6A, 6B/2000G131

X-ray crystallographic studies of prenyl transferases

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Introduction

Over 23,000 structurally diverse isoprenoids are produced in nature, most of which are essential components of cellular machinery and serve as visual pigments, reproductive hormones, defensive agents, constituents of membranes and signal transduction components. Prenyltransferases, so-called prenyl diphosphate synthases, catalyze the prenyl chain elongation of prenyl diphosphates that are the common precursors of the carbon skeletons for all isoprenoids. These enzymes can be classified into two major sub-groups according to the *cis*- and *trans*-isomerism of products in the prenyl chain elongation.

Undecaprenyl diphosphate synthase (UPS) catalyzes the *cis*-prenyl chain elongation onto *trans, trans*-farnesyl diphosphate (FPP) to produce undecaprenyl diphosphate (UPP), which is indispensable for the biosynthesis of bacterial cell walls. We report here the crystal structure of UPS from *M. luteus* B-P 26 as the first three dimensional structure in all *cis*-prenyl chain elongating enzymes, in order to understand the molecular mechanism of *cis*-prenyltransferases.

Materials and Method

UPS from *M. luteus* B-P 26 was overproduced, purified and crystallized as described [1]. Heavy atom derivatives were prepared by the soaking method. For both thimerosal and $KAu(CN)_2$ derivatives, the concentration of heavy atom compounds in the soaking buffer and the soaking periods were 1 mM and 1 day, respectively. Diffraction studies were performed at room temperature at the Photon Factory (BL6A and BL6B).

Results and Discussion

The overall structure of UPS from *M. luteus* B-P 26 was determined at 2.2 Å resolution by multiple isomorphous replacement with anomalous scattering (MIRAS) [2]. This enzyme acts as a homodimer of 29 kDa subunits under physiological conditions. The asymmetric unit contains one homodimer, in which each monomer is crystallographically independent. The front view of the dimeric form of UPS looks like the face of an elephant. The contact interface of the dimer is about 15 %. The topology diagram of the secondary structures shows that the monomer has six parallel β-strands (S1-S6) and seven α-helices (H1, H2, H3, H5, H6, H8 and H10). The β-strands form a central β-sheet core, which is surrounded by five of the seven β-helices (H1, H2, H3, H5 and H10). Additionally, there are three short 3_{10}-helices (H4, H7, and H9) in each monomer.

The fold of UPS is completely different from those of other isoprenoid biosynthesis-related enzymes. These enzymes, including franesyl diphosphate synthases, pentalenene synthases, 5-epi-aristolochene synthases, squalene cyclase and protein farnesyltransferase, have a common structural motif. This motif is called the isoprenoid synthase (or terpenoids synthase) fold, and has been believed to be included in all enzymes related to isoprenoid biosynthesis. It is composed of 10 to 12 mostly antiparallel α-helices. However, UPS of the present study has a central β-sheet core, which is a different from the "isoprenoid synthase fold." Additionally, it was found that UPS belongs to a new protein folding family, judging from the multiple alignment of 3D structural neighbours search. This suggests that the catalytic mechanisms of UPS, including substrate recognition, are also different from those of other enzymes related to isoprenoid biosynthesis. Conserved amino acid residues among *cis*-prenyl chain elongating enzymes are located around a large hydrophobic cleft in the UPS structure. A structural P-loop motif, which frequently appears in the various kinds of phosphate binding site, is found at the entrance of this cleft. The catalytic site is determined on the basis of these structural features and the reaction mechanism to synthesize the C_{55} prenyl chain product is proposed.

References

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