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Structural study on enzymes related to elongation and modification of prenyl chain

Masahiro Fujihashi*, Daisuke Sasaki, Kunio Miki
Department of Chemistry, Graduate School of Science, Kyoto University,
Sakyo-ku, Kyoto, Kyoto 606-8502, Japan

More than 50,000 structurally diverse isoprenoids, which are composed of C5 isoprene units, are widely distributed in nature. These isoprenoids compounds are essential components of life, such as steroids, hemes, carotenoids, vitamins, quinones, and membrane lipids. Linear prenyl diphosphate compounds are common precursors of all isoprenoids. We have determined the crystal structures related to elongation and modification of prenyl chain.

Two enzymes have been investigated[1,2]. The first enzyme is a prenyltransferase, hexaprenyl diphosphate synthase from *Micrococcus luteus* B-P 26 (*Ml*-HexPPs). This enzyme catalyzes consecutive head-to-tail condensations of three molecules of isopentenyl diphosphates (IPP, C_5) on a farnesyl diphosphate (FPP, C_{15}) to produce an (all-*trans*) hexaprenyl diphosphate (HexPP, C_{30}). Although typical *trans*-prenyltransferases function as homodimer, HexPPs is a heterodimeric enzyme. The small and large subunits of heterodimer are named HexA and HexB, respectively. Since the molecular mechanism of heterooligomeric *trans*-prenyltransferases is not yet clearly understood, particularly with respect to the role of the small subunits lacking the catalytic motifs conserved in most known *trans*-prenyltransferases, we have determined the crystal structure of *Ml*-HexPPs in ligand-free and inhibitor-bound forms. The X-ray diffraction datasets of ligand-free and inhibitor-bound crystals were collected in beamlines BL5A and NW12A, respectively. The ligand-free datasets were phased with single isomorphous replacement with anomalous scattering (SIRAS) method using a mercury derivative crystal at beamline BL17.

The structure of HexB consists of mostly antiparallel α-helices jointed by connecting loops, and very similar to a subunit of typical homooligomeric *trans*-prenyltransferase. The diphosphate part of the inhibitor 3-DesMe-FPP is bound around the two aspartate-rich motifs (FARM and SARM) and the other characteristic motifs in HexB. The structure of HexA is very similar to that of HexB, despite the quite low amino-acid sequence identity and the disparate polypeptide chain lengths between HexA and HexB. The hydrophobic-tail of 3-DesMe-FPP is accommodated in a large hydrophobic cleft starting from HexB and reaching to the inside of HexA. These structural features suggest that the condensation reactions are catalyzed on HexB and the product chain-length control are involved in both HexA and HexB.

The other investigated enzyme is geranylgeranyl reductase from *Sulfolobus acidocaldarius* (*Sa*-GGR)[2]. The enzyme is a flavoprotein and is involved in reduction of the double bonds on the isoprenoid moiety of archaeal membranes. The datasets were collected at beamline BL5A. Phasing was performed with molecular replacement method using the structure of GGR from *T. acidophilum* as a search model.

The determined structure shows *Sa*-GGR belongs to the *p*-hydroxybenzoate hydroxylase family in the glutathione reductase superfamily. This enzyme works as a monomer and is divided into three domains: FAD-binding, catalytic, and C-terminal domains. The catalytic domain has a large cavity surrounded by a characteristic YxWxFPx$_{7-8}$GxG motif and by the isoalloxazine ring of a FAD molecule. The cavity holds a lipid molecule, which is probably derived from *E. coli* cells used for overexpression. One of the two forms of determined structures clarifies the presence of an anion pocket holding a pyrophosphate molecule, which may hold the phosphate group of the natural ligands. Mutational analysis supports that the three aromatic residues of the YxWxFPx$_{7-8}$GxG motif hold the ligand at the appropriate position for reduction. Cys47, which is widely conserved in known GGRs, is located at the *si*-side of the isoalloxazine ring of FAD, and mutation of this residue significantly suppresses the enzymatic activity

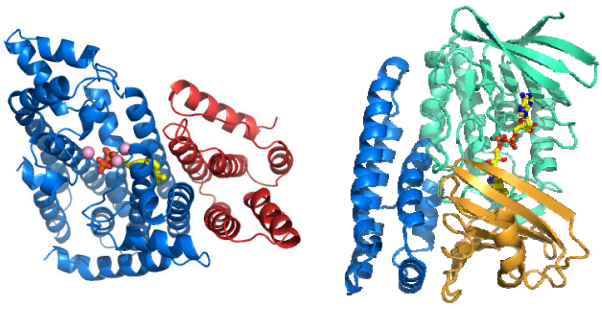

Figure left: HexPPs, right: GGR

References

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[2] D. Sasaki *et al.*, J. Mol. Biol. 409, 543-57 (2010).

*mfuji@kuchem.kyoto-u.ac.jp