Characterization and Cofactor Binding Mechanism of a Novel NAD(P)H-Dependent Aldehyde Reductase from Klebsiella pneumoniae DSM2026

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Introduction

1,3-Propanediol (1,3-PD) is widely used in the modern polyester industry, especially in the synthesis of polytrimethylene terephthalate (PTT) [13]. So far, 1,3-PD is manufactured mainly by chemical synthesis started from acrolein or ethylene oxide. However, the yield of 1,3-PD is relatively low (<40% for acrolein, about 80% for ethylene oxide), and a toxic intermediate is released during the production process. As a result, the production of 1,3-PD was shifted from chemical to biological synthesis [20]. Fermentation of glycerol into 1,3-PD has been widely studied with the genera Klebsiella [23] and Clostridium [5]. Klebsiella pneumoniae is mostly used because of its high productivity of 1,3-PD.

During the fermentative production of 1,3-propanediol under high substrate concentrations, accumulation of intracellular 3-hydroxypropionaldehyde will cause premature cessation of cell growth and glycerol consumption. Discovery of oxidoreductases that can convert 3-hydroxypropionaldehyde to 1,3-propanediol using NADPH as cofactor could serve as a solution to this problem. In this paper, the yqhD gene from Klebsiella pneumoniae DSM2026, which was found encoding an aldehyde reductase (KpAR), was cloned and characterized. KpAR showed broad substrate specificity under physiological direction, whereas no catalytic activity was detected in the oxidation direction, and both NADPH and NADH can be utilized as cofactors. The cofactor binding mechanism was then investigated employing homology modeling and molecular dynamics simulations. Hydrogen-bond analysis showed that the hydrogen-bond interactions between KpAR and NADPH are much stronger than that for NADH. Free-energy decomposition dedicated that residues Gly37 to Val41 contribute most to the cofactor preference through polar interactions. In conclusion, this work provides a novel aldehyde reductase that has potential applications in the development of novel genetically engineered strains in the 1,3-propanediol industry, and gives a better understanding of the mechanisms involved in cofactor binding.

Keywords: 1,3-Propanediol, aldehyde reductase, cofactor binding, free-energy decomposition, Klebsiella pneumoniae
this problem is to construct genetically engineered strains that overexpress 1,3-PDOR. However, no evident increase of production yield is achieved as expected, although the concentration of 3-HPA is successfully kept at a low level [25]. On the other hand, metabolic flux analysis of glycerol utilization suggests that the production yield of 1,3-PD could be increased by utilizing not only NADH from the glycolytic pathway (EMP pathway) but also NADPH from the pentose phosphate pathway (PP pathway) [26]. These results indicate that the production yield of 1,3-PD is limited by the amount of NADH rather than the activity of 1,3-PDOR. Therefore, it is necessary to obtain novel oxidoreductases that are able to convert 3-HPA to 1,3-PD using both NADH and NADPH as cofactors.

In proteomics study, a hypothetical oxidoreductase (HOR) is detected as the concentration of 3-HPA increases in K. pneumoniae [22]. HOR can catalyze the conversion of 3-HPA to 1,3-PD in order to protect cells against the toxic condition. In the meantime, an NADPH-dependent aldehyde reductase (YqhD) from E. coli has recently been reported to serve as an antidotal enzyme to physiologically protect cells against the toxic effect of aldehydes derived from lipid oxidation [17]. Inspired by these observations, we expected to discover a novel YqhD-type enzyme in K. pneumoniae that is able to convert 3-HPA to 1,3-PD using NADPH as cofactor. As shown in this work, a novel aldehyde reductase was successfully cloned and characterized, and its cofactor binding mechanism was investigated through hydrogen-bond analysis and free-energy decomposition.

Materials and Methods

Materials
The bacterial genomic DNA extraction kit, ExTaq DNA polymerase, 250 bp DNA Ladder, plasmid pMD 19-T, and IPTG were purchased from TaKaRa (TaKaRa Biotechnology (Dalian) Co., Ltd., China). Plasmid pET23a was donated by Prof. Debra Dunaway-Mariano (University of New Mexico, USA). Restriction enzymes and T4 DNA ligase were purchased from New England Biolabs (Beijing, Ltd, China). The primers used in this work were custom-synthesized at TaKaRa (Table 1).

1,3-Propanediol was purchased from Fluka (Germany). 3-HPA was synthesized according to the published method [8]. NADPH, NADH, and NADP⁺ were products of Roche (F. Hoffmann-La Roche, Ltd, Switzerland). All other reagents used were commercial products of the highest grade available.

Cloning, Expression, and Purification
The genomic DNA of K. pneumoniae DSM2026 was extracted according to the method provided by the kit. The gene yqhD was amplified using primers KpAR-F and KpAR-R. Then the PCR product was linked to plasmid pMD19-T. The recombinant pMD18-T-yqhD was transferred into E. coli DH5α using the methods of CaCl₂ and hot attack. The plasmid from E. coli DH5α was isolated with a TIANprep Mini plasmid isolation kit (Tiangen Biotechnology (Beijing) Co., Ltd., China). The gene sequencing was carried out at TaKaRa. The sequencing result was submitted to the GenBank database and the accession number is EU740388.

Because of the existence of an Ndel site in the original sequence, which would influence the subsequent gene expression, synonymous substitution was carried out employing the overlap extension PCR technique. First, primers KpAR-NF and KpAR-ssR were used to amplify the 5’ end of gene yqhD; KpAR-ssF and KpAR-BR for the 3’ end. Second, the full-size fragment was amplified using the two overlapping fragments as template and KpAR-NF, and KpAR-BR as primers. Then, the amplified DNA fragment was digested and ligated to the Ndel-BamHI-digested pET23a(+). The produced recombinant plasmid pET23a(+)−yqhD was transferred into E. coli BL21(DE3).

E. coli BL21(DE3) containing pET23a(+)−yqhD was harvested by centrifugation. The suspension was disrupted in an ice bath by sonication, and the supernatant was loaded onto a Q Sepharose FF ion-exchange column. The column was first equilibrated and then washed with buffer A (50 mM Tris-Cl buffer (pH 7.4) containing 0.1 mM MnCl₂ and 2 mM DTT). Proteins were eluted with buffer B (buffer A supplemented with 1 M KCl) using a linear gradient of 0 to 1 M. Active fractions were applied on a column of Sephacryl S-300, which was eluted with buffer A.

Enzyme Activity and Characterization
Enzyme activity in the physiological direction was determined at 340 nm by the initial rate of substrate-dependent NAD(P)H decrease. The assay mixture contained 27 mM 3-HPA, 0.37 mM

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>KpAR-F</td>
<td>5'-ATGAAATATTTCCAGCCTGACATTGCCATACCC-3'</td>
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</tr>
<tr>
<td>KpAR-R</td>
<td>5'-TTAGCCGTGCAGCTGTAATCTAC-3'</td>
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<td>Mutation site</td>
</tr>
<tr>
<td>KpAR-ssR</td>
<td>5'-CGGAGCATGTCGGCGTCCGCGATCTCTGT-3'</td>
<td>Mutation site</td>
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