Crystallization and preliminary X-ray crystallographic analysis of human geranylgeranyl pyrophosphate synthase*

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Abstract Human geranylgeranyl pyrophosphate synthase (GGPS) is an enzyme that catalyzes the synthesis of geranylgeranyl pyrophosphate (GGPP) from farnesyldiphosphate and isopentenyldiphosphate. Recombinant human GGPS was crystallized by the hanging-drop vapor diffusion method. Crystals were grown at 18℃ using PEG 4000 as precipitant. Diffraction data were obtained to a resolution of 2.8 Å from a single frozen crystal belonging to space group P1, with unit-cell parameters: $a=68.9$ Å, $b=107.7$ Å, $c=137.4$ Å, $\alpha=99.6°$, $\beta=97.6°$, $\gamma=97.8°$.

Keywords: human geranylgeranyl pyrophosphate synthase, crystallization, space group, unit-cell parameter.

Geranylgeranyl pyrophosphate synthase (GGPS) is a member of the prenyltransferase family and encodes a protein with geranylgeranyl pyrophosphate (GGPP) synthase activity. Prenyltransferases (prenyl diphosphate synthase) catalyze the condensation of isopentenyl diphosphate with allylic diphosphate to produce isoprenoids of various chain lengths, which are precursors for numerous types of isoprenoid compounds such as steroids, respiratory quinoes and prenylated proteins. These enzymes are classified according to the chain length of the final product and the geometry formed by the double bonds in a condensation reaction (*trans*- or *cis*- type). The short-, medium-, and long-chain prenyltransferase yields C10-25, C30-35, and C40-50 products, respectively[1].

Comparison of the amino-acid sequence has revealed that two highly conserved aspartate-rich motifs, FARM (the first aspartate-rich motif) and SARM (the second aspartate-rich motif), act as binding sites for allylic substrates and isopentenyl diphosphate respectively[2]. A crystal structure of avian farnesyl diphosphate synthase (FPS) revealed that α-helices constitute a reaction cavity in the center of a subunit of the enzyme, and that FARM and SARM exist in distinct α-helices and face each other on different sides of the rim of the cavity[3]. Several mutagenic studies have revealed that aromatic amino acids, frequently found at the fourth or fifth position before FARM and two amino acids inserted into FARM, are called the chain-length determinant region (CLD) of the short-chain enzymes, which fix the chain-length of products in short-chain (all-*trans*) prenyl diphosphate synthase. It is thought that these aromatic amino acids are involved in the formation of the bottom of a reaction cavity to prevent further elongation of the prenyl chain of the final products[4—7].

Geranylgeranyl pyrophosphate (GGPP) synthase (GGPS) catalyzes the synthesis of all-*trans*-geranylgeranyl pyrophosphate from farnesyldiphosphate (FPP) and isopentenyl diphosphate (IPP). GGPP, of which carbon chain length is C20, is used as precursor in the synthesis of carotenoids, geranylgeranylated protein, and chlorophylls. These GGPS are classified into three types based on their amino acid sequences: type-I (archaea) and type-II (plants and eubacteria) and the type-III GGPS found in eukaryotes (except plants). For type-I GGPS, a large amino acid occupies the 4th or 5th position prior to the first DDxxD motif to block further elongation of the final product; type-II GGPS contains two amino acids inserted within the first DDxxD motif, and type-III GGPS lacks any bulk amino acid at these positions. Mutagenic analysis showed that the histidine residue at the second position before the conserved G

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(Q/E) motif of the type-III geranylgeranyl pyrophosphate synthase from *Saccharomyces cerevisiae* affects the chain length of product and might function in a manner similar to those of the aromatic amino acids before FARM in some other short-chain enzymes[8].

The gene encoding GGPS was localized in human chromosome band 1q43[9]. GGPS mRNA is ubiquitously expressed in various human tissues and encodes a protein of 300 amino acids. Here we report the purification, crystallization and preliminary crystallographic studies of human GGPS in order to understand the function and properties of this protein more clearly.

1 Materials and methods

1.1 Protein expression and purification

The GGPS gene was amplified using the polymerase chain reaction (PCR) method. The forward primer 5′-CGGGATCCATGGAGAAGACTCAAGAAACAG-3′ and the reverse primer 5′-CCGCTCGAGTTATTCATTTTCTTCTTTGAAC-3′ were designed. The PCR product was digested with *Bam*H I and *Xho* I, purified and ligated into the *Bam*H I and *Xho* I restriction sites of the pGEX-6p-1 vector (Pharmacia). The recombinant plasmid was transformed into *Escherichia coli* strain BL21(DE3). The transformed cells were then cultured at 37 ℃ in LB medium containing 50 μg/mL Ampicillin. When the culture density reached an A_{600} of 0.6—0.7, 0.4 mmol/L isopropyl-β-D-thiogalactopyranoside (IPTG) was introduced and cell growth continued for 14—16 hours at 16 ℃. After cultivation, the cells were harvested by centrifugation at 5000 *g* for 10 min.

The bacterial cell pellet was resuspended in 1× PBS buffer (140 mmol/L NaCl, 2.7 mmol/L KCl, 10 mmol/L Na_2HPO_4, 1.8 mmol/L KH_2PO_4, pH 7.3) and was homogenized by sonication. The lysate was centrifuged at 20000 *g* for 30 min to remove the cell debris. The supernatant was applied to Glutathione Sepharose 4B matrix column and the contaminant protein was washed off with the 1× PBS buffer. The fusion GGPS protein was then cleaved by PreScission protease at 4 ℃ to remove the N-terminal glutathione S-transferase (GST) tag and the target GGPS protein was eluted with 1× PBS buffer. The protein was introduced into a Resource Q (Amersham Pharmacia, USA) ion-exchange chromatography column equilibrated with 20 mmol/L Tris pH 8.0 and eluted with a linear gradient of 0—500 mmol/L NaCl in the same buffer. The pooled fractions were further purified by gel filtration using a Superdex200 HR 10/30 (Amersham Pharmacia, USA) column equilibrated with 20 mmol/L Tris pH 8.0, 150 mmol/L NaCl. The purity of GGPS was estimated to be greater than 95% by SDS-PAGE.

1.2 Crystallization

The purified protein was dialyzed against crystallization buffer (5 mmol/L Tris-HCl pH 8.0, containing 20 mmol/L NaCl, 5 mmol/L $MgCl_2$, 2 mmol/L DTT) and concentrated to 15—20 mg/mL. Protein concentration was determined by absorbance at 280 nm, assuming an A_{280} of 1.040 for a 1.0 mg/mL solution. Crystallization trials were performed by the hanging-drop vapor-diffusion method at 18 ℃ in 16-well plates. Hampton Research Crystal Screen Kits I and II (Riverside, CA, USA) were used for initial screening crystallization trials. Micro crystals were obtained with reagent No. 17 of Crystal Screen I, which contains 30% PEG4000, 0.1 mol/L Tris HCl pH 8.5, 0.2 mol/L lithium sulfate. Well-diffracting crystals of GGPS were obtained by mixing 1.2 μL of the GGPS protein solution with 0.25 μL 30% (w/v) 1, 6-hexanediol as an additive and 1.0 μL reservoir solution (0.1 mol/L MES pH 6.2, 0.2 mol/L lithium sulfate, 15% PEG4000), and equilibrating with 200 μL of the reservoir solution. Crystals were obtained within 5 days (Fig 1).

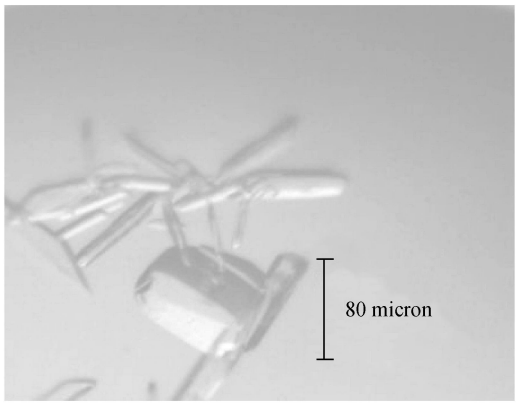

Fig. 1. A single crystal of GGPS.

1.3 X-ray crystallographic studies

A set of diffraction data was collected from a single GGPS crystal in house on the Rigaku MM-007 rotating copper anode X-ray generator operated at 40

kV and 20 mA (λ=1.5418 Å) with a R-AXIS IV++ detector. Crystals were picked up from the hanging drop with a nylon crystal loop (Hampton Research, USA) and then flash-frozen prior to data collection at 100 K using the reservoir solution as cryoprotectant. The crystal to detector distance was 150 mm. A total of 720 rotation images were collected with an oscillation angle of 0.5° with the exposure time of 300 seconds for each image. Data processing and scaling was performed using HKL2000 program and SCALEPACK[10].

2 Results and discussion

We initially obtained dozens of GGPS crystals using the reservoir solution containing 0.2 mol/L lithium sulfate and 15% PEG4000 in 0.1 mol/L MES pH 6.0, because there were too many crystals in a single drop, which influenced the quality of them, they were unsuitable for X-ray diffraction. Therefore, further crystallization optimization was performed by adding additives to the reservoir solution in order to increase the size of crystal and improve their quality. Fortunately, a few additives, especially 3%(w/v)1, 6-hexanediol have obvious effects on the crystal quality. Crystals grown from the optimized reservoir solution containing 0.1 mol/L MES pH 6.2, 0.2 mol/L lithium sulfate, 15%PEG4000 with 3%(w/v)1, 6-hexanediol as an additive were found to be more suitable for X-ray diffraction and diffracted to 2.8 Å resolution finally (Fig. 2).

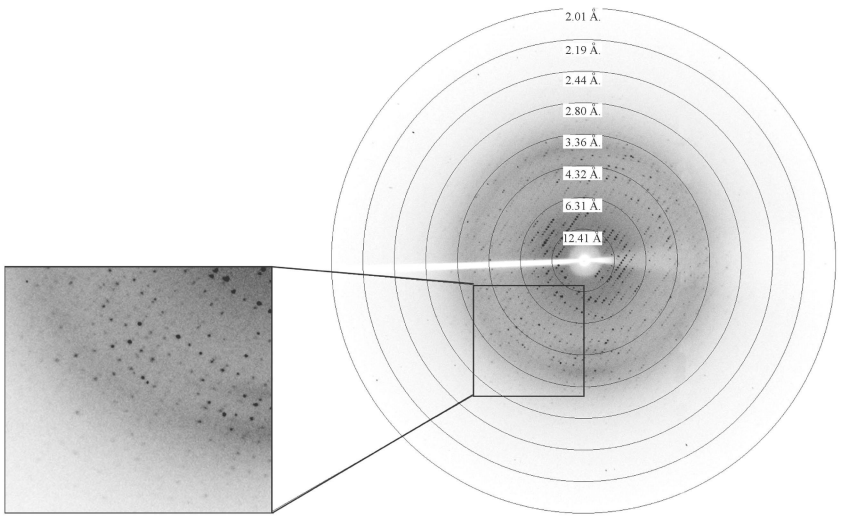

Fig. 2. A typical diffraction image of GGPS crystals.

The crystal belongs to a primitive triclinic lattice, with unit-cell parameters $a=68.9$ Å, $b=107.7$ Å, $c=137.4$ Å, $\alpha=99.6°$, $\beta=97.6°$, $\gamma=97.8°$. Self-rotation function and solvent content suggest that each asymmetric unit may contain twelve molecules. Assuming that there are twelve molecules per asymmetric unit (MW=35.3 kD), Mathews coefficient[11] is 2.4 $Å^3\ Da^{-1}$ with solvent content of 48.1% (Table 1). Complete data-collection statistics are shown in Table 2.

Table 1. The result of Matthews coefficient calculation

Nmol/asym	Matthews Coeff ($Å^3\ Da^{-1}$)	Solvent (%)
10	2.9	56.7
11	2.6	52.4
12	2.4	48.1
13	2.2	43.8
14	2.0	39.4

Table 2. Data collection and processing statistics

Parameters	GGPS crystals
Space group	P1
Unit-cell parameters	$a=68.9$ Å, $b=107.7$ Å, $c=137.4$ Å $\alpha=99.6°$, $\beta=97.6°$, $\gamma=97.8°$
Resolution (Å)	50.0—2.8
Total observations	300812
Unique reflections	92829
Redundancy	3.3(2.8)
Completeness (%)	96.9(94.4)
R_{merge}[a] (%)	10.0(46.2)
Average $I/\sigma(I)$	8.1(2.2)

a) $R_{merge}=\Sigma_h\Sigma_I|I_{ih}-\langle I_h\rangle|/\Sigma_h\Sigma_I\langle I_h\rangle$, where $\langle I_h\rangle$ is the mean of the observations I_{ih} of reflection h. Numbers in parentheses are corresponding values for the highest resolution shell.

The result of BLAST[12] showed that there were six homologous structures in PDBank. However, the highest sequence identity is only 29%. Besides, there are too many molecules (10 to 14) in one asymmetric unit of the GGPS crystal, which makes it difficult to get phasing using molecular replacement method. Crystallization of selenomethionine-labled GGPS and heavy atom derivative are now in progress.

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