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Rational design of xylose dehydrogenase for improved thermostability and its application in development of efficient enzymatic biofuel cell

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ABSTRACT

In this paper, the construction of 3D model structure of xylose dehydrogenase (XDH) by using homology modeling to guide the rational design of the enzyme for improving thermostability was reported. Three XDH mutants of NA-1 (+249L), NA-2 (G149P) and NA-3 (+249L/G149P) were designed and displayed on the surface of bacteria. Among them, bacteria displaying NA-1 (NA-1-bacteria) exhibited superior thermostability without compromising its activity and substrate specificity in comparison with its wild-type counterpart. NA-1-bacteria retained its original activity after incubation at room temperature for one-month with the half-life of 9.8 days at 40 °C. Finally, the NA-1-bacteria were applied to construct xylose/O_2 based biofuel cell with good performance including enhanced operational stability. Thus, the approach described here could be explored for engineering of other enzymes for improving certain characters without three-dimensional structure identified by experimental methods.

1. Introduction

As natural biocatalysts, most enzymes exhibit superior catalytic efficiency and substrate specificity. However, the poor thermostability of most enzymes originating from mesophilic bacteria limits its further real applications. As the traditional method for improving thermostability, directed evolution is challenged by the construction of a large scale mutation library and involvement of high throughput screening process [1] Rational design has been widely employed for analysis of the protein structures [2]. Homology models can be used to study protein–protein interaction, molecular docking and functional annotation of genes [3]. Thus, homology modeling provides a useful tool to study the structure of target protein without crystal structure, which refers to construct an atomic-resolution model of the target protein from its amino acid sequence and an experimental three-dimensional (3D) structure of a related homologous protein as the "template" [4,5]. Therefore, compared to amino acid sequence, the protein tertiary structure is well conserved [6]. Accordingly, proteins share common structural properties even though they have different amino acid sequences.

Xylose dehydrogenase (XDH) involving in D-xylose metabolism by an oxidative pathway in microorganisms has been identified in several prokaryotes [7,8]. Recently, XDH from oligotrophic freshwater bacterium *Caulobacter crescentus* NA1000 has been studied in detail, which enables to catalyze the direct oxidation of D-xylose to D-xylonolactone with NAD^+ as its cofactor [9], suggesting D-xylose metabolism occurring through a contrasting route different from the classical D-xylose metabolism pathway in most microorganisms [10,11]. XDH is of interest for xylose analysis in human nutrition, food technology as well as many bioprocesses. Owing to its good activity and substrate specificity, XDH has been successfully used in electrochemical biosensor [12] and biofuel cells [13] in our laboratory. Unfortunately, the recombinant wild-type XDH exhibited poor thermostability, which limited its potential applications. Therefore, it is highly desirous to improve this character. To date, some researchers have reported the molecular weight, enzyme activity, and substrate specificity of XDH originated from different resources [7,14], however, there is no report on its crystal structure and the improvement of enzyme stability. Therefore,

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homology modeling could be used to generate a reliable 3D structure of XDH by taking other members of redoxase family as the template. Furthermore, the simulation can guide the modification of XDH to improve its thermostability.

There are many cockamamie steps for isolation and purification of heterologous intracellular expressed proteins, which could greatly increase the cost of preparation and reduce the stability of protein. The microbial cell surface displaying of proteins is an alternative approach to address these issues. The system allows foreign peptides or proteins of interest to be displayed on the microbial surface through recombinant DNA biotechnologies by fusing them with appropriate anchoring motifs [15]. This method enables foreign proteins to directly interact with substrate without passing through the cell membrane. Moreover, it could improve stability of displayed proteins due to the immobilization on the surface of biomaterial support [16,17]. Each type of anchor protein has different characteristics and often leads to different effects on host cells. So it is vital to choose a suitable anchor protein. Microbial surface display has a vast sphere of biotechnological and industrial applications, such as live-vaccines [18], peptide or protein library screening [19], bioadsorbents [20], whole-cell biocatalysts [21], biosensors [22] as well as biofuel cells [23]. In our previous study, glucose dehydrogenase (GDH) (EC1.1.1.47) from *Bacillus subtilis* has been successfully displayed on the surface of bacteria, and mutants with significantly enhanced stability and specificity were also obtained [24]. The enzymatic activities of wild type and mutated GDHs could be directly detected using the whole cells as catalysts.

Eschericia coli cell-surface-displayed wild-type XDH from *C. crescentus* NA1000 using ice nucleation protein (INP) from *Pseudomonas borealis* as an anchor motif had been constructed in our laboratory before [25]. In the present study, model structure of XDH was obtained via homology modeling in order to improve the thermostability. Three mutant XDHs including NA-1 (+249L), NA-2 (G149P) and NA-3 (+249L/G149P) were displayed on the surface of *E. coli* . The kinetic constants of wild type and variants were determined. Among them, bacteria displaying NA-1 (+249L) (NA-1-bacteria) exhibited excellent thermostability with good activity and fair substrate specificity. Further, the NA-1-bacteria were applied in xylose/O_2 based biofuel cell (BFC), which exhibited good performance with enhanced stability in comparison with its wild type counterpart. Therefore, the current research provides a useful method to study the thermostability of certain protein without the 3D structure verified by experimental methods. To the best of our knowledge, this is the first attempt to use the structural simulation for improving the thermostability of dehydrogenase.

2. Materials and methods

2.1. Materials and chemicals

Isopropyl-β-D-thiogalactopyranoside (IPTG) was purchased from Sigma–Aldrich (St. Louis, MO USA). NAD^+ (the oxidized form of nicotinamide adenine dinucleotide) was obtained from Blue Season Biotechnology Company (Shanghai, China). D-xylose, L-arabinose, D-glucose and other monsugars as well as other regents were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Nafion (perfluorinated ion-exchange resin, 5 wt% solution in a mixture of lower aliphatic alcohols and water) was purchased from Aldrich (St. Louis, MO, USA), from which 0.05 wt% Nafion solution was prepared. All other reagents were of the highest grade and all solutions were prepared with ultrapure water.

2.2. Sequence alignment and homology modeling

Homology modeling was performed by Discovery Studio 3.5 (Accelrys, San Diego, USA). Redoxase from *Burkholderia thailandensis* (NCBI Accession No. WP_009888997.1) and GDH from *Bacillus megaterium* (NCBI Accession No. P40288) were used for multiple sequence alignment of XDH, which was carried out by CrustalW method [26]. Homology modeling was performed using MODELLER method, based on the alignment results as well as protein structures of redoxase (Protein Data Bank ID: 4IVU) and GDH (Protein Data Bank ID: 1RWB). The molecular dynamics cascades were used to minimize the structure violation of the steric interference.

2.3. Construction of expression vectors harboring XDH mutant encoding genes

E. coli DH5α and *E. coli* BL21 (DE3) were used to construct expression vectors and express recombinant proteins, respectively. The pTInaPbN-Xdh expression vector carrying the genes encoding wild type XDH and INP from *P. borealis* used here has been constructed in our previous study [25]. *E. coli* strains harboring recombinant plasmids were cultured in Luria-Bertani (LB) medium supplemented with kanamycin (30 μg/mL) at 200 rpm and 37 °C.

The sequences of PCR primers used in this study are shown in Table 1. The NA-1 variant was made by means of polymerase chain reaction (PCR), in which plasmid pTInaPbN-Xdh was used as a template. In order to generate plasmid harboring mutant gene of NA-1, plasmid pTInaPbN-Xdh was digested with *Bam* H I and *Hin* d III restriction endonucleases to produce cohesive ends. Meanwhile,

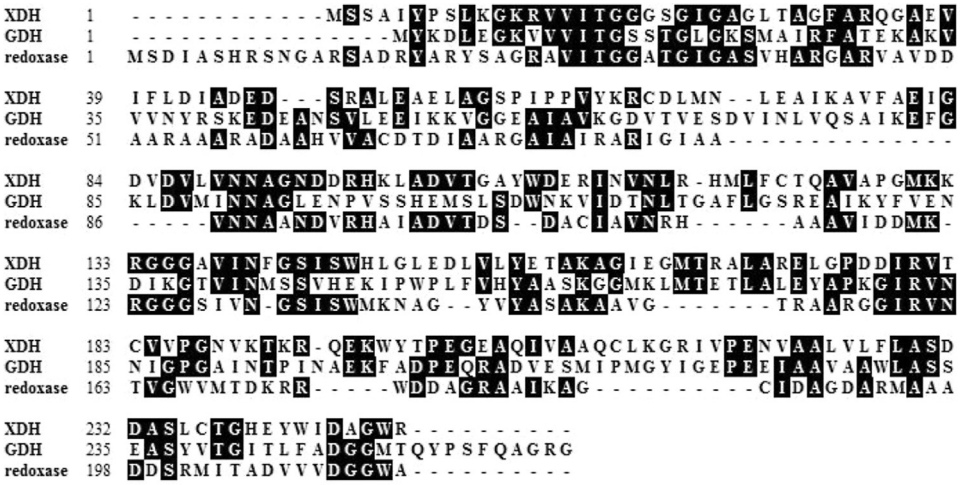

Fig. 1. Multiple sequence aligment of XDH from *C. crescentus* NA1000, GDH from *B. megaterium* and redoxase from *B. thailandensis*.