Stereoselective catabolism of β-1 type lignin-derived aromatic isomers in *Sphingobium* sp. strain SYK-6

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Research objective

Lignin, a plant cell wall component, is an aromatic polymer with complex linkages, and its abundance has made its use desirable. Currently, the metabolic capacity of bacteria is being utilized to develop technology for the conversion of heterogeneous low-molecular weight aromatic compounds obtained through the chemical depolymerization of lignin into raw materials for fabrication of functional polymers [1]. A remarkable number of bacterial catabolic systems essential for the development of this technology have been investigated. However, the catabolic system for converting lignin-derived dimeric compounds with stereoisomers is not well studied [2,3].

The β-1-type dimeric compound 1,2-diguaiacylpropane-1,3-diol (DGPD) exists as *threo* and *erythro* stereoisomers, each of which contains enantiomers (Fig. 1). In *Sphingobium* sp. strain SYK-6 cells, LigD and LigL, which are Cα-dehydrogenases involved in the degradation of β-O-4 dimeric compounds [4], play key roles in the oxidation of *threo*-DGPD (DGPD I and II) to the keto form (DGPD-keto I and II). Subsequently, DGPD-keto II is reduced to *erythro*-DGPD (DGPD IV) in the presence of NADPH by LdpB, another Cα-dehydrogenase, and DGPD IV is rapidly degraded by an SYK-6 cell extract.

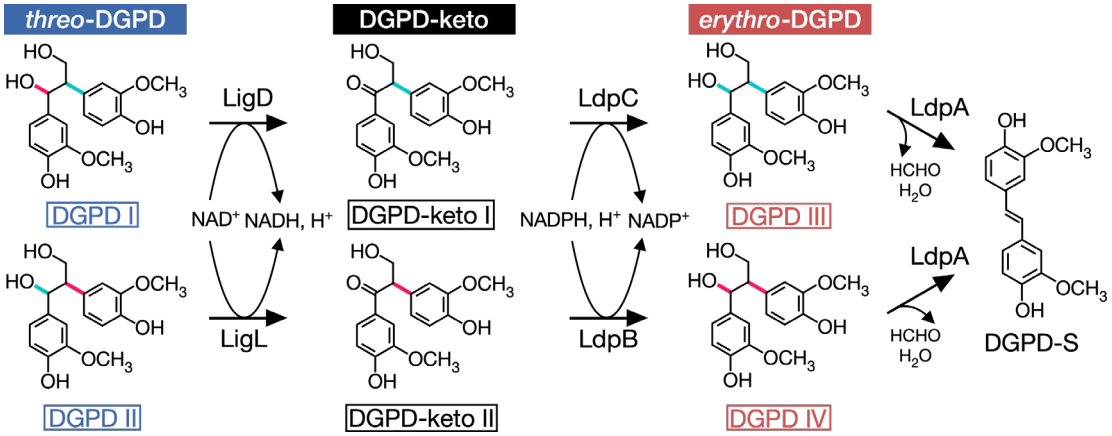

Fig. 1. The catabolic system for the conversion of the stereoisomers of β-1-type dimeric compounds in *Sphingobium* sp. SYK-6.

Thus, the DGPD stereoisomers are metabolized via a unique conversion system that inverts the stereochemistry of the Cα hydroxyl group of *threo*-DGPD. In this study, we aimed to determine the enzyme genes involved in the conversion of DGPD-keto I and *erythro*-DGPD

(DGPD III and IV) and their associated functions to gain insights into the catabolism of β-1-type dimeric compounds.

Methods

The β-1 dimeric compounds were provided by Dr. Shojiro Hishiyama of the Forestry and Forest Products Research Institute and Dr. Rui Katahira of the National Renewable Energy Laboratory. Cosmid clones were isolated from a genomic library of SYK-6 constructed in *Sphingobium japonicum* UT26S by selecting the transformants with *erythro*-DGPD conversion ability using HPLC analysis. Each gene was expressed using either the pET system in *E. coli* or the pQF system in SYK-6. The gene products were purified using Ni-affinity chromatography. The substrate conversion measurements and identification and quantification of products were performed by HPLC, LC-MS, and chiral HPLC. The enzymatic activity of LdpA was measured based on the increase in absorbance of the product DGPD-S at 333 nm. The enzymatic activity of LdpC was measured based on the decrease in absorbance of NADPH/NADH at 340 nm when DGPD-keto I and II were used as the substrates. Gene disruption was performed by homologous recombination. Complementary plasmids for the gene-disruption strains were prepared by introducing each gene into pJB861.

Results

Identification and functional analysis of the *erythro*-DGPD-converting enzyme gene

A cosmid clone that conferred the *erythro*-DGPD conversion ability to host cells was isolated from the genomic library of SYK-6. During this study, a group in the U.S. published a paper identifying an *erythro*-DGPD-converting enzyme gene *lsdE* from *Novosphingobium aromaticivorans* DSM 12444 [5]. In the resulting cosmid, a gene showed an 80% amino acid sequence identity with DSM 12444 *lsdE*, which was designated as *ldpA*. LdpA produced in *E. coli* was purified, and its enzymatic properties were investigated. LdpA catalyzed the Cγ deformylation of *erythro*-DGPD (DGPD III and IV) accompanied by dehydroxylation at the Cα-position, which led to the generation of a stilbene-type compound DGPD-S (Fig. 1). In contrast, LdpA did not show *threo*-DGPD (DGPD I and II) conversion ability. An *ldpA*-disrupted strain of SYK-6 was defective in *erythro*-DGPD conversion, indicating that *ldpA* was responsible for the conversion of *erythro*-DGPD. Additionally, DGPD-S was metabolized via vanillate.

Identification of the DGPD-keto I-converting enzyme gene

We hypothesized that Cα-dehydrogenase of the short-chain dehydrogenase/reductase (SDR) family, which LdpB belongs to, is involved in DGPD-keto I reduction. Since DGPD-keto I is converted in the presence of NADPH by the SYK-6 cell extract, six of the seven Cα-dehydrogenase genes were expressed in *E. coli*, and the resulting cell extracts were incubated with DGPD-keto I in the presence of NADPH. HPLC analysis showed that none of the recombinant strains exhibited DGPD-keto I conversion activity. An unexpressed gene in *E. coli* was expressed in SYK-6, and the gene product was purified using Ni-affinity chromatography. However, no DGPD-keto I conversion activity was observed for this purified

enzyme.

ldpB is located upstream of *ldpA* in the SYK-6 genome. In addition to *ldpB*, two other genes belonging to the SDR family are located around *ldpA*. Disruption strains for each gene were generated, and the conversion ability of each mutant was determined by incubating the respective resting cells cultured in LB with DGPD-keto I. The amount of DGPD-keto I converted by the *ldpC*-disrupted strain after 4 h of incubation was reduced to approximately 20% of that of the wild type. DGPD-keto I conversion ability was restored in the *ldpC*-disrupted strain complemented with *ldpC*. These results indicate that *ldpC* is primarily responsible for the conversion of DGPD-keto I in SYK-6 cells.

Enzymatic function of LdpC

The cell extract of *E. coli* expressing *ldpC* was incubated with DGPD-keto I in the presence of NADPH, and then the reaction mixture was analyzed using HPLC. The cell extract converted DGPD-keto I into *erythro*-DGPD (Fig. 1). In contrast, the cell extract showed no conversion activity against DGPD-keto II. The specific activity of purified LdpC for DGPD-keto I conversion in the presence of NADPH was 31 μmol/min/mg and that in the presence of NADH was lower by less than 3%. These results indicate that LdpC exhibits NADPH-dependent DGPD-keto I reduction activity.

Conclusion

The *threo*-DGPD isomers are oxidized by LigD and LigL in SYK-6 cells, followed by reduction via LdpC and LdpB, which invert the stereochemistry of the Cα hydroxyl group of *threo*-DGPD. Since LdpA is specific to *erythro*-DGPD, the SYK-6 cells can catabolize all stereoisomers of the β-1 type dimer by converting the *threo* form to the *erythro* form.

References

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