Journal of Academia and Industrial Research (JAIR)
Volume 6, Issue 9, February 2018

ISSN: 2278-5213

Research Article

Molecular Modeling and Docking of Mannitol Dehydrogenase Active Site and Residues Involved in the Interaction of D-mannitol

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Abstract

The protein sequence of mannitol dehydrogenase (MtDH) derived from *Caldicellulosiruptor hydrothermalis* was utilized for homology modeling using Swiss model. 3D structure revealed was docked with D-mannitol using AutoDock Vina software version 5.6. The results of presented homology modeling and docking studies revealed that the conserved residues of MtDH were interacting with D-mannitol. In this study we investigated the production of D-mannitol via MtDH and its interaction through homology and molecular docking procedure. The results of homology modeling and docking studies explained that the conserved residues of MtDH which are not directly involved and those directly involved in the oxidation reaction.

Keywords: *Caldicellulosiruptor hydrothermalis,* homology modeling, mannitol dehydrogenase, molecular docking.

Introduction

In 1920, D-mannitol was initially extracted from Manna ash tree exudates (*Fraxinus ornus*). Therefore, D-mannitol has numerous names along with mannite or mana sugar. It is a familiar as low-calorie sweetener because of its 1/2 relative sweetness evaluating to sucrose and different hexoses. Due to D-mannitol high demand, it was considered as an ideal sugar substitute in food, medicine, and pharmaceutical products, and has low solubility in water (around 18% w/v) in comparison to other isomers and consequently, its separation from sorbitol became a great deal easier after crystallization (Bhatt *et al.*, 2013). The commercial production of D-mannitol involves high-pressure hydrogenation of fructose using a nickel catalyst, a fairly costly and inefficient procedure (Heinen *et al.*, 2000; Van der Heijden *et al.*, 2002). To date, D-mannitol was synthesized by certain species of yeasts and fungi and a few bacterial species, mainly heterofermentative lactic acid bacteria (LAB) which produce D-mannitol but at very low levels without byproducts formation (sorbitol) (Saha and Racine, 2011).

Currently, the three dimensional structure measurements of proteins became an interesting approach within the biological techniques to estimate the catalytic mechanism of the protein or enzyme. Such accurate methods to design three dimensional (3D) protein designs depend on homology modeling which has been used in many sensible applications.

Modeling and docking are identified as proportional modeling of protein, which is designed to create the amino acid in figure of protein structure. It has been emerged over currently as the most accurate method in comparison to other methods (Chothia and Arthur, 1986; Koehl and Levitt, 1999). Homology modeling quality is usually reliant on the sequence alignments identity and template configuration. However, the structure constructed using homology modeling is same as spectroscopy or X-ray crystallography and nuclear magnetic resonance (NMR) (Sanchez and Sali, 1997). Homology modeling is a common application to determine the molecular docking for protein–protein interaction prediction and protein-protein docking (Gopal *et al.*, 2001).

In this study, we used various homologies and verification software to provide homology and modeling structure of MtDH from *C. hydrothermalis* and also to estimate the docking of D-mannitol into active site of MtDH enzyme to recognize the enzyme and substrate interaction. These studies have discussed to an in-intensity knowledge on how enzymes carry out chemical interaction and the position of substrate and enzyme in the active site (Klimacek *et al.*, 2003; Bubner *et al.*, 2008; Klimacek and Nidetzky, 2010; Hammes *et al.*, 2011). To date, most purposeful studies at the role of active site residues in enzymes have concentrated in molecular interactions directly concerned in enzymatic mechanism (Slatner *et al.*, 1999; Klimacek and Nidetzky, 2002; Klimacek *et al.*, 2003; Klimacek *et al.*, 2010).

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Conversely, active site residues that are not directly involved in the reaction chemistry are rarely assessed for their role in enzyme function. These residues are often highly conserved, indicating that they may also play an important role in enzyme function (Kavanagh *et al*., 2003; Kearse *et al*., 2012). Further experimental investigations of the functional importance of active site residues need more investigations. Here in this study, we presented the homology and structure modeling of MtDH from *C. hydrothermalis* which was used in production of D-mannitol from fructose in our previous studies. Significant structural and mechanistic studies have been carried out on this enzyme because of its industrial significance in the conversion of D-fructose to D-mannitol.

Materials and methods

Protein homology modeling and verification: SWISS-MODEL was using to design the 3D structure homology model of MtDH from *C. hydrothermalis* by gathering the information revealed from the template alignment. The mechanism of this system depends on searching of elevated individuality templates protein structures to construct models for evolutionary correlated proteins. The SWISS-MODEL is continuous system for updating the databases of homology models for groups of model organism proteomes of high biomedical concern. The Sequences of MtDH was inserted to SWISS-MODEL and the all options of model construction were chosen consistent with enzyme structure (Tetramer, homodimer). The model protein structure was subsequently retrieved from the protein data bank (PDB) through the template and the high comparable protein structures to the MtDH were chosen as a template.

Docking analysis: To design docking, the 3D model structure constructed using SWISS-MODEL was submitted to Auto DockVina software. Docking analysis is molecular modeling simulation software, which is particularly effectual for Protein-ligand docking. Chamira and Accelary Studio software was used to recognize and observed the molecular at an atomic structure and the outputs of the AutoDock Vina.

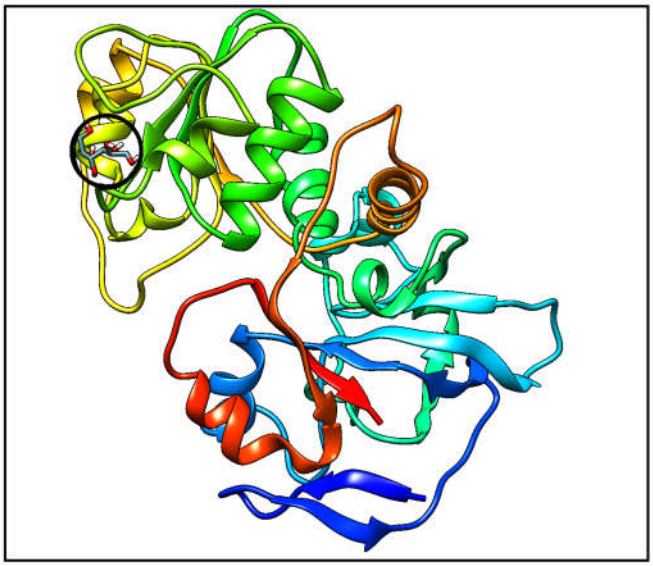
Three dimensional (3D) structure verification: A variety of verification methods were used to verify the modeled structure revealed from Swiss model. The favored regions, additional allowed regions, generously allowed regions, and disallowed region were checked and calculate through PROCHEK for the protein fold quality prediction. Furthermore, the enzyme structure was verified using Errat. In comparison to the database of consistent high-resolution structures, the error values were calculated depending on the information of non-bonded atom-atom interactions in the structure.

Additionally the MtDH structures were checked through Verifying 3D to determines the compatibility of an atomic model with its own amino acid sequence by assigning a structural class based on its location and environment (alpha, beta, loop, polar, nonpolar etc.) and comparing the results with superior structures.

Results and discussion

Mannitol dehydroegnase (MtDH) is a key enzyme which catalyzes the enzymatic reaction to reduce D-fructose to D-mannitol. MtDH belongs to the oxidoreductases family, particularly those MtDH which are acting on the hydroxyl group of donor accompany with NAD+ or NADP+ as acceptor (Stoop and Pharr, 1992). To the author acquaintance, this is the first report for docking of D-mannitol into the active site of MtDH form *C. hydrothermalis* stain. MtDH was characterized from *C. hydrothermalis* which was showed high affinity and specificity to D-mannitol and D-fructose, and the enzyme was NADH dependent. The molecular mass of the purified *C. hydrothermalis* MtDH was recorded as 36 kDa. The results of native molecular mass for *C. hydrothermalis* were 76.7 kDa. Figure 1 outlined the 3D of *C. hydrothermalis* MtDH which was functioned as homodimer with 2 subunits in the active site.

Fig. 1. Absolute 3D model structure-side view of MtDH from *C. hydrothermalis* (Homodimer).



Sequence alignment: The result of sequence alignment for *C. hydrothermalis* was reported from the model structure through SWISS MODEL. The results of *C. hydrothermalis* were modeled in (Fig. 2). The Swiss similarity result for selecting the template revealed that the most similar structure to *C. hydrothermalis* MtDH was the putative zinc-binding dehydrogenase enzyme family.

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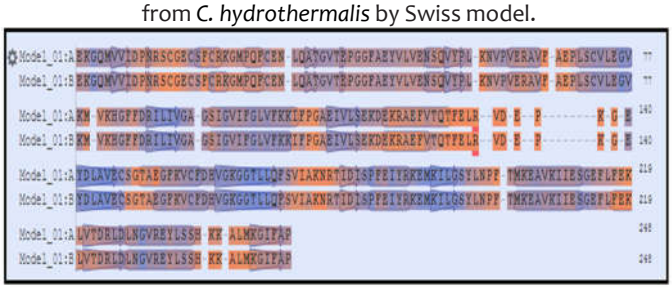
Fig. 2. Alignment of amino acid sequences template of MtDH from *C. hydrothermalis* by Swiss model.



Molecular docking: PROCHECK was used to check the model structure of *C. hydrothermalis* MtDH and to confirm 3D Errat (http://services.mbi.ucla.edu/ERRAT/) as shown in (Fig. 4). The Ramachandran plot for the two MtDHs structure suggested 85.5%, 12.5%, 0.9% and 1.1% for residues in most favored regions, additional allowed regions, generously allowed regions, and disallowed regions, respectively. The merged favored and allowed categories with the high percentage for model structure probable to materialize an accurate protein fold. The combined favored and allowed categories for *C. hydrothermalis* MtDH was 85.5% (Fig. 4). By justification of structures through verifying 3D of the residues, the enzyme had an overall average score of -0.27. The ternary structure of *C. hydrothermalis* with D-mannitol was utilized to identify the residues that responsible of molecular interactions with D-mannitol in the active site with atoms within (2.0 ˚A) which is creating the molecular interactions of important significance.

The previously proposed catalytic mechanism of MtDH was referenced to categorize the active site residues that directly involved in oxidation mechanism of the C2 alcohol of D-mannitol (Fig. 3). The 3D structure of *C. hydrothermalis* was submitted to AutoDock tools as PDB file to create the PDBQT file required for Vina software to perform docking analysis. Hydrogen atoms and grid box were added to the structure. The grid parameters were adjusted for number of comparable proteins in (x, y and z dimension) and middle grid box and the PDBQT files of protein structure were submitted to Vina software. Vina software was utilized to investigate and design the liking points of D-mannitol replications and coenzyme (NADH) and record their allocation in the regions of the center of the active sites. Active site residues with atoms within (3.5 A˚) of D-mannitol were considered to be making molecular interactions of significant interest (Fig. 4). Additionally, *C. hydrothermalis* MtDH included five catalytic residues Asp205, Arg203, Glu 187, Lys188 and Glu190 that were in direct contact with D-Mannitol through hydrogen bonding, whilst Asp189 is not involved in the chemical reaction.

Fig. 3. (A) Prediction result of MtDH from *C. hydrothermalis* by homology modeling. The Ramachandran plot showing 85.5% of the atom residing in the most favored region, 12.5% in allowed region and 0.9% in generously allowed region and 1.1% was in disallowed region. (B) Overall quality of structure using Errat was 86.387%.

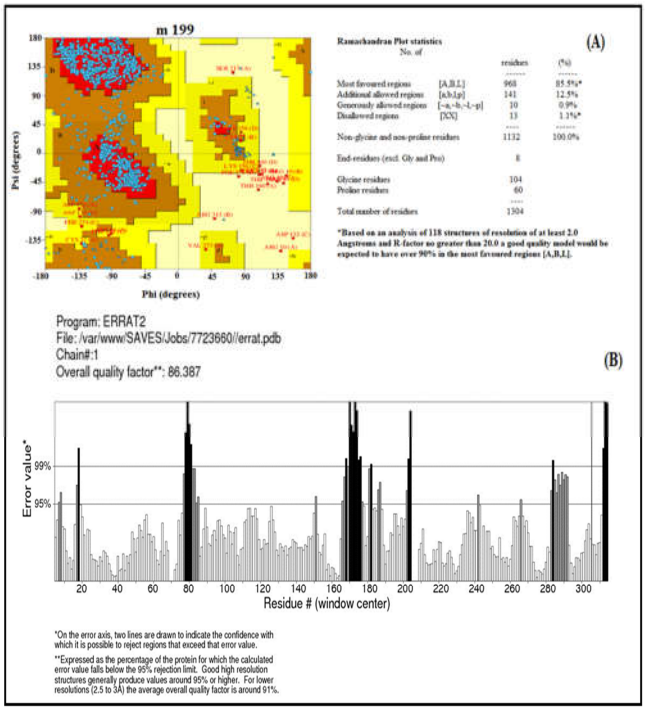
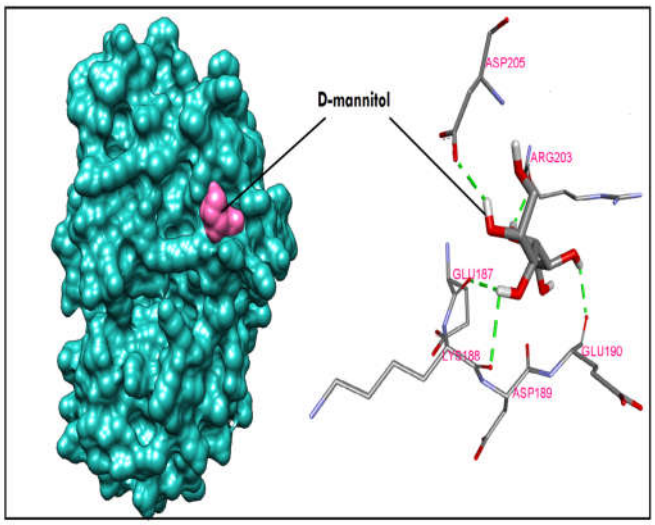


Fig. 4. The interaction between *C. hydrothermalis* MtDH and D-mannitol. Asp205, Arg203, Glu187, Lys188, Asp189 and Glu190 were the conserved amino acids involved in the catalytic mechanism. D-manniol molecule was shown in pink color.



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Conclusion

We concluded that our target MtDH *C. hydrothermalis* might utilize as catalytic key in D-mannitol production from D-fructose as he had distinguish possesses substrate specificity for D-mannitol. We have also identified several highly conserved active site residues which play important roles in D-mannitol catalysis pathway. The suitable grid score which prove that MtDH had effective interaction for binding to D-mannitol was -0.27.

Acknowledgements

This study was financially supported by the Support Project of Jiangsu Province (No. 2015-SWYY-009) and the project of outstanding scientific and technological innovation group of Jiangsu Province (Jing Wu).

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