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In Silico Optimization of Formate Formation by *Acetobacteriumium woodii's* formate Dehydrogenase

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Abstract

Formate dehydrogenase from *Acetobacteriumium woodii* is under investigation for industrial conversion of CO2 to formate. To have an economically viable biotechnological process for production of formate from CO_2 the conversion rate needs to be increased substantially. Here, we used structure prediction and molecular modelingfor predicting a reliable 3D structure of *A. woodii* FDH and following docking experiments we identified two critical residues as main formate binding sites in the enzyme. Our result will help to increase the efficacy of CO_2 fixation as a biotechnological approach to reduce the atmospheric concentration of this greenhouse gas as well as biologically producing formate as a safe way of H2 transport in biofuel application.

Keywords: Formate; *Acetobacteriumium woodii;* Formate dehydrogenase; Docking

Introduction

In September 2016 the concentration of atmospheric CO_2 has reached to 400 ppm (NASA). The increased in CO_2, alongside other greenhouse gases has profound effects in climate all over the world and threatens a sustainable life on the planet in long run. The reduction of atmospheric CO_2 is an interesting challenge for chemists as well as biochemists [1,2]. The biotechnological conversion of CO_2 to organic acids via enzymatic processes other than photosynthesis is an attractive alternative. One of these enzymatic solutions is conversion of CO_2 to formate catalyzed by the action of formate dehydrogenase (FDH; E.C.:1.2.1.2).

Although most known FDHs predominantly convert formate to CO_2, in acetogenic bacteria the tendency is toward reduction of CO_2 to formate [3]. The eminence of developing new biocatalysts with favorable kinetics towards formate production has motivated research in this area (Hartmann). The FDH of *Acetobacteriumiumwoodii* is one of the few characterized enzymes for this purpose. A. woodii is now being considered as a key player in biotechnological conversion of CO_2 and H2 to formate for safe transport of hydrogen gas [4]. Unfortunately, the crystal structure of this enzyme is not publicly available. Therefore,any attempt for improving the kinetics of this enzyme demands the prediction of 3 dimensional structureof the enzyme based on homology with other FDH's. Here, we sought to increase formate production by *A. woodii's* FDH by *in silico* modelling, molecular docking and simulated site-directed mutagenesis to enhance formate release from the active site.

Methodology

The ligand structures were obtained from Pubchem database (molybdopterin: CID_45266731 and formate: CID_283) and the sequence of formate dehydrogenase from *Acetobacteriumiumwoodii* was obtained from NCB database (accession # WP_041668236.1).The 3D structure of protein was predicted by a Meta prediction approach. To do this, we have used phyre2, a fold recognition algorithm to predict the model based on the identical folds from known homologues structures [5]. In addition, we have used I-tasser, an *ab inotio* based prediction web server [6]. The obtained structures were used as the template for final model prediction by Modeller V 9.12 software [7]. The predicted structure was verified by Prosa Z score [8] and solved in a water box including neutralizing ions using GROMACS 5.0 software [9]. The solved structure was used as the template for molybdopterin interaction prediction site by docking method using mole gro virtual docker software [10] by MolDock as scoring function. After docking energy minimization performed and hydrogen bonds optimized to reach highly possible accurate binding model.

Results and Discussion

Our approach for enhancing formate formation is based on decreasing the binding affinity of formatein the active site of the formate dehydrogenase enzyme, the catalytic equilibrium would be redirected to formate release and hence providing space for new CO_2 and H_2 substrate molecules. The critical step was to predict a reliable 3D structure. The predicted structure reached the score of -9.12 in Prosa Z scoring system which means that the predicted model has the X-ray quality. The quality of predicted model form described Meta prediction method is depicted in (Figure 1) (Table 1). Formation from CO_2 and H_2, so it would be a better candidate for being used in the formic acid production industry. For gaining this purpose, we attempt to reach a high quality theoretical model.

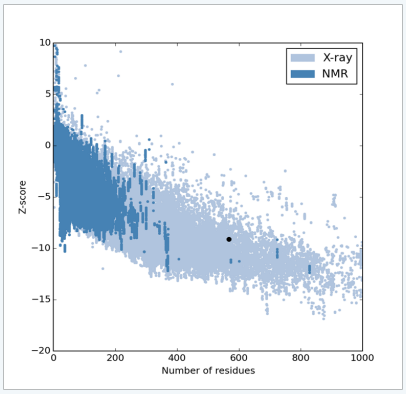

Figure 1: The prosa Z score of -9.12 indicates that the predicted model of formate dehydrogenase from Acetobacteriumiumwoodii has the X-ray quality.

Table 1: The binding affinity of formate to the native model.

Ligand	Molecule	Residue ID	Total	E Pair
Formate	Ala	398	-2.50444	-2.50444
Formate	Ala	404	-2.96195	-2.96195
Formate	Arg	403	-1.93383	-1.93383
Formate	Asp	401	-1.41473	-1.41473
Formate	Gly	402	-1.77861	-1.77861
Formate	His	392	-0.486014	-0.486014
Formate	His	399	-6.15139	-6.15139
Formate	Lys	396	-0.655467	-0.655467
Formate	Phe	397	-5.44195	-5.44195

The primary structure comparison in the conserved Molybdopterin-Binding (MopB) domain of the MopB superfamily of proteins, a large, diverse, heterogeneous super family of enzymes that, in general, bind molybdopterin as a cofactor is present in (Figure 2) (Table 2). This domain is the first 413 N-terminal residues of the enzyme. To investigate which amino acids are playing key role in ligand binding, we have docked molybdopterin to the Mop B domain of the enzyme. Molybdopterin is present in the catalytic site of enzyme and facilitate the electron transfer. The interaction of top 20 hits with Molybdopterin-Binding domain was investigated and observed that most of the hits were located in one position of the Mop B, so with the most probability this position is the most possible site for ligand binding. The ligand map and the location of binding position is depicted in (Figure 3) (Table 3). Also the structure of formate was docked in the predicted active site position in the presence of Molybdoptein. The ligand map and the position of formate in the active site is depicted in (Figure 4.) Based on the ligand map, it can be understood that residues F397 and H399 are key amino acids in formate binding while they are not directly engaged in the binding with molybdopterin. So, performing substitution in these positions are favorable for decreasing the binding affinity of formate to the active site. For gaining this purpose, we have designed several FDH mutant models by replacing F397 and H399 with different type residues and similar in steric condense.

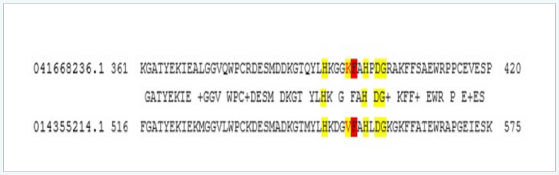

Figure 2: Sequence alignments at Mo-Binding site of FDH.

Table 2: The ligand binding energy decreased 3 units by replacing Phe 397 with Ser. The binding affinities are in MolDock scale.

Ligand	Molecule	ResidueID	Total	EPair
Formate	Ala	398	-2.50444	-2.50444
Formate	Ala	404	-2.96196	-2.96196
Formate	Arg	403	-1.93383	-1.93383
Formate	Asp	401	-1.41473	-1.41473
Formate	Gly	402	-1.77861	-1.77861
Formate	His	392	-0.82733	-0.82733
Formate	His	399	-7.60252	-7.60252
Formate	Lys	396	-0.655467	-0.655467
Formate	Ser	397	-2.49989	-2.49989

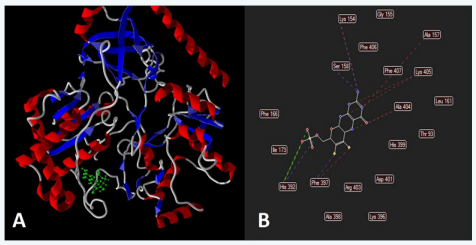

Figure 3A: the binding lication of molybdopterin in MopB domain of formate dehydrogenase from Acetobacteriumiumwoodii.

Figure 3B: The ligand map of molybdopterin indicates the key binding residues.

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Table 3: The ligand binding energy decreased 6.1 unit by replacing His 399 with Met. The binding affinities are in MolDock scale.

Ligand	Molecule	ResidueID	Total	EPair
Formate	Ala	398	-2.50444	-2.50444
Formate	Ala	404	-2.96195	-2.96195
Formate	Arg	403	-1.93383	-1.93383
Formate	Asp	401	-1.41473	-1.41473
Formate	Gly	402	-1.77861	-1.77861
Formate	His	392	-0.486014	-0.486014
Formate	Lys	396	-0.655467	-0.655467
Formate	Met	399	18.6045	18.6045
Formate	Phe	397	-5.44195	-5.44195

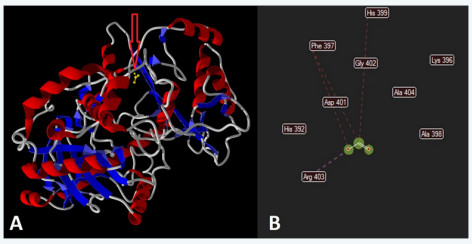

Figure 4A: the binding position of Formate in the active site of formate dehydrogenase from Acetobacteriumiumwoodii.

Figure 4B: The ligand map of formate in contact with the native model.

We have selected residues with similar steric condense to apply the minimum possible change in the conformation of protein and avoid alteration in the secondary structure pattern of the substitution location. Phe397 made hydrophobic interaction with the carbon atom of formate. For debilitation of Phe 397-C1 binding, we have replaced Phe 397 with Serine and the results of docking indicated that the binding affinity decreased significantly (3 units in Mol Dock scale). Also His 399 made a steric interaction with C1 from formate. We have replaced it with Methionine and found that the binding affinity decreased 6.1 unit in molDock scale.

Docking simulation of formate revealed new critical residues as targets for protein engineering experiments. Previous attempts for improving FDHs efficacy were concentrated on NAD binding residues [11], or cofactor regeneration for making a bifunctional enzyme [12]. Also the catalytic efficiency of formate dehydrogenase from *Candida boidinii* was increased in a study before [13]. Moreover, by site saturation mutagenesis the catalytic activity of *Candida methylica* formate Dehydrogenase was increased [14]. Therefore, our approach opens new area for protein engineering of FDH with aim of increasing the formate formation capacity of A. woodii's FDH.

Conclusion

Macromolecular simulation of formate Dehydrogenase from *Acetobacteriumiumwoodii* indicated that Phe 397 and His 399 are key amino acids which bind to the catalytic product formate. Replacing Phe 397 with Ser and His 399 by Met theoretically will decrease the avidity of formate and makes better condition for its release from the active site.

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