Allosteric pathways in imidazole glycerol phosphate synthase

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AUTHOR SUMMARY

Allostery is a fundamental property that allows the regulation of function and dynamic adaptability of enzymes and proteins. Gaining a detailed understanding of allosteric mechanisms would provide more precise control of enzyme function and roadmaps for the expansion of drug discovery beyond the active site responsible for catalytic activity. Here, we combine molecular dynamics (MD) simulations, nuclear magnetic resonance (NMR), and correlation analysis of protein motions based on network theory methods (i.e., the studies of graphs as a representation of relations between discrete objects such as the amino acid residues in proteins) and elucidate the allosteric mechanism of the enzyme imidazole glycerol phosphate synthase (IGPS) from the thermophile *Thermotoga maritima*. IGPS is a heterodimeric enzyme that catalyzes the hydrolysis of glutamine (Gln) (Fig. P1). IGPS is not present in mammals but is involved in essential biosynthetic pathways of microorganisms. In particular, many plant pathogens and, importantly, opportunistic human pathogens such as *Cryptococcus*, *Candida*, and *Ajellomyces* have an IGPS that is highly homologous to the *T. maritima* enzyme. The allosteric IGPS complex is, thus, a potential target for antifungal, antibiotic, and herbicide development. We have elucidated the allosteric pathway that correlates the IGPS effector and active sites. Such insight should provide useful information for the design of molecules that might hamper the activity of the enzyme glutaminase in plant or human pathogens by interrupting the allosteric signal propagation of IGPS.

IGPS is a V-type enzyme; i.e., an allosteric enzyme in which the binding affinity for the ligand (e.g., Gln in IGPS) in the active site is unaffected by the presence of the effector (e.g., PRFAR; i.e., N′-[(5′-phosphoribulosyl)formimino]-5-aminoimidazole-4-carboxamideribonucleotide, in IGPS) in the allosteric site. However, hydrolysis of Gln is accelerated 5,000-fold upon PRFAR binding 25 Å away from the active site (1). How does the effector binding affect the catalytic activity occurring more than 25 Å away? A recurring feature among enzymes in the Gln amidotransferase family, such as the glutaminase domain of IGPS, is the so-called "oxyanion strand," a β-strand adjacent to the Gln-binding site (the nucleophilic cysteine, *h*C84) that can stabilize a tetrahedral intermediate during Gln hydrolysis, assisting the enzymatic activity (Fig. P1). In the unbound IGPS complex from *T. maritima* (2), the oxyanion strand is constituted of the highly conserved sequence *h*P49-*h*G50-*h*V51-*h*G52 (49-PGVG), with an improper (inactive) conformation in which the NH group of *h*V51 points out of the Gln-binding site (Fig. P1). Our previous ^{1}H, ^{15}N NMR analysis (3) revealed conformational exchange motions of *h*G50 on the micro/millisecond time scale upon titration with PRFAR, supporting the hypothesis that a conformational change of the 49-PGVG backbone is associated with the allosteric transition that stimulates the glutaminase reaction. How can the effector binding perturb the motion of the oxyanion strand adjacent to the active site? What are the specific interactions spanning the effector and the active sites that are affected by the PRFAR binding?

To address these questions, we explore the effect of PRFAR on correlated motions of amino acid residues and use this information to evaluate how effector binding affects the protein network of the unbound (apo) enzyme. We find that PRFAR binding induces changes in fundamental interactions correlating the effector and active sites that are essential for the allosteric mechanism. The resulting protein motions partially promote rotation of the conserved oxyanion strand, responsible for the inactive-to-active allosteric transition (Fig. P1). The analysis thus shows evidence that PRFAR binding alters the community structure of interactions in the inactive IGPS, along with the collective pathways linking the effector and active sites. This study provides valuable insight

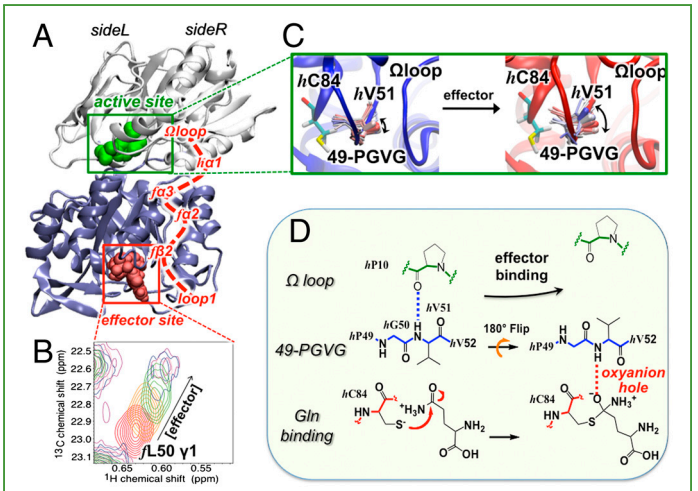

Fig. P1. Allosteric mechanism of the imidazole glycerol phosphate synthase (IGPS). (*A*) IGPS allosteric pathways (dashed red line) linking the effector (red) and active (green) sites, including the flexible loop1; the *f*β2 strand; the *f*α2, *f*α3, and *h*α1 helices; and the Ω loop. (*B*) ^{1}H-^{13}C NMR spectral overlays of the titration of effector into IGPS, showing large chemical shift perturbation for *f*L50 in the *f*β2 strand upon effector binding in the allosteric site. (*C*) Partial reorientation of the 49-PGVG backbone adjacent to the active site triggered by effector binding. Thermal fluctuations of the NH amide group of *h*V51 (sticks colored from red to blue for 0–50 ns). (*D*) Schematic representation of the oxyanion hole formation during the glutamine hydrolysis reaction.

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into the mechanism of information transfer in this V-type allosteric enzyme.

The rearrangement of the communication pathways induced by PRFAR involves the flexible loop1 and the hydrophobic region ($f\beta2$) near the allosteric site (Fig. P1); ionic interactions in the interdomain region next to loop1 (sideR), including residues in the $f\alpha2$, $f\alpha3$, and $h\alpha1$ helices; as well as a hydrogen bond between the Ω loop and the conserved 49-PGVG sequence (oxyanion strand) adjacent to the glutaminase active site. The proposed allosteric mechanism is supported by the computational analysis of early protein dynamics in conjunction with NMR chemical shifts and relaxation dispersion experiments probing millisecond protein motions. Single amino acid residues, such as fL50, fR59, fE91, and hP10, play an important role in the allosteric mechanism and should be good candidates for unique mutagenesis studies. The combined approach we describe is an effective strategy for exploring the signaling pathways in any other allosteric systems.

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