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Structural study on cis-type prenyl chain elongating enzyme

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Structural study on cis-type prenyl chain elongating enzyme

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Abbreviations

DMAPP: dimethylallyl diphosphate
DTT: dithiothreitol
FPP: farnesyl diphosphate
FPS: farnesyl diphosphate synthase
GGPP: geranylgeranyl diphosphate
GPP: geranyl diphosphate
HepPP: heptaprenyl diphosphate
HexPP: hexaprenyl diphosphate
HEPES: $N$-2-hydroxyethylpiperazine-$N'$-2-ethanesulfonic acid
IP: imaging plate
IPP: isopentenyl diphosphate
IMSS: Institute of Materials Structure Science
KEK: High Energy Accelerator Research Organization
MIR: multiple isomorphous replacement
MIRAS: multiple isomorphous replacement with anomalous scattering
MR: molecular replacement
NCS: non-crystallographic symmetry
PEG400: polyethylene glycol 400
PF: Photon Factory
r.m.s.d: root-mean-square deviations
SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis
Tris: tris(hydroxymethyl)aminomethane
UPP: undecaprenyl diphosphate
UPS: undecaprenyl diphosphate synthase
Abstract

Over 23,000 structurally diverse isoprenoids are produced in nature, most of which play various roles in the cellular machinery. 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. Undecaprenyl diphosphate synthase (UPS), which belongs to this family, catalyzes the cis-prenyl chain elongation onto trans, trans-farnesyl diphosphate (FPP) to produce undecaprenyl diphosphate (UPP). This enzyme is indispensable for the biosynthesis of bacterial cell walls, and functions as homodimer composed of two 29 kDa subunits.

UPS from Micrococcus luteus B-P 26 was crystallized by the sitting drop vapor diffusion method at 20 °C using ammonium sulfate and lithium sulfate as precipitants. These crystals belong to the monoclinic space group C2 with unit-cell dimensions \( a=127.2 \, \text{Å}, b=60.2 \, \text{Å}, c=75.7 \, \text{Å} \), and \( \beta=105.6 \, ^\circ \). The crystal structure was solved using mercury and gold derivatives and refined to crystallographic \( R \) of 19.0 % and \( R_{\text{free}} \) of 24.6 % for the final atomic model, including 38 water molecules and two sulfate ions. The stereochemistry of final model is reasonable, no residues being unfavorable regions in the Ramachandran plot. The root-mean-square deviations from standard values were 0.006 Å and 1.120 ° for bond lengths and angles, respectively.

The crystal structure determined is the first one among cis-prenyltransferases and is completely different from the so-called “isoprenoid synthase fold” that is believed to be a common structure for the enzymes relating to isoprenoid biosynthesis.
In addition, it was found that UPS is classified into a new protein folding family.

A large cleft, which is composed of hydrophobic residues, was found on the UPS structure. Conserved amino acid residues among cis-prenyl chain elongating enzymes are located around this cleft. At the entrance of this cleft, four arginine residues form a positive charged cluster. In addition, a structural P-loop motif, which frequently appears in the various kinds of phosphate binding site, is found at this entrance. The motif binds to a sulfate ion, which was used for crystallization. Based on these structural features and site-directed mutagenesis studies performed by our collaborators, the hypothetical substrate-binding model of this enzyme was constructed. In this model, the P-loop motif and the hydrophobic cleft recognize the diphosphate head and the hydrophobic tail of allylic substrate, respectively. The homoallylic substrate IPP is suggested to bind with close residues from the structural P-loop motif. The chain length of the final product is assumed to be determined by the size of the hydrophobic cleft.
1 Introduction

1-1 Isoprenoids

Isoprenoid compounds are the most structurally diversified family in natural products (Figure 1-1). Over 23,000 individual isoprenoid compounds have been characterized, and hundreds of new structures are reported each year. They serve as hormones, vitamins, visual pigments, pheromones, electron carriers, constituents of membranes, photoreceptors and in many other roles. All of these isoprenoid compounds are derived from linear prenyl diphosphates, which are synthesized by sequential condensations of isopentenyl diphosphate (IPP) with allylic prenyl diphosphate (Ogura & Koyama, 1998). These condensation reactions are catalyzed by a family of prenyltransferases.

1-2 Prenyltransferase family

Prenyltransferases can be classified into two sub-families according to the cis- and trans-isomerism of products in the prenyl chain elongation (Ogura & Koyama, 1998) (Figure 1-2). trans-Type prenyltransferases can be sub-divided into three subgroups (Prenyltransferase-I, II and III) according to their requirement for enzymatic activity and the chain length of the final product. Enzymes for cis-type prenyl chain
elongation is classified as Prenyltransferase-IV.

Prenyltransferase-I synthesizes short chain prenyl diphosphates such as farnesyl diphosphate (FPP, \(C_{15}\) compound) and geranylgeranyl diphosphate (GGPP, \(C_{20}\)). These enzymes work as homodimer under the physiological condition, and require only divalent ions (\(Mg^{2+}\) or \(Mn^{2+}\)), which are required by all prenyltransferases, for their catalytic reaction (Ogura & Koyama, 1998). Products were used for the primer substrate for the biosynthesis of steroid and carotenoid as well as the allylic substrate for prenyltransferase II, III and IV.

Prenyltransferase II synthesizes medium chain prenyl diphosphates, especially (all-trans)-hexaprenyl diphosphate (HexPP, \(C_{30}\)) (Fujii et al., 1982) and (all-trans)-heptaprenyl diphosphate (HepPP, \(C_{35}\)) (Takahashi et al., 1980). They consist of heterodimer, neither of them has any catalytic activity by itself (Fujii et al., 1982).

Prenyltransferase III synthesizes (all-trans)-long chain prenyl diphosphates such as octaprenyl diphosphate (\(C_{40}\)) (Fujisaki et al., 1986) and solanesyl (nonaprenyl) diphosphate (\(C_{45}\)) (Ohnuma et al., 1991). These enzymes work as homodimer like the cases of prenyltransferase-I, and require the additional protein activation factors for their catalytic reactions (Ohnuma et al., 1991). These activation factors are assumed to remove the hydrophobic long chain product for the catalytic turnover.

Prenyltransferase IV does not catalyze the trans-type prenyl chain elongation, but elongates the chain length of cis-prenyl diphosphates. In many cases, the product chain length of prenyltransferase-IV is longer than that of prenyltransferase III.
Undecaprenyl diphosphate (C\textsubscript{55}) synthase, which is a typical enzyme of this family, has been partially purified and characterized from several bacteria (Takahashi & Ogura, 1982; Fujisaki et al., 1986; Allen et al., 1976). Rubber transferases from higher plants, which synthesize the natural rubber, also belong to this family (Ogura & Koyama, 1998). Enzymes belonging to this family require phospholipid or detergents for their enzymatic reactions in addition to magnesium ion (Muth & Allen, 1984). They work as homodimer under the physiological conditions (Muth & Allen, 1984).

1-3 Molecular mechanism of \textit{trans}-Prenyltransferase

Multiple alignment of the amino acid sequences of \textit{trans}-type prenyltransferases (prenyltransferase I, II and III) has shown the presence of seven conserved regions in the primary structure (Koyama \textit{et al.}, 1993; Ogura & Koyama, 1998) (Figure 1-3). The second and the sixth conserved regions from the N-terminal (region II and VI, respectively), have characteristic aspartate-rich DDXXD motifs. Crystal structure of FPS from avian indicated that these motifs were located at the C-terminal of \alpha-helices and formed the entrance of the large cleft (Tarshis \textit{et al.}, 1994). Mutated FPS structures bound with allylic substrates showed that the conserved DDXXD motif in region II plays dominant roles to bind an allylic substrate (in case of this enzyme, dimethylallyl diphosphate (DMAPP) or geranyl diphosphate (GPP))
through two magnesium ions (Tarshis et al., 1996). Site-directed mutagenesis of aspartate residues in region IV results in up to $10^5$-fold decrease of $k_{cat}$ values and 10-fold increase of $K_m$ values for IPP compared to the wild type (Koyama et al., 1996). The product chain length is regulated by the depth of the central cavity of this enzyme, substitution from phenylalanine located on the bottom of the cavity to alanine elongates the chain length of the final product. (Ohnuma et al., 1996a; Ohnuma et al., 1996b; Tarshis et al., 1996). As described above, the molecular mechanisms of FPS including substrate recognition and chain length regulation have been well studied.

In addition, the Se-methionyl FPS from Bacillus stearothermophilus was crystallized (Nakane et al., 1993), and diffraction data at 2.4 Å resolution were collected at the wavelength of peak, edge and remote of Se under both cryo and non-cryo conditions. (Figure 1-4) The crystal structure analysis of this enzyme is in progress. Comparison between FPS’s from avian and B. stearothermophilus will gave deep insights into the molecular mechanism of trans-prenyltransferase.

1-4 Other enzymes related to isoprenoid biosynthesis

Several crystal structures of enzymes related to the isoprenoids biosynthesis have been determined, such as pentalenene synthase from Streptomyces UC5319 (Lesburg et al., 1997), 5-epi-aristolochene synthase from Nicotiana tabacum (Starks et al., 1997), squalene cyclase from Alicyclobacillus acidocaldarius (Wendt et al., 1997),
protein farnesyltransferase from rat (Long et al., 1998; Long et al., 2000) and Rab protein geranylgeranyltransferase from rat (Zhang et al., 2000) (Figure 1-5). These structures showed the presence of the common structural motif, which was composed of 10 to 12 mostly antiparallel α-helices, called “isoprenoid synthase fold (or terpenoids synthase fold)” (Sacchettini & Poulter, 1997; Wang & Ohnuma, 1999). This fold is believed to be common fold in all enzymes related to isoprenoid biosynthesis.

1-5 cis-Prenyltransferase

Little is known about the molecular mechanism of cis-prenyltransferase (prenyltransferase IV), compared to the case of trans-prenyltransferases (prenyltransferase I, II & III). Molecular cloning of the gene for undecaprenyl diphosphate synthase (UPS), which catalyzes the cis-prenyl chain elongation onto farnesyl diphosphate (FPP, C_{15}) to produce undecaprenyl diphosphate (UPP, C_{35}), has recently been carried out on an enzyme from Micrococcus luteus B-P 26 (Shimizu et al., 1998). The UPS gene from Escherichia coli as well as from other bacteria was also identified shortly afterwards (Apfel et al., 1999; Kato et al., 1999), and the Saccharomyces cerevisiae RER2 gene has been identified to encode a cis-prenyltransferase that is essential for the biosynthesis of dolichols (Sato et al., 1999). Comparison of the deduced primary structures of the cis-prenyltransferases reveals the presence of three conserved regions, which lack the DDXXD motif and are completely
dissimilar to those of the trans-prenyltransferases (Figure 1-6) (Carattoli et al., 1991; Joly & Edwards, 1993; Koyama et al., 1993; Song & Poulter, 1994; Koyama et al., 1996; Apfel et al., 1999; Chen et al., 1994).

From the viewpoint of drug development, the physiological functions of cis-prenyl chain compounds are quite important and attractive. UPP, which is the product of UPS, works as a glycosyl carrier lipid for the biosynthesis of peptidoglycan (Figure 1-7). O-antigen, which is an important component of the outer membrane of gram-negative bacteria, is synthesized from UPP. In many cases, bacteria lacking the O-antigen are killed by complements even in the absence of a specific antibody (Goldman et al., 1984). cis-Prenyl chains are also indispensable for the biosynthesis of glycoprotein in eukaryotes. Deficiency of these cis-prenyltransferases results in the inhibition of cell wall biosynthesis in E. coli and an abnormal accumulation of ER and Golgi membranes in yeast S. cerevisiae (Kato et al., 1999; Sato et al., 1999).

1-6 UPS from Micrococcus luteus B-P 26

In order to reveal the molecular mechanism of cis-prenyltransferase (prenyltransferase IV), I tried to determine the crystal structure of undecaprenyl diphosphate synthase (UPS) from Micrococcus luteus B-P 26. This enzyme catalyzes the sequential cis-addition of isopentenyl diphosphate (IPP, \( C_5 \) compound) onto farnesyl diphosphates (FPP, \( C_{15} \)) to produce the undecaprenyl diphosphate (UPP, \( C_{35} \)) (Figure
Magnesium ion and detergent are required for the catalytic reaction same as other cis-prenyltransferases. The $K_m$ values for FPP and IPP are $8.3 \pm 1.3 \, \mu M$ and $2.3 \pm 2.8 \, \mu M$, respectively (Fujikura et al., 2000). This enzyme works as homodimer under the physiological condition, and no cofactor is found. The molecular mass of the subunit is about 29 kDa, and it composed of 249 amino acids (Shimizu et al., 1998). Here, I report the crystal structure of this enzyme as a first three-dimensional structure of cis-prenyltransferase in the world.
Figure 1-1 Isoprenoids biosynthesis pathway
Farnesyl Diphosphate (FPP; C15)

Isopentenyl Diphosphate (IPP; C5)

Undecaprenyl Diphosphate Synthase
One of the cis-prenyltransferase

Heptaprenyl Diphosphate Synthase, Octaprenyl Diphosphate Synthase etc...

trans-prenyltransferase

trans-prenyl Diphosphate

trans-type Prenylchain Elongation

Prenyltransferase IV

Prenyltransferase II and III

Figure 1-2 Reaction catalyzed by cis- and trans- prenyltransferases. Both type of enzymes catalyze the sequential condensations between allylic substrates and isopentenyl diphosphates. As shown above, prenyltransferase IV catalyze the cis-elongation, whereas prenyltransferase II and III elongate the trans-chain. Prenyltransferase I also catalyze the trans-elongation, but they do not use the farnesyl diphosphate as a primer. Farnesyl diphosphate is a typical product of prenyltransferase I.
Figure 1-3  Multiple amino sequence alignment of 9 trans-prenyltransferases. Sequences: farnesyl diposphate synthase (FPS) from *Bacillus steatorrhophilus* (FPS BST), FPS from *Escherichia coli* (FPS EC), FPS from *Avian* (FPS Avian), FPS from *Saccharomyces cerevisiae* (FPS Yeast), geranylgeranyl diposphate synthase from *Sulfobolus acidocaldarius* (GGPS Sacid), octaprenyl diposphate synthase from *E. coli* (OctPPS EC), hexaprenyl diposphate synthase from *Micrococcus luteus* B-P 26 (HexPPS Mutae), heptaprenyl diposphate synthase from *Bacillus steatorrhophilus* (HeppPPS BST), heptaprenyl diposphate synthase from *Bacillus subtilis* (HeppPPS Sbub). Seven conserved regions, I to II, are colored yellow.
Figure 1-4  A Crystal of Se-Methionyl FPS from *B. Stearothermophilus.*
Figure 1-5  Crystal structures of enzymes related to isoprenoid biosynthesis.  (a) farnesyl diphosphate synthase from Avian, (b) pentalenene synthase from *Streptomyces*, (c) 5-epi-aristolochene synthase from *Nicotiana tabacum*, (d) squalene cyclase from *Alicyclobacillus acidocaldarius*, (e) protein farnesyltransferase from rat, (f) Rab protein geranylgeranyltransferase from rat.  These figures and figures 3-10, 12, 13, 15-18, 20, 24 are prepared using *MOLSCRIPT* (Kraulis, 1991) and *Raster3D* (Merritt & Bacon, 1997; Merritt & Murphy, 1994).
A comparison of amino acid sequences in 18 proteins that have sequence homology with UPS from Micrococcus luteus B-P 26. The proteins from *M. luteus*, *E. coli*, *Haemophilus influenzae* and *Streptococcus* demonstrate UPS activity (Apfel et al., 1999; Kato et al., 1999; Shimizu et al., 1998). Yeast RER2p and Srt1p have the cis-prelyltransferase activity (Sato et al., 1999). The others are the potential cis-prelyltransferases homologous with *M. luteus* UPS. Green indicate the conserved regions (Koyama, 1999).
Chlamydophila pneumoniae, Rickettsia prowazekii, Chlamydia trachomatis, Treponema pallidum, Mycobacterium leprae, Mycobacterium tuberculosis.

Micrococcus luteus B-P 26

Yeast Srt6p

Yeast Srt2p

Escherichia coli

Haemophilus influenzae

Streptococcus pneumoniae

Bacillus subtilis

Synechocystis PCC6803

Anabaena variabilis

Mycobacterium tuberculosis

Mycoplasma leprae

Treponema pallidum

Methanococcus jannaschi

Helicobacter pylori

Chlamydia trachomatis

Rickettsia prowazekii

Helicobacter pylori, J99

Micrococcus luteus B-P 26

Yeast Srt6p

Yeast Srt2p

Escherichia coli

Haemophilus influenzae

Streptococcus pneumoniae

Bacillus subtilis

Synechocystis PCC6803

Anabaena variabilis

Mycobacterium tuberculosis

Mycoplasma leprae

Treponema pallidum

Methanococcus jannaschi

Helicobacter pylori

Chlamydia trachomatis

Rickettsia prowazekii

Helicobacter pylori, J99

Micrococcus luteus B-P 26

Figure 1-6 (continued)
Figure 1-7 Physiological functions of cis-prenyl chain compounds in prokaryotes and eukaryotes. (left) Undecaprenyl diphosphate (UPP) functions as the carrier lipid for the synthesis of sugar chain in prokaryote cell. These sugars are used for the cell wall biosynthesis. Defection of UPP synthase is lethal for the cell growth. (right) Dolichyl phosphate (Dol-P) functions as the carrier lipid for the synthesis of sugar chain in the eukaryote. These sugars are used for protein glycosylation.
Figure 1-8  Reactions catalyzed by UPS.  The C₅ compound IPP is condensed 8 times onto FPP (C₁₅ Allylic compound), step by step.  As a result, the C₅₅ compound UPP is produced.
2 Materials and Methods

2-1 Overexpression and Purification

The recombinant UPS from *M. luteus* B-P 26 was overproduced in *E. coli* cells and purified as previously described (Shimizu et al., 1998). The cells were disrupted by sonication, and the supernatant obtained after ultracentrifugation was fractionalized by 30-60% saturation ammonium sulfate. The protein was further purified by ÄKTA explorer 10S system with MonoQ and Superdex 200 HR (Amersham Pharmacia Biotech). All purification steps were carried out at 293 K, but the sample was stored at 277 K. The purity of the UPS sample was judged by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis).

2-2 Crystallization

Crystallization of UPS was carried out by the sitting-drop vapor diffusion method at 293 K. The purified UPS from *M. luteus* was dialyzed against 20 mM Tris-HCl, 100 mM sodium chloride and 1 mM DTT, and concentrated to about 15mg/ml using Centriprep-10 (Amicon). The concentration of UPS was measured by UV absorbance at 280 nm ($\varepsilon_{280}\text{nm, } 1\text{cm} = 1.23$)
Crystal form I

The reservoir solutions of form I crystals were composed of 0.1 M citrate-Na, 0.5 M ammonium sulfate and 1.0 M lithium sulfate. The mixture of protein solution (1.5 µl) and the same volume of the reservoir solution were equilibrated against the reservoir solution (100 µl) at 293 K. An assembly of form I crystals grew in about 6 weeks to an approximate size of 1.2 mm × 0.8 mm × 0.2 mm (Fujihashi et al., 1999).

Crystal form II

The reservoir solutions of form II crystals were composed of 0.1 M HEPES-NaOH pH 7.5, 2.0 M ammonium sulfate and 2.0 % (v/v) PEG400. 1 mM 4-Bromoisopentenyl diphosphate, which is an inhibitor of UPS, and 5 mM magnesium chloride were added to UPS solution. The mixture of protein solution with inhibitor (1.5 µl) and the same volume of the reservoir solution were equilibrated against the reservoir solution (100 µl) at 293 K. Crystals of form II grew to an approximate size of 0.3 mm × 0.2 mm × 0.1 mm.

2-3 Preparation of heavy atom derivatives

Heavy atom derivatives for multiple isomorphous replacement (MIR) analysis were prepared by soaking method at 293 K. Native form I crystals were transferred
into the soaking buffers, which are composed of 0.1 M citrate-Na, 0.5 M ammonium sulfate, 1.0 M lithium sulfate and heavy atom reagents shown in Table 2-1. Heavy atom compound concentrations of soaking buffer and soaking periods were also shown in Table 2-1.

2-4 Data collection and processing

The single form I crystals, with maximal size of 1.1 mm $\times$ 0.5 mm $\times$ 0.2 mm, were cut out from the assembly. Both form I and II crystals were sealed in thin wall glass capillaries with the drop of their mother liquor. Intensity data were collected using Cu $K\alpha$ radiation generated by RIGAKU ultraX 18 rotating-anode generator and synchrotron radiation at BL-6A and BL-6B beam lines of Photon Factory (PF), Institute of Materials Structure Science (IMSS), High Energy Accelerator Research Organization (KEK). Recorded data were processed and merged using the program $DENZO$ and $SCALEPACK$, respectively (Otwinowski & Minor, 1997). For subsequent calculations, data were reduced using program $TRUNCATE$ in the $CCP4$ program suite (Collaborative Computational Project, Number 4, 1994).

Data collection using Cu $K\alpha$ radiation

The rotating-anode generator was operated at a high voltage of 40 kV and a
current of 100 mA. Diffraction intensities were recorded on R-AXIS IV image plates. Conditions of data collection are shown in Table 2-1.

**Data collection using synchrotron radiation**

The synchrotron radiation was monochromatized to 1.000 Å with a Si (111) monochromator and a 0.1 mm (BL-6A beamline) and a 0.2 mm (BL-6B beamline) aperture collimators were used. A screenless Weissenberg camera for macromolecular crystals was used with a cylindrical cassettes of radius 429.7 mm at BL-6A, and of radius 573 mm at BL-6B (Sakabe *et al.*, 1995). Diffraction intensities were recorded on an 200 mm × 400 mm image plates read on Fuji BAS 2000 at BL-6A, or 400 mm × 800 mm image plates read in drum type imaging plate reader, IPR4080 (Sakabe *et al.*, 1996), at BL-6B.

2-5 Phasing

**Form I crystal**

Form I crystal were phased by multiple isomorphous replacement with anomalous scattering. Thimerosal (a kind of mercury compound) derivative and gold(I) potassium cyanide (KAu(CN)₂) derivative are found to be effective for form I
crystal. The refinement of heavy atom parameters and calculation of MIRAS phases were performed using the program MLPHARE in the CCP4 package (Collaborative Computational Project, Number 4, 1994). MIRAS phases were calculated at 3.1 Å and 3.2 Å resolutions with thimerosal and KAu(CN)\(_2\) derivatives, respectively. Non-crystallographic two-fold axis was calculated from these 6 heavy atom positions using program FINDNCS in the CCP4 program package (Collaborative Computational Project, Number 4, 1994). The MIRAS phase was improved by solvent flattening, histogram mapping, multi-resolution modification and non-crystallographic symmetric (NCS) averaging using the program DM in the CCP4 package (Collaborative Computational Project, Number 4, 1994).

**Form II crystal**

The form II diffractions were phased by the molecular replacement (MR) method using program AmoRe from the CCP4 program suite (Navaza, 1994; Collaborative Computational Project, Number 4, 1994). The form I crystal structure was used as a search model, except for the solvent and the sulfate molecules. This phase was calculated from 10 Å to 4.0 Å resolution.
2-6 Structure refinement

The Cα atoms of crystal form I were traced on the MIRAS phased electron density map using the program O (Jones et al., 1991). Phases calculated from the partially constructed model were combined with MIRAS phase using the program SFALL and SIGMAA in the CCP4 package (Read, 1990; Collaborative Computational Project, Number 4, 1994). The constructed atomic model were refined using the program X-PLOR with simulated annealing from 3000 K to 300 K, atomic B factor refinements and bulk solvent corrections at 50.0 Å to 2.2 Å resolution (Brünger, 1992; et al., 1990; Jiang & Brünger, 1994). The stereochemistry of the final model was analyzed using program PROCHECK (Laskowski et al., 1993).

The atomic coordinates of UPS from Micrococcus luteus B-P 26 have been deposited in the Protein Data Bank, www.rcsb.org (PDB ID 1F75).

2-7 Structure analysis.

Accessible surface area was calculated using SURFACE in the CCP4 package (Collaborative Computational Project, Number 4, 1994). A 3D-structural homology search was performed using the DALI algorithm (Holm & Sander, 1995). The monomer and the dimer of poly-glycine model coordinates were submitted to the DALI server (http://www2.ebi.ac.uk/dali/dali.html). The superimposition of the dimer was performed using the program LSQMAN (Holm & Sander, 1995).
<table>
<thead>
<tr>
<th>Dataname</th>
<th>Soaking</th>
<th>X-ray</th>
<th>Detector</th>
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<td></td>
<td>reagent</td>
<td>Source</td>
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<td></td>
<td>concentration (mM)</td>
<td>period (hours)</td>
<td>(Å)</td>
</tr>
</tbody>
</table>

**Native (form I)**

- **ups1**: Native, ultra18², 1.542, Raxis IV, 200
- **ups3**: Native, PF-6B, 1.000, large-IP, 573
- **ups13**: Native, PF-6B, 1.000, large-IP, 573

**Derivative (form I)**

- **hgu1**: HgCl₂, 1.0, 17.0, PF-6B, 1.000, large-IP, 573
- **thimu1**: Thimerosal (Hg), 1.0, 17.5, PF-6B, 1.000, large-IP, 573
- **thimu2**: Thimerosal (Hg), 1.0, 20.5, PF-6A, 1.000, small-IP, 429.7
- **thimu3**: Thimerosal (Hg), 1.0, 20.5, ultra18², 1.542, Raxis IV, 150
- **thimu4**: Thimerosal (Hg), 1.0, 20.0, ultra18², 1.542, Raxis IV, 150
- **ptu1**: K₂PtCl₄, 1.0, 27.0, ultra18², 1.542, Raxis IV, 150
- **ptu2**: K₂PtCl₄, 1.0, 27.0, ultra18², 1.542, Raxis IV, 150
- **ptu3**: K₂PtCl₄, 0.10, 18.5, PF-6B, 1.000, large-IP, 573
- **ptu4**: K₂PtCl₄, 0.010, 22.0, PF-6A, 1.000, small-IP, 429.7
- **bripu1**: Br-IPP, 1.0, 24.0, PF-6A, 1.000, small-IP, 429.7
- **bripu2**: Br-IPP, 1.0, 24.0, PF-6A, 1.000, small-IP, 429.7
- **uu1**: UO₂(CH₃COO)₂, 1.0, 13.0, PF-6A, 1.000, small-IP, 429.7
- **auu1**: K[Au(CN)₂], 1.0, 14.5, PF-6A, 1.000, small-IP, 429.7
- **hgiu1**: K₂HgI₄, 1.0, 12.0, PF-6A, 1.000, small-IP, 429.7
- **ptdnu1**: Pt(H₂NC₂H₄NH₂)Cl₂, 1.0, 17.5, PF-6A, 1.000, small-IP, 429.7
- **cdu1**: CdCl₂, 1.0, 15.5, PF-6A, 1.000, small-IP, 429.7
- **smu1**: SmCl₃, 1.0, 12.5, PF-6A, 1.000, small-IP, 429.7
- **iru1**: IrCl₄, 1.0, 18.0, PF-6A, 1.000, small-IP, 429.7

**Co-crystal (form II)**

- **upbrip1**: co-crystallization¹, PF-6A, 1.000, small-IP, 429.7
- **upbrip2**: co-crystallization¹, PF-6A, 1.000, small-IP, 429.7

¹Co-crystallization with Br-IPP
²Rigaku ultra 18X rotating anode-generator
3 Results and discussion

3-1 Crystallization

Purity of the UPS sample for crystallization was over 95%, judging from the SDS-PAGE. Colorless assemblies of form I crystals were appeared in about 6 weeks to an approximate size of 1.2 mm × 0.8 mm × 0.2 mm (Figure 3-1) by the sitting drop vapor diffusion method at 293 K using ammonium sulfate and lithium sulfate as precipitants (Fujihashi et al., 1999).

Form II colorless crystals were appeared to an approximate size of 0.3 mm × 0.2 mm × 0.1 mm (Figure 3-2) by the sitting drop vapor diffusion method at 293 K using ammonium sulfate as a precipitant and PEG400 as an additive.

3-2 Xray diffraction data

Crystal form I

A single crystal of form I was separated from the assembly and sealed in a glass capillary with the mother liquor. Diffraction data were collected on imaging plates R-AXIS IV with Cu Kα radiation by ultra 18X anode generator (Rigaku Co. Ltd.), running at 40kV, 100mA. The diffraction data were processed, merged and reduced as space group P1 using the programs DENZO, SCALEPACK and TRUNCATE, respectively (Otwinowski & Minor, 1997; Collaborative Computational Project,
Number 4, 1994). The pseudo-precession images (Figure 3-3), which were displayed using program HKLVIEW in the CCP4 program suite (Collaborative Computational Project, Number 4, 1994), indicated the crystal belongs to the monoclinic space group \( C2 \) with unit-cell parameters \( a=127.2 \, \text{Å}, \ b=60.2 \, \text{Å}, \ c=75.7 \, \text{Å} \) and \( \beta=105.6 \, ^\circ \) (Otwinowski & Minor, 1997). The diffraction data were re-processed as the determined space group \( C2 \) for subsequent calculations. Assuming one dimer (29 kDa \( \times \) 2) per asymmetric unit, the Matthews content, \( Vm \), is calculated to be 2.4 \( \text{Å}^3 \text{Da}^{-1} \) and the solvent content of the crystal to be 48.8%, which are within the range commonly observed for protein crystals (Matthews et al., 1968).

The crystals diffract to 1.95 Å resolution using synchrotron radiation at the BL-6B beamline at PF, KEK, Japan (Figure 3-4). The data were processed and merged at 2.2 Å resolution. The statistics of intensity data of native and heavy atom derivatives were summarized in Table 3-1.

## Crystal form II

Form II crystals were also sealed in a glass capillary with its mother liquor. The crystals diffract to 3.2 Å resolution using synchrotron radiation at the BL-6A beamline at PF, KEK, Japan. As shown in Figure 3-5, the mosaicity of this crystal is quite high. The diffraction data were processed by the same way as form I crystals, indicating form II crystals belong to the primitive orthorhombic space group with unit-cell parameters \( a = 88.00 \, \text{Å}, \ b = 98.94 \, \text{Å} \) and \( c = 55.88 \, \text{Å} \) (Figure 3-6). The \( b \) axis is parallel with the \( 2_1 \)-screw axis by the systematic absences of the reflections.
(Figure 3-7). However, because of the lack of data completeness around $a^*$ and $c^*$ axes, it cannot be determined that both $a$ and $c$ axes are parallel with $2_1$-screw axis or not. Thus, this crystal belongs to the space group $P2_2_1_2_1$, $P2_1_2_2_2$ or $P2_1_2_1_2_1$. The space group could be determined as described in section 3-4. Assuming one dimer in the asymmetric unit, the Matthews content, $V_m$ is calculated to be $2.1 \text{ Å}^3 \text{Da}^{-1}$ and the solvent content of the crystal to be 41%, which are within the range commonly observed for protein crystals (Matthews et al., 1968).

3-3 Structure determination

The crystal structure of UPS was determined using form I crystal.

Phasing of crystal form I

Phasing for the crystal form I was accomplished by the multiple isomorphous replacement method with anomalous scattering using thimerosal and KAu(CN)$_2$ derivatives (Table 3-2). Four mercury atoms and two gold atoms were found in the asymmetric unit of thimerosal and KAu(CN)$_2$ derivatives, respectively (Table 3-3) (Figure 3-8). The two of four mercury positions gave major sites (Hg1 and Hg2) and the remaining positions gave minor sites (Hg3 and Hg4). The major two mercury atom sites were almost the same as the two gold atom positions (Table 3-3). The electron density map with initial MIRAS phases was improved by the histogram
mapping, multi-resolution modification at 3.1 Å. The improved map enabled us to construct the mask of protein region and incorporate the several α-helices and β-strands (Figure 3-9). The electron density modification with non-crystallographic symmetry averaging did not afford significant improvements of the quality.

Model building of crystal form I

Because no three-dimensional structures similar to UPS were available, the model building was started from the construction of poly-alanine model. Introductions of individual residues for UPS were started from 210W and 224W based on the large electron density of tryptophan’s side-chain. The model refinement and phase combinations were then performed step by step, and almost all residues could be reasonably substituted. The mercury atoms (Hg1 and Hg2) at the major sites and gold atoms (Au1 and Au2) of derivatives were bound to 235C of each monomer, and mercury atoms (Hg3 and Hg4) at the minor positions to 212C. On this stage, the crystallographic $R$ and free $R_f$ factors with XYZ coordinates refinement from 10 Å to 2.6 Å resolution were 31.2 % and 40.2 %, respectively.

Water molecules were assigned to residual density peaks in the Fo-Fc maps. Two of these residual density peaks were about three times higher than the other, and related by the non-crystallographic two-fold axis. These electron density peaks were surrounded by several nitrogen atoms and no oxygen atoms. These positions were suitable to bind with negatively charged molecules. In addition, these electron density peaks were remained even in the Fo-Fc map, which was drown by the model
containing the water molecules at those positions. Based on these features, it is reasonable to assign sulfate ions to these peaks. These sulfate ions were suggested to come from the crystallization buffer.

Finally, all the residues were incorporated, except for the N-terminal residues 1M to 13N of one monomer and 1M to 18A of the other monomer, residues from 74S to 85V of both monomers, and the C-terminal 243H to 249L of both monomers. The electron densities corresponding to residues 86N to 90M of both monomers were very broad. The final crystallographic $R$ and free $R$ values with simulated annealing, $B$-factor and bulk solvent refinements from 50 Å to 2.2 Å resolution were 19.6 % and 24.6 %, respectively (Table 3-4). The molecular packing in the crystal is shown in Figure 3-10.

The stereochemistry of the final refined model was reasonable, no residues lied at the unfavorable regions in the Ramachandran plot (Figure 3-11a), the main chain stereochemical parameters were inside or much better regions (Figure 3-11b and c), and the side-chain geometry was within expected regions for chi-1 and chi-2 stereochemical parameters. The root-mean-square deviations from standard values were 0.006 Å for bond length and 1.12 ° for bond angles, respectively (Table 3-4).

3-4 MR phasing of crystal form II

The form II crystal was phased by the molecular replacement method using the
form I crystal structure of UPS as a search model. The rotation search was calculated using 10.0 Å to 4.0 Å resolution range gave two clear solutions (Rot1 and Rot2, Table 3-5), which were related by non-crystallographic symmetry. The translation search of the space group $P2_12_12_1$ for each solution of rotation function gave a clear solution (Trans d-1, Table 3-5), but those of the space group $P22_2_1$ and $P2_12_12_1$ gave no solution. This indicates that the form II crystals belong to the primitive orthorhombic space group $P2_12_12_1$. No unfavorable molecular contacts were observed in the crystal packing (Figure 3-12). After the rigid-body refinement, the correlation and crystallographic $R$ factors were 63.6 and 36.4%, respectively (Table 3-5).

Because of the lack of the completeness and the high mosaicity of the diffraction data, the electron density map was very poor. The atomic model could not be incorporated into the electron densities, and it was unclear that Br-IPP is bound to UPS or not. Several refinements including positional refinement were performed, but no significant improvement was observed. Crystallization and data collections of form II crystal are in progress.

3-5 Overall Structure of UPS

The overall structure of UPS from *M. luteus* B-P 26 was determined at 2.2 Å resolution as the first three-dimensional structure in all *cis*-prenyl chain elongating enzymes. The asymmetric unit contains one physiological homodimer, in which each
monomer is crystallographically independent. The front view of the dimeric form of UPS looks like the face of an elephant (Figure 3-13). 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) (Figure 3-14). The β-strands form a central β-sheet core, which is surrounded by five of the seven α-helices (H1, H2, H3, H5 and H10). Additionally, there are five short 3_10-helices (H3, H4, H7, H8 and H9) in each monomer. The Cα trace of the UPS monomer is shown in Figure 3-15.

Dimer interface

The contact interface of the dimer is about 15 % of the total surface of UPS. As shown in Figure 3-16, considerable numbers of hydrophobic residues are found in the subunit-subunit interface. The hydrophobic surface composed of these residues is buried in the dimer structure. Hydrophilic interactions such as hydrogen bonds and charged interactions are found in the peripheral of the interface. Ion-bond is found only between guanidinium group of 151R and carboxyl group of 191D.

Comparison to related enzymes

The fold of UPS is completely different from those of other isoprenoid biosynthesis-related enzymes. These enzymes, including FPS (Tarshis et al., 1994; Tarshis et al., 1996), pentalenene synthases (Lesburg et al., 1997), 5-epi-aristolochene
synthases (Starks et al., 1997), squalene cyclase (Wendt et al., 1997), protein farnesyltransferase from rat (Long et al., 1998; Long et al., 2000) and Rab protein geranylgeranyltransferase from rat (Zhang et al., 2000), have a common structural motif (Figure 3-17). This motif is called “isoprenoid synthase fold (or terpenoids synthase fold)” (Sacchettini & Poulter, 1997; Wang & Ohnuma, 1999), 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 “isoprenoid synthase fold.” Additionally, it was found that UPS belongs to a new fold family of proteins, judging from the multiple alignment of 3D structural neighbours search (Figure 3-18). This implies that the catalytic mechanisms of UPS, including substrate recognition, are also different from those of other enzymes related to isoprenoid biosynthesis.

3-5 Substrate Binding Site

Both FPP and IPP, the substrates of UPS, consist of negatively charged diphosphates and hydrophobic carbon chains. Thus, it is clear that the diphosphates must be recognized by the positively charged residues, and the carbon chains must be bound to the hydrophobic residues. As shown in Figure 3-19 and 20, UPS has a large cleft on its molecular surface that is surrounded by the S2 and S4 strands and the H2 and H3 helices. Most of the conserved amino acid residues among
cis-prenyltransferases are located in this cleft (Figure 3-20). The interior of the cleft mainly consists of hydrophobic residues and four arginine residues (R33, R42, R197 and R203) located at the entrance form a positively charged cluster. This cleft seems to be suitable for recognition of the substrates, as hereafter discussed.

**Structural P-loop motif**

At the entrance of this cleft, a common motif for phosphate recognition called a “structural P-loop” was found. Structural P-loop motifs are found in many phosphate-binding enzymes such as nucleotide triphosphate hydrolase, phosphofructokinase, c-AMP binding domain and sugar phosphatase (Kinoshita et al., 1999). This motif generally consists of four residues located in the N-terminal of an α-helix, in which glycine at the N-terminal is followed by three strongly conserved residues among related enzymes with similar functions. In the UPS structure, the P-loop motif is composed of 30G, 31N, 32G and 33R located at the N-terminal of the H1 helix. In the P-loop sequence, 30G, 31N, and 33R are strictly conserved among cis-prenyltransferases, as shown in Figure 3-21. On the other hand, Gly and Arg are alternatively located at the positions corresponding to the 32nd residue (in the *M. luteus* UPS sequence) (Figure 3-21), which is located close to the 42nd residue in the three-dimensional structure (Figure 3-22). Interestingly, in many cis-prenyltransferases that have Gly at the position corresponding to the 32nd, Arg is always located at the position corresponding to the 42nd, whereas the residue is not
specified when the residue corresponding to the 32nd is Arg (Figure 3-21). In UPS from *M. luteus*, 42R is one of the arginine residues in the positively charged cluster at the entrance of the cleft, and it is close to 32G as shown in Figure 3-22. When 32G is replaced with arginine in cis-prenyltransferases, the guanidinium group of the replaced arginine (32R) may occupy the space for the guanidinium group of 42R (Figure 3-23). This indicates that Arg at the 32nd or 42nd position in the three-dimensional structure is complementarily conserved among cis-prenyltransferases. In other words, at the entrance of the cleft, an arginine residue is conserved in the restricted position in order to recognize the diphosphate group of the substrates for this enzyme.

**A sulfate ion**

A sulfate ion used in crystallization as a precipitant binds to the four residues of the P-loop motif (Figure 3-22) as described in section 3-3. The electron density for this ion is larger than that of water and it is not located near the main chain O atoms but is surrounded by the main chain N atoms of the P-loop residues and also by the guanidinium group of R33. These main chain N atoms of 30G, 31N, 32G and 33R and the guanidinium group of 33R could be hydrogen bonded to the O atoms of the sulfate ion. It has been reported that the main chain N atoms of the four residues of the P-loop motif usually bind to the phosphate group of the substrates of many phosphate-binding enzymes (Kinoshita et al., 1999). These observations suggest that the diphosphate group of the substrate is electrostatically recognized by the P-loop
motif in the same position where the sulfate ion is located.

**Flexibility around the cleft**

The catalytic site might be highly flexible, which is necessary for substrate recognitions, catalytic reactions and product releases. The residues from 74S to 85V could not be incorporated even in the finally refined model because of their invisible electron densities. As deduced from the positions of 73F and 86N, these undefined residues are assumed to be located near the cleft (Figure 3-20). The superposition of the two equivalent monomers in the dimer shows that most of the Cα atoms are well superimposed on each other (r.m.s.d. of Cα atoms = 0.49 Å) except for the region from 86N to 95F, which is adjacent to the disordered loop (Figure 3-24). It is noteworthy that the residues between 71Y and 91L, which are highly conserved among cis-prenyltransferases (Apfel et al., 1999), show a high mobility (average B factor = 66.8 Å²) in the UPS structure. Additionally, one of the cluster arginines, 42R, which is a residue complementarily conserved with 32G and in close contact with the sulfate ion, also has a large B-factor (B = 74.8 Å²). Each 42R in the two monomers shows different features in binding with the sulfate ion. The guanidinium group of 42R in one monomer is hydrogen-bonded to the O atoms of the sulfate ion. On the other hand, no atoms of 42R in the other monomer interact directly with the sulfate ion. However, the electron density of the side chain of 42R is very broad, suggesting the existence of several side chain conformers. The guanidinium group of 42R in one
such conformer can bind to the sulfate. The high flexibility around this cleft must play an essential role in catalytic reactions.

**Site-directed mutagenesis**

Site-directed mutagenesis analysis also supports that this cleft is the catalytic site of UPS (Fujikura *et al.*, 2000). Substitutions of 77N significantly decrease the $k_{cat}$ value and that of 78W increase the $K_m$ value for FPP. These results indicate that 77N plays an important role in catalytic reaction, and 78W is the binding residue with FPP (Fujikura *et al.*, 2000). Although these two residues could not be incorporated into the final refined structure, positions of 73F and 86N suggest that 77N and 78W must locate at the front of this cleft (Figure 3-20).

### 3-6 Catalytic mechanism

A hypothetical substrate-binding model is shown in Figure 3-25. In this model, the diphosphate group of FPP binds to the guanidinium group of 33R and to the main chain N atoms of 30G, 31N, 32G and 33R, which form the P-loop motif in the present UPS structure. The carboxyl group of 29D also binds to this diphosphate group through the magnesium bridge. In site-directed mutagenesis studies (T. Koyama *et al.*, personal communication), replacements of 33R to other residues
increase the $K_m$ value for FPP, and that of 29D results in the significant decrease of $V_{\text{max}}$ value. The hydrophobic carbon chain of FPP may be recognized by the hydrophobic cleft. On the other hand, the diphosphate group of the substrate IPP binds to the guanidinium groups of 197R and 203R and to the carboxyl group of 216E of the other monomer through the magnesium bridge. Substitution of 197R, 203R and 216E results in the increase of $K_m$ value for IPP and significant decrease of the $V_{\text{max}}$ value (T. Koyama et al., personal communication). Dimerization of UPS must be indispensable for the catalytic reaction, because the IPP binding site located at the dimer interface is composed of the residues of both monomers.

A plausible mechanism of the enzymatic condensation between FPP and IPP is as follows (Fig. 3-26). The reaction may be triggered by the release of the diphosphate group of FPP as previously observed in the FPS reaction (Poulter & Rilling, 1978). This release produces the allylic farnesyl cation, where the positive charge is located at C1. The $\pi$ electron at C4’ of IPP might attack this positive charge, and then the covalent bond between C1 and C4’ could be formed. A strongly conserved residue 74S is assumed to abstract a C2’ proton of IPP. This residue could reasonably interact IPP, although the position of 74S could not be incorporated into the refined model. Site-directed mutagenesis on 74S leads to decrease of $V_{\text{max}}$ values (T. Koyama et al., personal communication). By repetition of this stereochemically controlled reaction, the $cis$-prenyl chain must be elongated until the hydrophobic carbon chain reaches the fixed length of C55 by moving into the inside of the hydrophobic cleft (Figure 3-25). The final product UPP may be bound to the cleft with bending
conformation, and the exact chain length may be determined by the size of this hydrophobic cleft.
Figure 3-1  Assembly of form I crystals of UPS from *M. luteus*. 
Figure 3-2  Form II crystal of UPS from *M. luteus*.
Figure 3-3  Pseudo precession photographs. These images were prepared from the data of ups1 (Table 3-1) processed as P1. Axes described following are indexed as space group C2.  

(a) A plane perpendicular to c* axis. The red line represents the mirror plane and is parallel to a* axis.  

(b) A plane perpendicular to b* axis. The oblique and horizontal lines represent the a* and c* axes, respectively. The small red circle indicates the 2-fold axis along with crystallographic b* axis. These images were prepared using HKLVIEW in the CCP4 program package (Collaborative Computational Project, Number 4, 1994).
Figure 3-4 Oscillation photographs of ups13 (crystal form I, table 3-1). These images were taken at BL-6B beamline at PF.
Figure 3-5  Oscillation photographs of upbrip2 (crystal form II, table 3-1). These images were taken at BL-6A beamline at PF. (a) whole image and (b) enlarged one.
Figure 3-6  Pseudo precession photographs of crystal form II. These images were prepared from the data of upbrip2 (crystal form II, table 3-1) processed as $P1$.  (a) (2 k l) plane.  Mirror planes are observed along with both horizontal ($b^*$) and vertical ($c^*$) lines.  (b) (h 20 l) plane.  Mirror planes are observed along with both horizontal ($a^*$) and vertical ($c^*$) lines.  These figures were prepared as Figure 3-3.
Figure 3-7  (a) (0 k l) plane of pseudo precession photograph of upbrip2 (crystal form II, table 3-1). Systematic absences are observed along with the horizontal (b*) line. This image was prepared as Figure 3-6.  (b) Systematic absences along with b* axis.
Figure 3-8  Harker sections of difference Patterson maps between (a) ups13 (native) and thimu2 (derivative), (b) ups13 (native) and auu1 (derivative). The peaks correspond to heavy atom sites were indicated by circles.
Figure 3-9  Initial electron density map phased by MIRAS and improved by density modification.  (a) Electron density of unit-cell. Yellow lines indicate the unit-cell. Electron densities of UPS and mercury atoms of derivative are shown in cyan and red, respectively.  (b) Electron density of a helix.
Figure 3-10 UPS molecules packing into the form I crystal. The green line indicate the unit cell of the crystal. Figures (a) and (b) are related with 90 rotation along with horizontal axes. (c) Stereo figure.
Rhamachandran Plot

Figure 3-11 Analysis of the stereochemistry using PROCHECK (Collaborative Computational Project, Number 4, 1994). (a) Ramachandran plot. Only Ala-17 of a monomer and Glu-201 of the other monomer falls in generously allowed region. (b) Main chain and (c) side chain parameters. All parameters are inside or better expected regions.
Main-chain parameters

(a) Ramachandran plot quality assessment

(b) Peptide bond planarity - omega angle st dev

(c) Measure of bad non-bonded interactions

(d) Hydrogen bond energies

(e) Zeta angle st dev

(f) H-bond energy st dev

(g) Overall G-factor

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Figure 3-11 (continued)
Side-chain parameters

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Figure 3-11 (continued)
Figure 3-12  UPS molecules packing into the form II crystal. These figures were prepared as figure 3-10. (a) and (b) These figures are related with 90° rotation along with horizontal axes. (c) Stereo figure.
Figure 3-13  Overall structure of UPS from *M. luteus*. A front view *(a)* and a top view *(b)* of the dimer structure (ribbon model). One monomer of UPS is shown by red helices (α- and 3₁₀- helices) and yellow arrows (β-strands), and the other monomer by blue helices and green arrows. Helices (H1 - H10) and strands (S1 - S6) are labeled together with the sulfate ions found in the crystal structure.
Figure 3-13 (continued)
Figure 3-14  A topology diagram of the secondary structure of the UPS dimer. α- and 3₁₀-helices of one monomer and those of the other monomer are colored pink, orange, blue and purple, respectively. β-strands of one monomer and those of the other one are colored yellow and green, respectively. Numbers surrounded by rectangle indicate the residue numbers.
Figure 3-15  A stereo view of the Ca trace of the UPS monomer, with every tenth amino acid numbered.
Figure 3-16 A stereo view of the interface of the UPS dimer. Brown lines indicate the Cα trace of the monomer.
Figure 3-17  Crystal structures of enzymes that have the “isoprenoid synthase fold” and UPS.  (a) FPS from Avian, (b) pentalenene synthase from Streptomyces, (c) 5-epi-aristolochene synthase from Nicotiana tabacum, (d) squalene cyclase from Alicyclobacillus acidocaldarius, (e) protein farnesyltransferase from rat, (f) Rab protein geranylgeranyltransferase from rat, (h) UPS from M. luteus.  Except for UPS, no enzyme have β-sheet in the core.
Figure 3-18  Crystal structures of UPS and enzymes relatively similar to UPS folding judged by Dali algorithm.  (a) Carboxyl methylesterase from *Salmonella Typhimurium* (PDB 1CHD),  (b) d-glyceraldehyde-3-phosphate-dehydrogenase from *Palinurus versicolor* (PDB 1DSS) , (c) enzyme IIb of the cellobiose-specific phosphotransferase system from *Escherichia coli* (PDB 1IIB), (d) xylose isomerase complex with xylose and MnCl$_2$ from *Streptomyces rubiginosus* (PDB 4XIS).  These four enzymes do not work as homodimer, thus monomer structures are shown in these figures.  (e) UPS from *M. luteus*.  Structures shown in figures (a) to (d) are dissimilar to UPS structure.
Large Cleft

View from this direction

Red...Negative Charge
Blue...Positive Charge

Figure 3-19 Molecular surface of UPS from *M. luteus*. Red and blue indicate negative and positive charges, respectively. A large hydrophobic cleft are surrounded by pink circle, and the entrance is positively charged. (a) Front view (almost same direction with figure 3-13 (a)). Green arrows indicate the direction of (b) (top view) and (c) (bottom view). These figures were prepared using GRASP (Nicholls & Honig, 1991)
Figure 3-20  Space filling model of the UPS dimer.  C atoms are indicated in yellow and gray, in which the former color corresponds to conserved residues and the latter non-conserved ones among the cis-prenyltransferase family.  These conserved residues form a large hydrophobic cleft indicated by black dotted circle.  N, O and S atoms are colored in blue, red and green, respectively.  White line indicates the interface of the dimer, where the left and right monomers are named A and B.  White dotted line shows the disordered residues from 74S to 85V.
Figure 3-21  A comparison of amino acid sequences in 13 proteins that have sequence homology with UPS from *M. luteus* B-P 26 (Shimizu *et al.*, 1998; Apfel *et al.*, 1999). Only residues corresponding to 28 to 46 of *M. luteus* UPS are presented. The proteins from *M. luteus*, *E. coli* and *Haemophilus influenzae* demonstrate UPS activity (Shimizu *et al.*, 1998; Apfel *et al.*, 1999; Kato *et al.*, 1999). RER2p from *S. cerevisiae* has the *cis*-prenyltransferase activity (Sato *et al.*, 1999). The others are the potential *cis*-prenyltransferases homologous with *M. luteus* UPS.

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<td><em>Saccharomyces cerevisiae</em> (Rer2p)</td>
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<td><em>Saccharomyces cerevisiae</em> (Srt1p)</td>
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<td><em>Caenorhabditis elegans</em></td>
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Figure 3-22  Stereo view of the electron density map around the structural P-loop motif and the sulfate ion. The final refined structure is superimposed. This figure was prepared using program O (Jones et al., 1991).
Figure 3-23 Three-dimensionally conserved guanidinium group of arginine. The positively charged guanidinium group of either (a) 32R (in case of 42G) or (b) 42R (in case of 32G) binds to the diphosphate head of the substrate instead of the sulfate ion in the crystal structure.
Figure 3-24 Superimposition of monomer A and B. Red and blue lines trace the Cα atoms of A and B subunits, respectively. The transparent ribbon model show the original (before superimposition) dimer structure. The left and right circle surround the residues from 86N to 95F and 242R, respectively. Except for these two regions, all Cα atoms are well superimposed.
Figure 3-25  Stereo view of a hypothetical FPP and IPP binding model with UPS. For FPP and IPP, C, O and P atoms are shown in green, magenta and orange, respectively. White line indicates the interface of the dimer, where the left and right envelopes (colored gray) are the surfaces of the monomers A and B, respectively. Among two C2’ protons of IPP, the abstracted one by UPS is colored in dark red. This figure was prepared using MOLSCRIPT (Kraulis, 1991), GRASP (Nicholls & Honig, 1991) and Raster3D (Merritt & Bacon, 1997; Merritt & Murphy, 1994)
Figure 3-26  Schematic presentation of UPS binding with FPP and IPP to propose the catalytic mechanism. The UPS structure is shown in green.
Table 3-1  Data Statistics

<table>
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<tr>
<th>Dataname</th>
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<th>$R_{\text{merge}}$</th>
<th>Completeness $I/\sigma(I)$</th>
<th>$R_{\text{iso}}$</th>
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<td></td>
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<td>$a$(Å)</td>
<td>$b$(Å)</td>
<td>$c$(Å)</td>
<td>$\beta$ ( )</td>
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<td>50-3.3 (3.42-3.30)$^3$</td>
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$^1$ $R_{\text{merge}} = \Sigma |I| - <I>/ \Sigma <I>$, where $I$ is the observed intensity and $<I>$ is the average intensity over symmetry equivalent measurements.

$^2$ $R_{\text{iso}} = \Sigma |F_{PH}| - |F_P| / \Sigma |F_P|$, where $F_{PH}$ and $F_P$ are the derivative and native structure factors, respectively.

$^3$ Values in parentheses are for the outmost resolution shell.

$^4$ $C$ monoclinic

$^5$ $P$ orthorhombic
Table 3-2  X-ray data collection and phasing statistics

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<td>1.000</td>
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<td>C₂</td>
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<td>c (Å)</td>
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<td><strong>Phasing Statistics</strong></td>
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<td>0.82 / 0.74</td>
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<td>CullisR acentric / centric</td>
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<td>CullisRanomalous</td>
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<td>Mean figure of merit (20.0-3.1 Å)</td>
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¹ C₉H₉HgNaO₂S
² Values in parentheses are for the outmost resolution shell.
³ Rmerge = Σ |I - <I>| / Σ <I>, where |I| is the observed intensity and <I> is the average intensity over symmetry equivalent measurements.
⁴ Riso = Σ|FPH - |FP|| / Σ|FP|, where FPH and FP are the derivative and native structure factors, respectively.
⁵ Phasing power = < |FH| / ||FPH| - |FP|| >, where FH, FPH and FP are the heavy atom, derivative and native structural factors, respectively.
⁶ CullisR = < ||FPH| - |FP+FH|| > / < ||FPH|| - |FP|| >
⁷ CullisRanomalous = < |FPH(+) - FPH(-)| - 2 × FH × sin αp > / < |FPH(+) - FPH(-)| >, where αp is the protein phase.
<table>
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<th>x</th>
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<th>z</th>
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<th>occupancy (anomalous)</th>
<th>B-factor</th>
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Data: thimu2

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## Table 3-4  Refinement statistics

### Data Statistics

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<td>unique</td>
<td>23530</td>
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<tr>
<td><strong>Completeness (%)</strong></td>
<td>83.7 (56.8)</td>
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<td>&lt;I/ó(I)&gt;</td>
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<tr>
<td><strong>R_{merge} (%)</strong></td>
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### Refinement Statistics

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### Model Statistics

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1 Values in parentheses are for the outmost resolution shell.

2 \( R_{merge} = \sum |I_i - <I_i>| / \sum <I_i> \), where \( I_i \) is the observed intensity and \( <I_i> \) is the average intensity over symmetry equivalent measurements.

3 \( R = \sum |F_{obs} - |F_{calc}|| / \sum |F_{obs}| \). \( R_{free} \) is the same as \( R \), but for a 5% subset of all reflections that were never used in crystallographic refinement.
Table 3-5  Solutions of molecular replacement

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<th>γ</th>
<th>ı</th>
<th>ı’</th>
<th>R</th>
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<table>
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<th>ı</th>
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<th>R</th>
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<tr>
<td>Rigid Body</td>
<td>α</td>
<td>β</td>
<td>γ</td>
<td>ı</td>
<td>ı’</td>
<td>R</td>
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<td>43.25</td>
<td>312.57</td>
<td>0.1251</td>
<td>0.4494</td>
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</table>

1^Euler angle
2^Fractional coordinates
3^Correlation coefficient between observed $F$ and calculated $F$
4^Crystallographic $R$ value
References


Collaborative Computational Project, Number 4, (1994). Collaborative Computational


Koyama, T., Obata, S., Osabe, M., Takeshita, A., Yokoyama, K., Uchida, M., Nishino, T.


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