

Structure of Mammalian Protein Geranylgeranyltransferase Type-I

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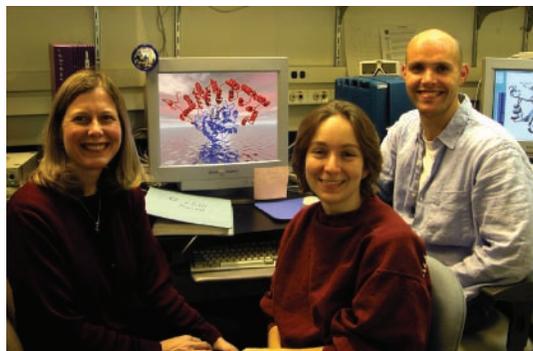
Protein geranylgeranyltransferase type-I (GGTase-I) is an essential enzyme in eukaryotes. GGTase-I catalyzes an essential reaction in which a specific lipid group, acting as a substrate, becomes covalently attached to proteins involved in cell growth and differentiation, allowing these proteins to associate with the cell membrane. We present the first structural information for mammalian GGTase-I, including a series of substrate and product complexes that delineate the path of the chemical reaction. These structures reveal that all protein prenyltransferases share a common reaction mechanism and identify specific amino acid residues that play a dominant role in selecting the correct lipid substrate. Protein prenyltransferase inhibitors are under evaluation in phase III clinical trials for the treatment of cancer and show promise for the treatment of parasitic infections, including malaria.

Over 100 proteins involved in cell growth and differentiation require the covalent attachment of an isoprenoid lipid, a process called prenylation, for membrane association and proper function. The three known enzymes that catalyze protein prenylation are GGTase-I, protein farnesyltransferase (FTase), and Rab GGTase. GGTase-I performs the bulk of cellular prenylation, and inhibiting its function has dramatic biological effects, such as blocking cell growth and promoting apoptosis, the natural death of a cell.

The structure of GGTase-I was determined by a method called single isomorphous replacement with anomalous scattering (SIRAS). There are six GGTase-I molecules (each is 91 kilo-Daltons in size), or approximately 33,000 non-hydrogen atoms, in each asymmetric unit. The overall structure of GGTase-I is shown in **Figure 1A**. The α subunit is composed of α -helical pairs, forming a crescent that wraps around the compact α - α barrel of the β subunit, and the active site opens into the central funnel-shaped cavity of the β subunit. At the rim of the active site is a catalytic zinc ion.

We have captured four crystal structures representative of the GGTase-I reaction cycle (**Figure 2**). The first step in the reaction cycle is the binding of the 20-carbon (20-C) lipid substrate geranylgeranyl diphosphate (GGPP, **Figure 1B**). GGPP binds in the GGTase-I active site with the first three isoprene units of the lipid group arranged along a straight line and the fourth isoprene unit turned approximately 90 degrees relative to this axis (**Figure 1C**). This structure permitted us to identify residues that are responsible for selecting the correct lipid substrate. To

test this hypothesis, a single point mutation was constructed, which converted the lipid specificity of FTase (15-C lipid substrate) to that of GGTase-I (20-C lipid substrate) (**Figure 1C**). Following GGPP binding, the next step in the reaction cycle is substrate



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peptide binding (**Figure 2-2**). GGase-I recognizes peptides that contain a C-terminal $\text{Ca}_1\text{a}_2\text{X}$ box, defined by the cysteine (C), two aliphatic residues (a_1a_2), and a variable C-terminal residue (X), which determines whether the protein is a substrate for GGase-I, FTase, or both. The $\text{Ca}_1\text{a}_2\text{X}$ box binds in an extended conformation, with the cysteine coordinating the catalytic zinc and hydrogen bonds that anchor the carboxyl-terminus. After catalysis, the peptide-prenyl product is retained by the enzyme (**Figure 2-3**). Comparing complexes 2 and 3 illustrates that the lipid substrate undergoes a conformational change during the transition state that brings it in-line for catalysis. GGPP binding partially displaces the product from the active site: The $\text{Ca}_1\text{a}_2\text{X}$ peptide adopts an alternate conformation, and the product lipid group translocates (to make room for the incoming GGPP) into a shallow, solvent-accessible groove, called the “exit groove” (**Figure 2-4**). The dissociation of the product, which is accelerated by fresh substrate peptide, allows the reaction cycle to repeat.

The structural snapshots of the GGase-I reaction cycle are consistent with the unusual reaction mechanism proposed for FTase, thereby indicating that this cycle is a common feature of the protein prenyltransferase family. Overall, these structures, when contrasted with FTase, reveal the dominant structural features responsible for substrate selectivity. Protein prenyltransferases are promising targets for chemotherapy drugs, but further development may require designing drugs that are highly selective for one enzyme. This work, along with our earlier work on FTase, provides a foundation for structure-based design and the optimization of drugs that are specific to a particular protein prenyltransferase.

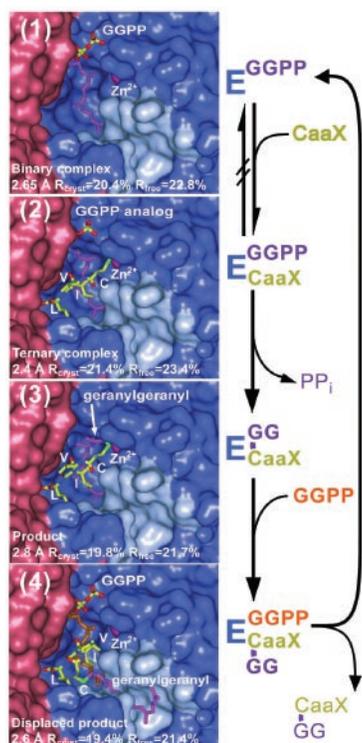


Figure 2. Structures of the GGase-I reaction cycle. The GGase-I active site is shown in stereo as a molecular surface, with the α subunit colored red, the β subunit blue, and the exit groove highlighted in cyan. Complex 1: The enzyme with bound GGPP. Complex 2: Ternary complex with 3'azaGGPP analog and $\text{Ca}_1\text{a}_2\text{X}$ peptide substrate (only $\text{CVIL}_{\text{COOH}}$ shown). Complex 3: Prenylated peptide product complex. Complex 4: Displaced prenylated peptide product and GGPP.

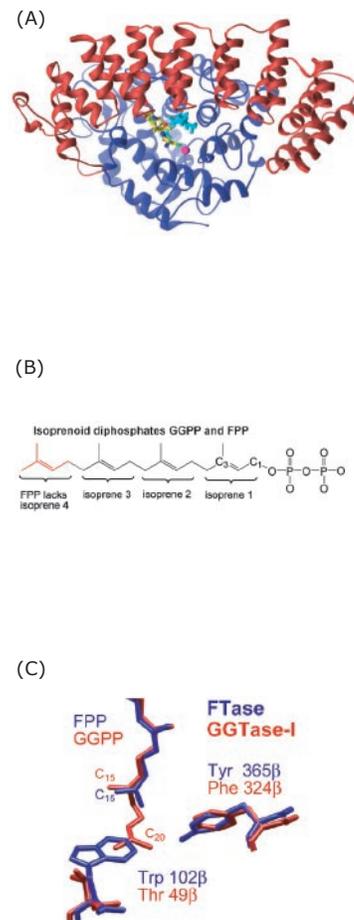


Figure 1. (A) GGase-I ternary substrate complex. The GGase-I consists of a 48 kilo-Dalton (kDa) α subunit (red) and a 43 kDa β subunit (blue). The non-reactive 3'azaGGPP (cyan) binds similarly to GGPP in the active site. The $\text{Ca}_1\text{a}_2\text{X}$ portion of the KKKSKTKCVIL peptide substrate (yellow) binds against the isoprenoid, with the cysteine sulfur coordinating the catalytic zinc ion (magenta). (B) Geranylgeranyl diphosphate (GGPP) has four isoprene units; farnesyl diphosphate (FPP) has three. (C) Comparison of lipid substrate binding in FTase (blue) and GGase-I (red). In FTase, the larger tryptophan residue fills the space where the additional five carbons of GGPP bind in GGase-I and is one of the prime determinants of lipid substrate specificity. Mutation of the tryptophan residue to the equivalent GGase-I threonine residue resulted in an FTase mutant that prefers the 20-C GGPP as a lipid substrate over the typical 15-C FPP lipid substrate.