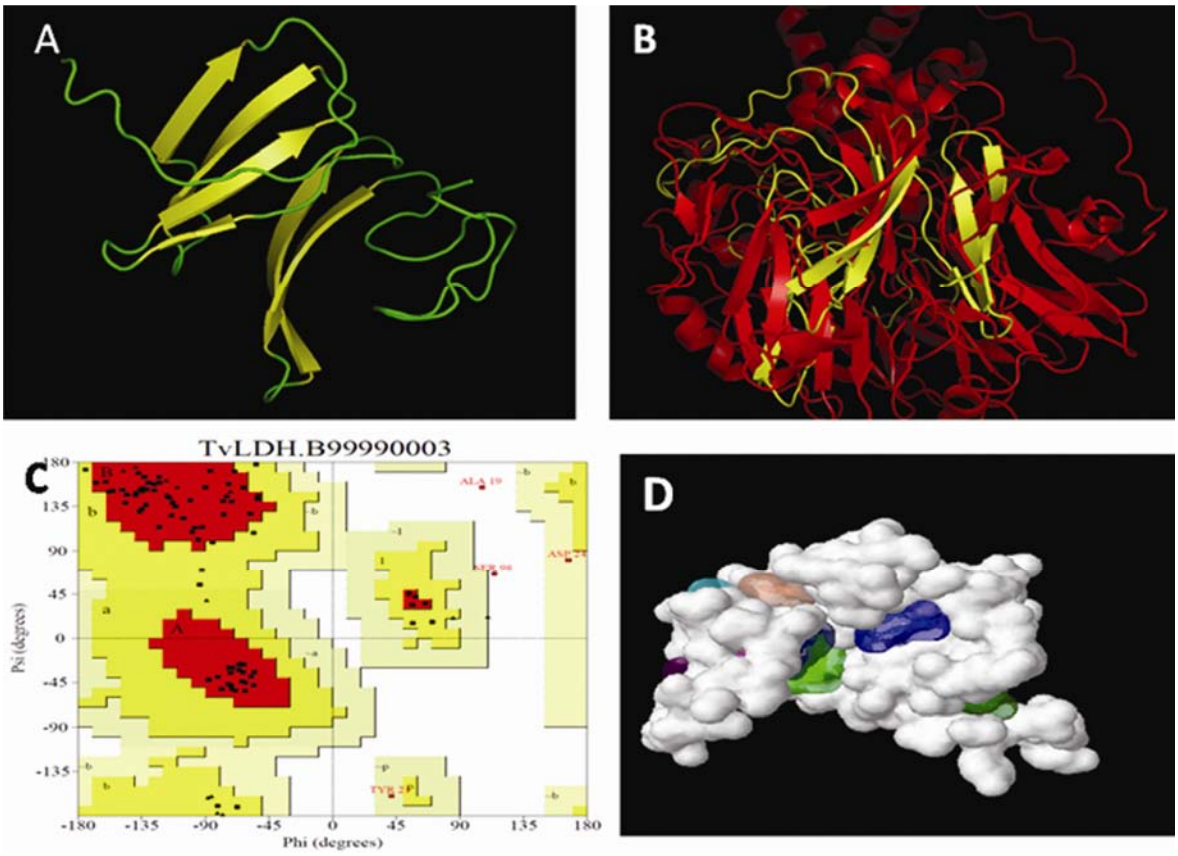

Fig. 1 — (A) Modeled structure of ADH of *A. aceti;* (B) Superimposition of target and template; (C) Ramachandran plot for the modeled ADH enzyme; (D) Active sites for the modeled ADH

shown in Fig. 1-C. The active sites for the newly modeled ADH of *A. aceti* found using the q site finder is shown in the Fig. 1-D.

The stereochemistry of the newly modeled protein structure is analyzed from the Ramachandran plots. The Ramachandran plot statistics is shown in the Table 2.

The 82% of the residues are observed in the most favoured region and 2.5% of the residues are noticed in the disallowed region. The Ramachandran plot statistics confirms the higher strength of the newly constructed ADH model of *A. aceti***.** Interactions of the newly modeled protein with ethyl alcohol are shown in the Fig. 2.

Docking of the newly modeled protein with ethyl alcohol results in binding affinity of -2.6 kcal/mol. The low free energy indicates the more viability of the substrate to form the product. The higher the binding affinity between the substrate and the enzyme, better is the catalysis. The binding affinity indicates the amount of energy that is required for the conversion of the substrate to product. The binding affinity of

Table 2 — Ramachandran statistics of modeled alcohol dehydrogenase.

S.No.	Ramachandran statistics	Number of residues (%)
1	Residues in most favoured region	82.7
2	Residues in additional allowed Region	12.3
3	Residues in generously allowed region	2.5
4	Residues in disallowed region	2.5

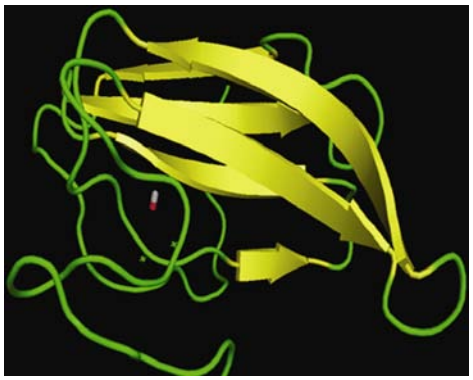

Fig. 2 — Binding interactions of ethyl alcohol to ADH of *Acetobacter aceti*

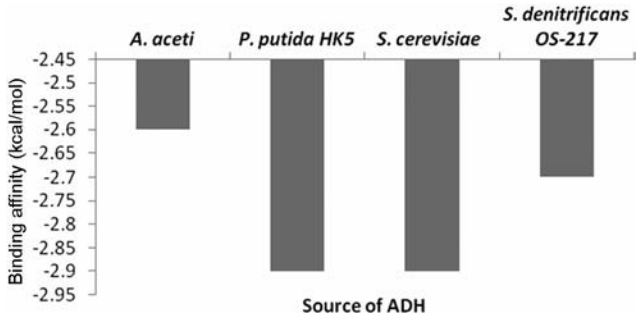

Fig. 3 — Binding affinities of ethyl alcohol (ligand) to ADH

ethyl alcohol ligand *with* the ADH *of Pseudomonas putida* HK5, *Saccharomyces cerevisiae* and *Schewanella denitrificans* OS-217 are shown in the Fig. 3.

The higher binding affinities of ethyl alcohol to the ADH of *Pseudomonas putida* HK5 and *Saccharomyces cerevisiae* clearly indicates need of higher energy to form the product. This confirms the good catalytic activity of the modeled ADH of *A. aceti to* ethyl alcohol when compared with the ADH of *Pseudomonas putida* HK5, *Saccharomyces cerevisiae* and *Schewanella denitrificans* OS-217.

The chart representing the binding affinities of ethyl alcohol to ADH of *Acetobacter aceti, Pseudomonas putida* HK5, *Saccharomyces cerevisiae* and *Schewanella denitrificans* OS-217 is shown in Fig. 3.

Conclusion

ADH plays a key role in the catalytic activity in oxidation reactions in microbial fuel cells. A new model proposed in this research paper for ADH enzyme from *A. aceti* and validated with different techniques. The resulted low binding energy in the experiment confirms its excellent catalytic activity compared with the same enzyme from other bacteria used in this study. Thus the experiment confirms that the binding affinity can be used as a tool to screen the enzymes for biological fuel cell applications. This will pave a new path for exploring the features of the enzymes including specificity and enzyme kinetics. Further, investigations shall be made to analyze the interactions of these enzymes with different substrates and their stereochemistry.

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