Crystallography

5A/2010

Structure of a heterotetrameric geranyl pyrophosphate synthase from mint (*Mentha piperita*) reveals intersubunit regulation

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Introduction

Linear prenyl pyrophosphates (LPPs) are the precursors for the more than 55,000 terpenes (isoprenoids) that have been identified in organisms. Many are essential for important biological processes such as protein prenylation (Ras, Rab, Rho), proper functioning of the electron transport chain (quinine, heme a), glycoprotein biosynthesis (dolichol), and the metabolism of growth hormones (gibberellin, cytokinin, sterol). Because the lengths of LPPs determine their distinct physiological roles, the production of LPPs is precisely regulated by their respective prenyltransferases (PTSs), groups of highly conserved enzymes in the cells. PTSs can be further distinguished into homomeric and heteromeric protens based on their subunit compositions. In contrast to the other well studies of homomeric PTSs, heteromeric PTSs have so far only been identified in a few prokaryotes and eukaryotes. Thus far, no structure of heteromeric PTS is available. Our aim was to solve the first structure of heteromeric PTSs and gain insight into the molecular mechanism of inter-subunit interaction in heteromeric PTSs.

Result and Discussion

Heteromeric PTSs are composed of two different types of subunits: one is significantly homologous (50% identity) to homomeric PTSs, while the other is less homologous (15% identity) and lacks the DD(X)$_n$D motif, and is recognized as a non-catalytic subunit. The non-catalytic subunit is not only required for enzymatic activities of the heteromeric PTSs, but also serves to modify catalytic fidelity or to promote catalytic activity in plants. *Mentha piperita* geranyl pyrophosphate synthase (Mp GPPS), involved in the biosynthesis of essential oil (menthol) in mint glandular trichomes, is such a two-component heteromeric PTS consisting of a large and a small subunit, denoted LSU and SSU, respectively. LSU is a PTS-like protein with about 75% sequence identity to plant geranylgeranyl pyrophosphate synthase (GGPPS), and presumably its structure is highly similar to that of *Sinapis alba* GGPPS (Sa GGPPS). SSU is less similar in sequence to other PTS proteins and lacks the essential catalytic amino-acid residues found in other PTSs.

We determined the crystal structure of a new (LSU·SSU)$_2$-type heterotetrameric Mp GPPS, composing of two LSU·SSU heterodimers (Fig. 1). The LSU and SSU in each LSU·SSU heterodimer are related by a pseudo-dyad axis, and two LSU·SSU dimers form a tetramer (LSU·SSU)$_2$ about a third dyad, all of which are parallel to one another.

The LSU and SSU of Mp GPPS are responsible for catalysis and regulation, respectively. Whereas no activity was detected for individually expressed LSU or SSU, the intact (LSU·SSU)$_2$ tetramer produced not only C_{10}-GPP at the beginning of the reaction but also C_{20}-GGPP at longer reaction times. The activity for synthesizing C_{10}-GPP and C_{20}-GGPP, but not C_{15}-FPP (farnesyl pyrophosphate), reflects a conserved active-site structure of the LSU and the closely related homodimeric Sa GGPPS. Furthermore, using a genetic complementation system, we showed that no C_{20}-GGPP is produced by the mint GPPS *in vivo*. Presumably through protein-protein interactions, the SSU remodels the active-site cavity of LSU for synthesizing C_{10}-GPP, the precursor of volatile C_{10}-monoterpenes.

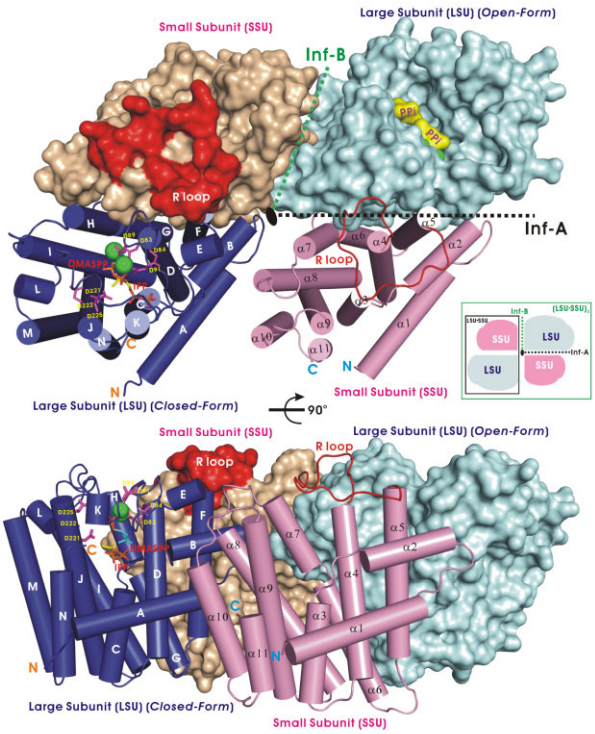

Fig. 1. Architecture of the (LSU·SSU)$_2$-type heterotetrameric Mp GPPS.

References

[1] T. H. Chang et al, *Plant Cell* 22, 454 (2010).

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