ALLOSTERIC CONTROL OF PHOSPHOGLYCERATE DEHYDROGENASE IN SERINE BIOSYNTHESIS-TWELVE DOMAIN TETRAMERS

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Phosphoglycerate dehydrogenase catalyzes the 1st step of the serine biosynthetic pathway, oxidizing 3-phosphoglycerate to 3-phosphohydroxypyruvate using NAD/NADH. The enzyme is allosterically regulated in a V_{max} manner by the pathway's end product, serine. The structure of PGDH:NAD:Ser revealed a homotetramer. Each subunit consisted of 3 domains, nucleotide (N), substrate (SUB) and serine (SER) binding. The 4º structure resembled an elongated toroid with 2 interfaces formed by the association of N-domains into a dimer accompanied by interfaces through the SER-domains. A cleft between N- and SUB-domains forms PGDH's active site and the Ser site is across two SER-domains 33Å away. The mechanism of Ser inhibition was investigated by point mutations disrupting subunit interfaces and enzyme truncations. Introduction of a Trp at the tetrameric interface abolished Ser inhibition but as revealed by a 4Å structure did not alter the 4º structure. The removal of W139 at the dimer interface reduced catalytic activity 600 fold and cooperativity of Ser inhibition. The 2.07Å structure showed a 42º rotation of the N-domain, 180º nicotinamide ring re-orientation and loss of subunit contacts at the N-interface. Removal of the Ser-domain resulted in a catalytically active species displaying kinetic and ligand binding parameters similar to native PGDH. From the catalytic standpoint the N-domain is essential whereas the SER-interface is necessary solely for regulation. We concluded subtle changes in N-domain hydrophobic subunit contacts played a critical role in stability and transmission of ligand binding and inhibition.
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Keywords: ALLOSTERIC ENZYME, PROTEIN DOMAINS, CONFORMATIONAL CHANGE

CRYSTAL STRUCTURE OF CLASS II RIBONUCLEOTIDE REDUCTASE: HOW AN ALLOSTERICALLY REGULATED MONOMER MIMICS A DIMER

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Ribonucleotide reductases (RNRs) catalyze the conversion of ribonucleotides to deoxyribonucleotides, an essential step in DNA biosynthesis and repair. We have determined the crystal structure of class II (adenosylcobalamin-dependent) ribonucleoside triphosphate reductase (RTPR) from *Lactobacillus leichmannii* in the apo enzyme form and in complex with adeninylpentylcobalamin at 1.75 and 2.0 Å resolution, respectively. This monomeric, allosterically regulated, class II RNR retains all the key structural features associated with the catalytic and regulatory machinery of oligomeric RNRs. Surprisingly, the dimer interface responsible for effector binding in class I RNR is preserved through a single 130 residue insertion in the class II structure. Thus, *L. leichmannii* RNR is a paradigm for the simplest structural entity capable of ribonucleotide reduction, a reaction that links the RNA and DNA worlds.

Keywords: ALLOSTERIC REGULATION METALLO-ENZYMES EVOLUTION

STRUCTURAL BASIS FOR ALLOSTERIC SUBSTRATE SPECIFICITY REGULATION IN ANAEROBIC RIBONUCLEOTIDE REDUCTASES

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In addition to their fascinating radical chemistry, ribonucleotide reductases (RNRs) are characterized by their ability to reduce all four ribonucleotides to deoxyribonucleotides. Substrate specificity is governed by a sensitive allosteric system whereby the dNTP products change the affinity for the NDP or NTP substrates. In addition, overall activity is upregulated by ATP and downregulated by dATP. These two allosteric functions have been localized to two separate sites. The broadly similar kinetic effects observed in all three RNR classes, in spite of otherwise rather large differences in sequence and cofactor requirements, have been used to argue for a common evolutionary origin.

The class III, anaerobic RNRs are structurally related to class I, aerobic enzymes despite having no significant overall sequence homology. However, the allosteric specificity site has fundamentally different features to those in class I, despite the similar allosteric behavior.

We present structures of a class III RNR in complex with four dNTPs, allowing a full comparison of the protein conformations. Discrimination between effectors is achieved by two side chains, Gln114 and Glu181, from separate monomers. Large conformational differences in the active site (loop 2), in particular Phe194, are observed between the complexes. Subtle differences in base size and hydrogen bonding pattern at the effector site are communicated to significant conformational changes in the active site. We propose that the altered overlap of Phe194 with the substrate base governs hydrogen bonding patterns with fixed hydrogen bonding groups in the active site and discuss the significance for evolution.

Keywords: RIBONUCLEOTIDE REDUCTASE ALLOSTERIC REGULATION CONFORMATIONAL CHANGES

REGULATION OF GTPase ACTIVITY OF HETEROTRIMERIC G PROTEINS

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The α subunits of heterotrimeric G proteins (Gα) are activated by GTP, enabling them to bind and regulate effector enzymes or ion channels. The intrinsic GTPase activity of Gα converts it to an inactive state with low affinity for effector. The intrinsic rate of catalysis is slow, 0.04 s^{-1}, which allows a Gα to remain active for 15 s. Regulators of G protein Signaling (RGS) that act as GTPase activating proteins for Gα increase the rate of hydrolysis 5-100 fold. Structures of Gα bound to non-hydrolysable GTP analogs suggest that the kinetic barrier to GTP hydrolysis is due to a conformational transition, which may be catalyzed by RGS proteins. However, structures of RGS-Gα complexes provide little insight into this dynamic process. We have introduced a series of alanine mutants into conformationally mobile segments of the protein surrounding the catalytic site. We find that GTPase activating mutants decrease the enthalpy and entropy for the transition state relative to the ground state, as do RGS proteins. Both appear to reduce the conformational heterogeneity of the G protein active site and interact more tightly to the sissile phosphate. The structures of two distant members of the RGS family, RGS4 and rgRGS are shown to have divergent structures and act by different mechanisms to accelerate the GTPase rate of their respective substrates, Giα and G13α.

Keywords: G PROTEINS GTPASE ACTIVATING PROTEIN CATALYSIS