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RESEARCH ARTICLE

Formate Dehydrogenase, Molecular Modeling and Docking with NAD^+ Interpreting the Interaction with Active Site

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Abstract

Cofactor regeneration is the hot research topic in recent studies. It has been developed by using various methods. The enzymatic reaction is the common procedure used in industrial cofactor regeneration. Formate dehydrogenase (FDH) is an enzyme that catalyses the oxidation of formate into carbon dioxide (CO_2). It is NAD^+ dependant enzyme and is responsible for regeneration of NADH from NAD^+. The present study investigates about the regeneration of NADH from NAD^+ by formate dehydrogenase, its action of the enzyme through homology and molecular docking, exploring the active site interaction. We have investigated formate dehydroenase from *Ogataea parapolymorpha* DL-1. The protein sequence of FDH was processed for homology modeling using Swiss model. 3D structure revealed was docked with NAD^+ using AutoDock Vina software. The results of presented homology modeling and docking studies revealed that the conserved residues of FDH interact with NAD^+ were Pro 68, Arg 258, Asn 119, Asn 228 and His 97. The cofactor NAD^+ has good interaction with FDH showing grid score of -60.23, which is the appropriate score for binding.

Keywords: Homology modeling, AutoDock Vina, formate dehydrogenase, docking analysis.

Introduction

Exploit of biocatalytic processes become widely used procedure in food and pharmaceutical production (Koeller and Wong, 2001; Schmid *et al.,* 2001; Schoemaker *et al.,* 2003). Cofactors such as NAD^+ and NADH are compounds that are important for several enzymatic reactions. In recent years, researchers developed cofactor regeneration using many methods. Enzymatic reactions usually are the best choice for cofactor regeneration (Chenault *et al.,* 1988; Chenault and Whitesides, 1987). It is more economical, practical, controllable and scalable. Formate dehydrogenase (EC 1.2.1.2, FDH) is an important enzyme for industrial NADH regeneration from NAD^+. It catalyses the oxidation of formate into CO_2. Most of the oxidoreductases require expensive cofactors such as NAD(H) or NADP(H) for their catalytic activity. FDH is widely used due to its irreversible reaction and wide range of pH (Tishkov *et al.,* 2006; Liu and Wang, 2007; Schrittwieser *et al.,* 2011; Wu *et al.,* 2013). It also has been used in production of other chemicals such as L-lactic acid and aromatic alpha-keto esters (Jayabalan *et al.,* 2012; Kratzer *et al.,* 2008). FDH is widely presented in methanol-assimilating bacteria and yeasts. It was produced from *Pseudomonas* sp. 101 (PseFDH), which was thoroughly studied and the crystal structure was resolved, in addition kinetic studies, thermal stability and protein modification were studied (Karzanov *et al*., 1989; Demchenko *et al*., 1990; Tishkov *et al*., 1991; Tishkov *et al*., 1993a,b; Lamzin *et al*., 1993; Filippova *et al*., 2006).

Formate dehydrogenase also was produced from *Candida boidinii* (CboFDH), it was studied through structural determination, molecular modification, active site studies, immobilization and its application (Bommarius *et al*., 1995; Sakai *et al*., 1997; Slusarczyk *et al*., 2000; Labrou and Rigden, 2001; Ansorge-Schumacher *et al*., 2006; Schirwitz *et al*., 2007). In addition, FDH was produced from *Pseudomonas oxalaticus* (Muller *et al*., 1978), *Mycobacterium vaccae* N10 (Galkin *et al*., 1995), *Maollaxella* sp. C1 (Asano *et al*., 1988) and *Bacillus* sp. F1 (Ding *et al*., 2011). In a recent report, a new formate dehydrogenase from a methanol-assimilating yeast *Ogataea parapolymorpha* DL-1 was cloned and characterized by our group and exploited in regeneration of NADH from NAD^+ (Yu *et al*., 2013).

Three dimensional structure of protein determination is an interesting technique in the biological field to understand the function and mechanism of the protein or enzyme. Homology modeling of protein is an accurate method to generate trusted three dimensional (3D) protein structure models and has been recently used in many practical applications. It is also known as comparative modeling of protein, which is designed to construct the amino acid in form of protein structure. It has been emerged over recent years as the most precise method comparing to other methods (Chothia and Arthur, 1986; Koehl and Levitt, 1999).

Homology modeling quality is usually dependent on the template structure sequence alignments identity. However, the structure constructed using homology modeling is similar to spectroscopy or X-ray crystallography and nuclear magnetic resonance (NMR) (Sanchez and Sali, 1997). Homology modeling can be use in molecular docking protein–protein interaction prediction and protein-protein docking (Gopal *et al*., 2001). In this study, we presented homology and structure modeling of FDH from a methanol-assimilating yeast *Ogataea parapolymorpha* DL-1, which was used in regeneration of NADH from NAD^+ and docking of NAD^+ into active site of FDH enzyme to understand the enzyme-substrate interaction by using various homologies, docking and verification software. In addition, genome comparison was conducted.

Materials and methods

Sequence similarity: Sequence of FDH from *Ogataea parapolymorpha* DL-1 was retrieved from National Center for Biotechnology Information (NCBI) database. The sequence was compared with similar formate dehydrogenase enzymes from other sources using NCBI tool. In addition, the sequences were submitted to ClustalW2 web services for sequence alignments(http://www.ebi.ac.uk/Tools/clustalw2/index.html). The best template protein structure was selected by submitting the FDH sequence into Swiss model services, where, the high identity structure to FDH from *O. parapolymorpha* DL-1 was selected as template.

Protein homology modeling and verification: FDH from *O. parapolymorpha* DL-1 3D structure homology model was generated by exploiting the information revealed from the template alignment by using Swiss model. Swiss model is fully automated homology-modeling server. The quality of the 3D model structure constructed by Swiss model was examined by using verification web services and software. To predict the protein fold, PROCHEK analysis were used to calculate the favoured regions, additional allowed regions, generously allowed regions and disallowed regions. Further, the structure was examined through Verify 3d to determine the compatibility of an atomic (3D) model with its own amino acid sequence by assigning a structural class based on its location and environment and comparing the results to structures with high resolution structures. In addition, the structure was verified using Errat, which the error values was calculated based on the statistics of non-bonded atom-atom interactions in the structure and comparing to a database of trusted high-resolution structures.

Docking analysis: To conduct docking studies, the 3D model structure constructed using Swiss model was submitted to AutoDock Vina software. It is molecular modeling simulation software, which is especially effective for Protein-ligand docking.

To understand and observed the molecular at an atomic structure, the outputs of the AutoDock Vina were processed by using Chamira and Accelary Studio software.

Results and discussion

Formate dehydrogenase is widely used in regeneration of NADH from NAD^+ due to its wide range of pH and its irreversible reaction function, therefore be coupled with any other dehydrogenase. In addition, FDH can provide 100% of product in ambient condition. FDH usually use in enzymatic reaction with other enzymes that make use of the generated NADH. In a previous study by our group, we characterized a new FDH from methanol-assimilating yeast *O. parapolymorpha* DL-1, which it had relatively high optimum temperature at 65°C suggesting that this enzyme had promising thermal stability. The enzyme showed wide pH optimum of catalytic activity from pH 6.0 to 7.0 (Yu *et al*., 2013). FDH from *O. parapolymorpha* DL-1 has not been crystallized. To model FDH from *O. parapolymorpha* DL-1, the amino acid sequence was submitted to Swiss model, the result revealed that the crystal structure of FDH from *Candida boidinii* PDB ID 2J6I was the appropriate template according to the sequence identity result. Schirwitz *et al*. (2007) crystallized the FDH from *Candida boidinii* and deposited the derived atomic models in the PDB with accession codes 2J6I. As shown in Fig. 1, the similarity of sequences between FDH from *O. parapolymorpha* DL-1 and template FDH from *Candida boidinii* 2J6I was 83.38%. The quality and accuracy of the 3D structure generated by homology modeling is extremely dependent on the sequence identity between target and template protein. However, identity of sequences more than 50% revealed that models have high reliable with overall root-mean-square deviation RMSD around 1 Å and few error in side chain (Baker and Sali, 2011). The comparison between FDH from *O. parapolymorpha* DL-1 and template FDH from *Candida boidinii* 2J6I sequences revealed high identity above 83% indicating expected high reliable structure.

Fig. 1. Alignment of amino acid sequences of FDH from *O. parapolymorpha* and template FDH from *Candida boidinii* PDB ID 2J6I through Swiss model.

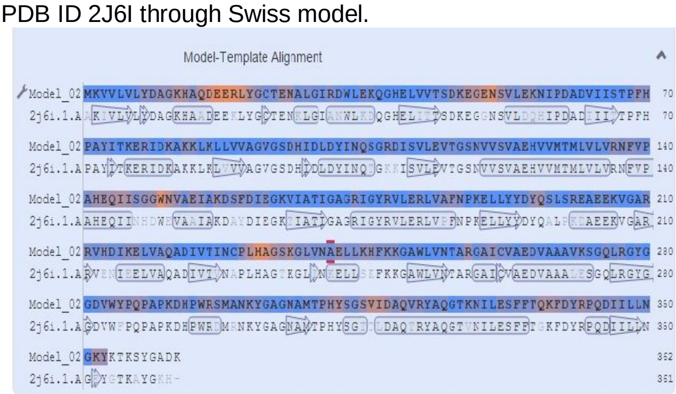
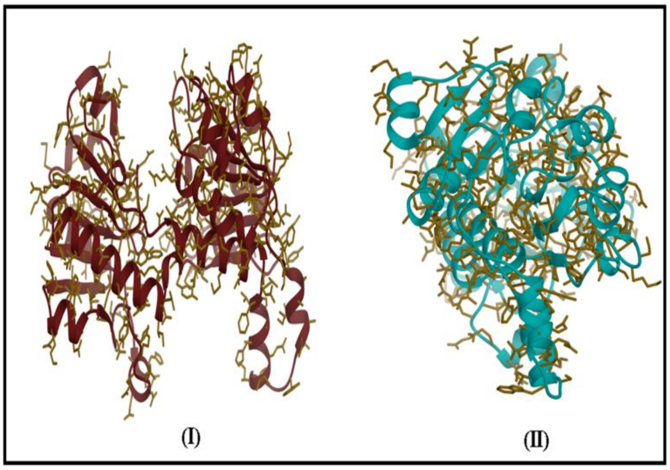
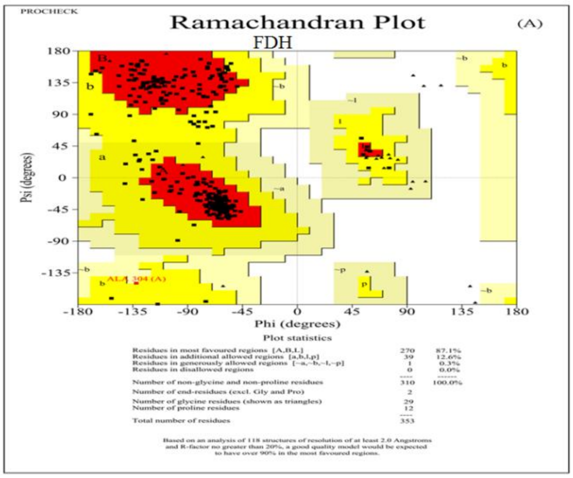


Fig. 2. Complete model structure of FDH from *Ogataea parapolymorpha* DL-1. (I) Dimer FDH (II) Structure side view.



As in a previous study for characterization of *Ogataea parapolymorpha* DL-1 FDH, the molecular mass of the purified enzyme was 40 kDa in each monomer and the result of native molecular mass was 78 kDa suggesting that the enzyme functions as dimer (Yu *et al*., 2014). The results of molecular mass of FDH was taken in consideration when the structure modeled by using Swiss. As shown in Fig. 2, the Formate dehydrogenase from *O. parapolymorpha* DL-1 was modeled into dimer structure. As shown in Fig. 3A, the Ramachandran plot for FDH suggested 87.1%, 12.6%, 0.3% and 0.0% for residues in most favoured regions, additional allowed regions, generously allowed regions and disallowed regions, respectively. The combine favoured and allowed categories with high percentage for model structure prospective to appear a good protein fold. The combined favoured and allowed categories for FDH were 99.7%. Thus, it was high quality structure in term of protein fold as expected. As shown in Fig. 3B, Errat was used to calculate error values based on the statistics of non-bonded atom-atom interactions in the structure (compared to a database of reliable high-resolution structures). The Errat overall quality for FDH from *O. parapolymorpha* was 95.942%. Verify 3d was used to determine the compatibility of an atomic model (3D) with its own amino acid sequence by assigning a structural class based on its location and environment (alpha, beta, loop, polar, non-polar etc.) and compared the results with high reliable 3D structures. By validation of FDH from *O. parapolymorpha* DL-1 structure through Verify 3d, 92.35% of the residues had an average 3D-1D score >= 0.2 for formate dehydrogenase. As reported by Takeshita *et al*. (2000), the production of allitol from D-psicose required an NADH as cofactor for ribitol dehydrogenase (RDH). Using of NADH directly as raw substrate without regeneration in the reaction is not considered for industrial uses. The regeneration of NADH using FDH is widely used in biological production. To understand the mechanism of FDH in regeneration of NADH, the NAD^+ was docked into the active site of FDH.

Fig. 3A. Structure prediction and validation result of FDH from *O. parapolymorpha* DL-1 by homology modeling, a Ramachandran plot showing 87.1% of the atom residing in the most favored region, 12.6% in allowed region, 0.3% in generously allowed region and 0.0% in disallowed region. B. Overall quality of structure using Errat and it was 95.942%.



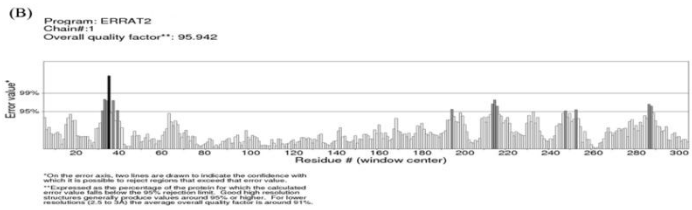


Fig. 4. Prove analyzing, the whole structure for the Z score and it was 33 % for outliers.

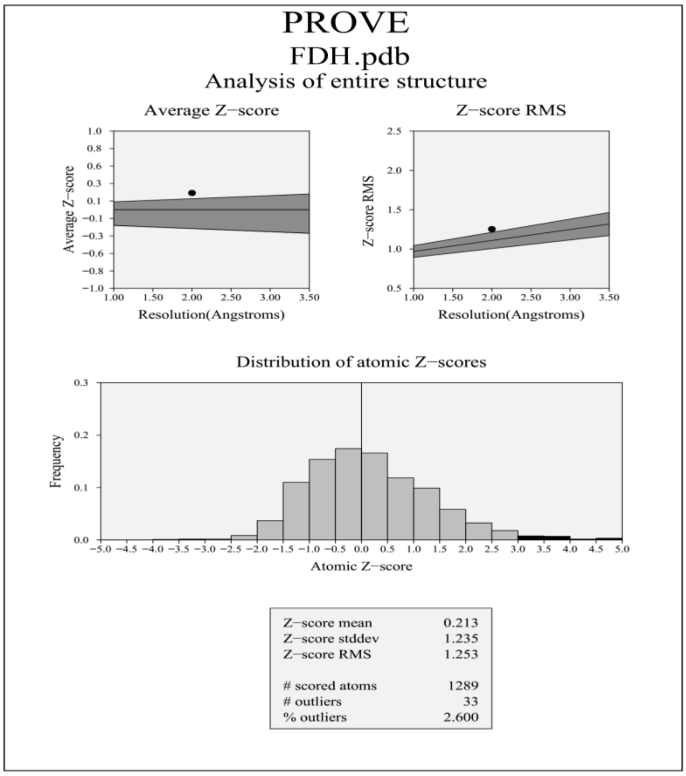
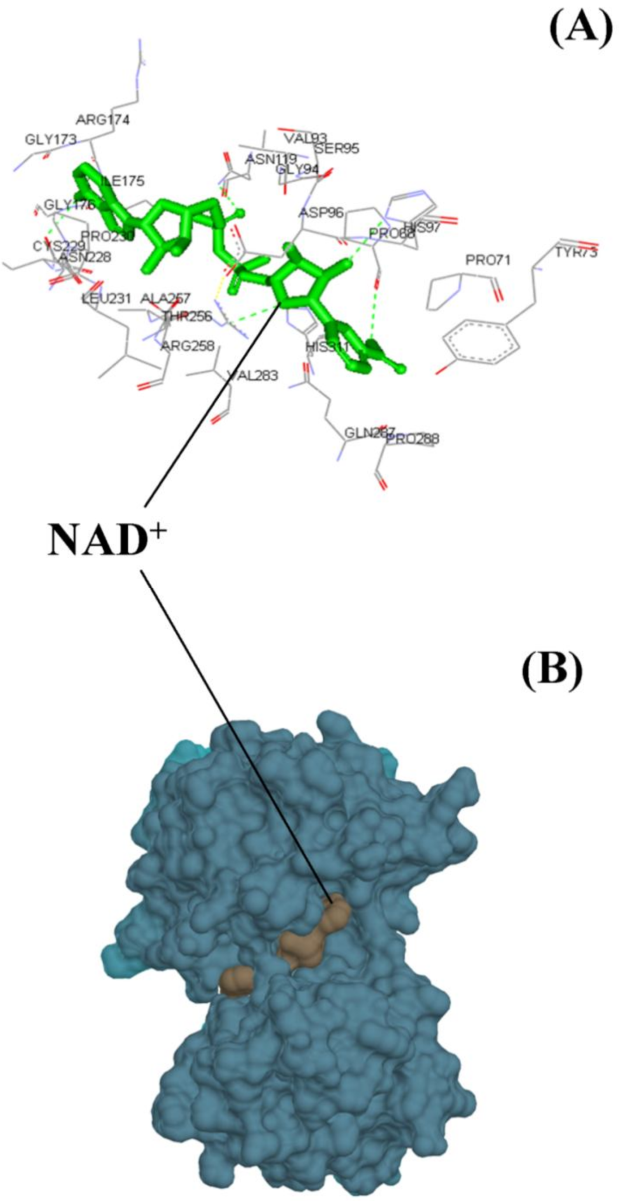


Fig. 5A. Interaction between FDH and NAD$^+$. Pro 68, Arg 258, Asn 119, Asn 228 and His 97 can be seen interacted with the NAD$^+$ molecule. B. NAD$^+$ molecule binds into the pocket shape of active site.



The 3D structure of FDH from *O. parapolymorpha* DL-1 revealed from Swiss was submitted to AutoDock tools as PDB file to create the PDBQT required for Vina software to conduct docking analysis. Hydrogen atoms and grid box were added to the structure. Grid parameters were put for the number of protein in x, y and z dimension and center grid box. The PDBQT files of protein and ligand were submitted to Vina software. The best docking model of FDH with NAD$^+$ was selected with low affinity, rmsd lower bound 0.0 and rmsd upper bound 0.0 form number of NAD$^+$ replications distributed around the active site revealed by Vina software.

As shown in Fig. 5A, the catalytic residues that interact with NAD$^+$ were Pro 68, Arg 258, Asn 119, Asn 228 and His 97 with distance of 3.14, 3.19, 3.18, 3.13 and 3.19 Å respectively. The similarity between FDH from *O. parapolymorpha* and other FDHs revealed that these catalytic residues have catalytic activity in active site of the FDHs. Schirwitz *et al*. (2007) reported that Pro 68, Asn 119, and Arg 258 were indicated as functionally relevant residues in FDH from *Candida boidinii*. To calculate the interaction energy, the 3D structure and NAD$^+$ molecule were submited to the Pose and Rank web service, which NAD$^+$ was indicated has a good interaction with FDH showing grid score of -60.23. The surface shape of the active site of FDH from *O. parapolymorpha* interacting with NAD$^+$ was observed which forms a pocket interaction shape (Fig. 5B), which demonstrated the interaction of NAD$^+$ and FDH in pocket form. The same orientation of active site for FDH from *Candida boidinii* was observed in deep cleft between domains.

Conclusion

The homology modeling and docking studies were investigated and reported in this study for formate dehydrogenase from *Ogataea parapolymorpha* DL-1. The results revealed that FDH has an effective interaction toward NAD$^+$ with grid score of -60.23, which is considered as an appropriate score for binding. After surfacing the 3D-structure of enzyme regions interacting with NAD$^+$, we observed that FDH forms a pocket interaction shape with NAD$^+$. The shape of FDH active site comprised with Pro 68, Arg 258, Asn 119, Asn 228 and His 97.

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