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Structure and Mechanism of Action of Isopentenylpyrophosphate-Dimethylallylpyrophosphate Isomerase

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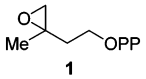
The mevalonate and isoprene biosynthesis pathways result in the synthesis of a wide variety of compounds, including sterols, dolichols, ubiquinones, heme a, farnesyl pyrophosphate, geranylgeranyl pyrophosphate, and many terpenes and other natural products. The structures of the enzymes catalyzing the biosynthesis of such metabolites are of considerable general interest,[1] not least from the perspective of drug design. For example, inhibitors of the enzyme hydroxymethylglutaryl CoA reductase, statins,[2] are important as cholesterol lowering drugs, while farnesyl pyrophosphate synthase inhibitors are important in treating osteoporosis, Paget's disease, and hypercalcemia due to malignancy.[3] The enzyme isopentenyl pyrophosphate-dimethylallyl pyrophosphate isomerase (EC 5.3.3.2)[4] catalyses the isomerization of isopentenyl pyrophosphate (IPP) to form dimethylallyl pyrophosphate (DMAPP), which then condenses with further IPP molecules to form FPP, which is used in, for example, protein prenylation and in cholesterol and ergosterol biosynthesis. There is, therefore, some interest in determining the structure of this enzyme, because it is a potential drug target.

In previous work,[5] we and others[6] reported the crystallographic structure of an IPP/DMAPP isomerase from *E. coli*, a relatively small enzyme which appears to represent the minimal core structure required to catalyze IPP isomerization. The enzyme required one Mg^{2+} or Mn^{2+} to fully fold, and we proposed a tentative structure-based model for isomerization.[5] It is, however, of course of interest to try to obtain additional structural information, on enzyme−inhibitor complexes, to more rigorously test any such mechanistic interpretations, as well as to facilitate future drug design. There are several IPP/DMAPP isomerase inhibitors known in the literature,[7−9] and one of these, the epoxide of isopentenyl pyrophosphate (**1**):



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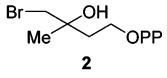

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is also known to be a potent stimulator of $\gamma\delta$ T cells.[10] In addition, the bromohydrin of isopentenyl pyrophosphate (**2**) is also a potent $\gamma\delta$ T cell activator[11] and is being developed for use in treating multiple myeloma, renal carcinoma, and non-Hodgkin's B cell lymphoma.[12] Because both compounds **1** and **2** are known to stimulate $\gamma\delta$ T cells and because the oxirane is a potent isomerase inhibitor,[7] we investigated the possibility that the bromohydrin (**2**, BH) compound might also be an inhibitor. This was found to be the case, with the bromohydrin pyrophosphate[13] having a K_i of ca. 1.4 μM (Supporting Information). Using this inhibitor, we were able to obtain crystals of an isomerase−inhibitor complex and solve its structure, using basically the methods we reported previously for the apo and Mg^{2+} or Mn^{2+} complexes.[5,14]

We show in Figure 1A the crystal structure of the isomerase−BH complex. The overall backbone fold is very similar to that obtained previously with the metal (Mg^{2+} or Mn^{2+}) bound isomerase.[5] However, there are now two metal sites observed, not just the single one seen previously. The first site is occupied by Mn^{2+} (pink), coordinated, as in the case of the metal-only complex, by three histidine and two glutamate residues, Figure 1A. The second site, which has not been seen previously, contains Mg^{2+} (cyan) which is coordinated to two pyrophosphate oxygens, the carbonyl group of the highly conserved residue 67, a carboxylate oxygen of E87, and two water molecules, forming an MgO_6 octahedral coordination sphere, as shown in Figure 1B. Both ends of the bromohydrin pyrophosphate inhibitor are "tethered" to the

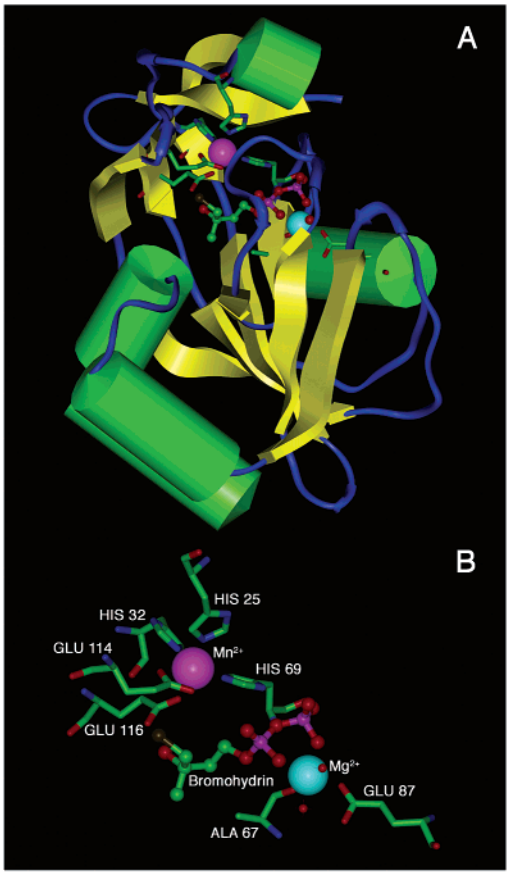

Figure 1. Isomerase−bromohydrin structure (A) and active site region (B).

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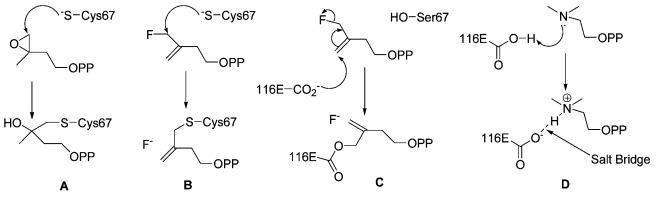
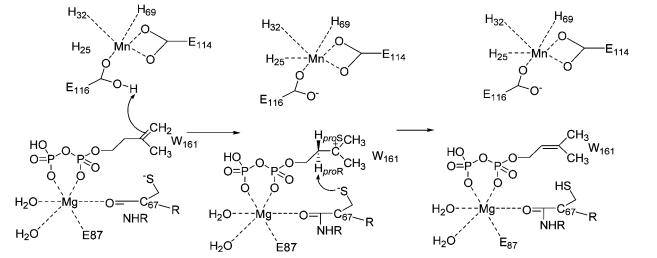


Figure 2. Mechanistic proposals for enzyme inhibition.

protein. At the proximal end, the pyrophosphate group is coordinated via P_9O_8 and P_5O_4 to Mg^{2+}. It is further stabilized by three ionic interactions, with K55, R51, and R83 (not shown). On the distal end of the molecule, the bromohydrin hydroxyl group is involved in a hydrogen bond to the E116 carboxyl (O−O distance $\approx$ 2.3 Å), which itself is coordinated to Mn^{2+}. The crystal structure suggests the mechanism for IPP/DMAPP isomerization[7−9,15] shown below in which the carboxylic acid group of E116 protonates the C3−C4 double bond, forming a carbocation. The thiolate or thiol of C67 (in the wild-type enzyme) then removes a proton from C2, resulting in the isomerization of IPP into DMAPP. The side chain of W161 can be expected to participate in the stabilization of this carbocation intermediate via cation−π interactions. Molecular



modeling of IPP based on the bromohydrin structure reveals that the *pro*-R ^{1}H can be removed by the C67 thiolate[16,17] and that the *re* face of IPP is protonated, resulting in the expected antarafacial [1,3] transposition.[16] The model also implies that proton exchange with solvent ^2H_2O[18] at C4 of IPP occurs via the E116 carboxylic acid group. Because both the E116 and the C67 conserved active site residues are now found to be bound to metals (E116 via its carboxyl to Mn^{2+}, C67A via its carbonyl to Mg^{2+}), it seems that both metals may play a role in catalysis. For example, the reaction may be facilitated by conversion of the E116 carboxyl group to a carboxylate, resulting in an electroneutral Mn^{2+} bis-carboxylate, while Mg^{2+} plays a crucial role in stabilizing the substrate or inhibitors. We should also note that it appears that both *R* and *S* forms of the inhibitor bind to the enzyme, because introduction of both enantiomers into the refinement resulted in a structure having an almost 1:1 occupancy of both forms. In both cases, however, the BH hydroxyl group appears to be involved in a short hydrogen bond to E116, and the structures of the pyrophosphate and C1−C3 fragments are the same in both enantiomers.

The bromohydrin−isomerase structure shown in Figure 1 also leads to a plausible explanation of other, published enzyme inhibition and mutagenesis results.[5,7−9] For example, in the case of the oxirane inhibitor,[1] the position of the oxide (based on docking to the bromohydrin structure) clearly enables the nucleophilic attack of the wt C67, shown in Figure 2A, resulting in Cys alkylation, as found experimentally.[7] Similarly, irreversible inhibition by 3-fluoromethyl-3-butenyl pyrophosphate, shown in Figure 2B, is clearly possible, based on the experimental isomerase−inhibitor structure, involving once again nucleophilic attack at C5 by cysteine. In the case of the C67S mutant, the weaker nucleophilic nature of the Ser OH does not result in serine alkylation. Rather, E116 is alkylated,[15] either via an S_N2 reaction with the allylic fluoride or by an S_N2' reaction, the latter possibility being outlined in Figure 2C. Finally, the potent inhibition by *N*,*N*-dimethylaminoethyl pyrophosphate[8,9] can be readily appreciated because the ammonium group can be expected to be involved in a strong electrostatic interaction with the carboxylate of E116, as well as being stabilized by binding to the second metal site, containing Mg^{2+}, together with the arginine and lysine groups present in this region. The inactivity of the E116Q and E87Q mutants[5] can also be readily explained because E116 is used to protonate IPP, while the conserved E87 is essential for Mg^{2+} binding. This isomerase−inhibitor structure is thus of considerable interest because it provides the first detailed structural insights into the mechanism of action of IPP/DMAPP isomerase, which may be of use in the development of new drugs which inhibit the mevalonate/isoprene pathway.

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Supporting Information Available: K_i determination (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) The bromohydrin of IPP was made by the addition of bromine water to IPP (Echelon Bioscience Inc., P.O. Box 58537, Salt Lake City, UT 84158−0537) at pH 7, ref 11. Excess bromine was removed using a stream of N_2, and the product was characterized by 500 MHz ^{1}H NMR spectroscopy.
(14) Isomerase crystals were grown by equilibration of a protein solution (6 mg/mL) against PEG2000 (16%), ammonium sulphate (100 mM), and $MnCl_2$ (10 mM) buffered to pH 5.5 with Tris/maleate. The crystals were then soaked in a solution of **2** for 2 h. A single crystal was then flash frozen, and diffraction data was collected using a Mar345 imaging plate system, from Marresearch, equipped with Osmic optics and running on an FR591 rotating anode generator. Diffraction data (1.93 Å) were processed with the MarFLM suite: space group $P2_12_12_1$ (two molecules in the asymmetric unit) with cell parameters a = 69.3, b = 72.6, and c = 92.5 Å. The final model was refined to an R value of 20.14% (26 583 reflections) and a R_{free} of 25.74% using Shelx197 (Sheldrick, G. University of Göttingen, Germany). Both enantiomers of **2** were included in the refinement. PDB submission: RCSB017448, PDB code 1N2U.
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