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CRYSTALLOGRAPHIC STUDIES OF RIBONUCLEOTIDE REDUCTASE FROM *SALMONELLA TYPHIMURIUM*. CLUES ABOUT THE ALLOSTERIC REGULATION

M. Uppsten M. Farnegard H. Eklund U. Uhlin
Bmc Department of Molecular Biology, SLU Box 590 UPPSALA S-751 24 SWEDEN

Ribonucleotide reductases (rnrs) catalyse the conversion of ribonucleotides to deoxyribonucleotides and is thus an interesting target for drug design. Structural studies of rnrs by xray crystallography can be of great help in understanding the mechanism. Class I enzymes consist of two homodimeric proteins, in class Ia R1 and R2 and in Ib R1E and R2F.
Ribonucleotide reductases are allosterically regulated enzymes and interact with both positive and negative effector molecules. In the class Ia enzymes two allosteric sites distinct from the active site are located in the large subunit R1. One of them, the overall activity site determines if the enzyme is active or not and apart from this a second site, the specificity site, is found. The specificity site ensures balanced dNTP pools by regulating which ribonucleotide is to be reduced. This regulation is thought to be performed through conformational changes in the large subunit.
The structure of the large subunit, class Ib RNR from Salmonella typhimurium, R1E has been solved in complex with two different specificity effectors, an ATP derivate AMP-PNP and dATP. The structure reveals a similar structure to the R1 from E.coli and the most striking difference is that the overall activity site is absent in R1E.
The two effectors bound to R1E show somewhat different interactions with the enzyme. Together the two structures give insight to how the regulation of the specificity of RNR class Ib is performed.

Keywords: ALLOSTERIC REGULATION RIBONUCLEOTIDE REDUCTASE DNA SYNTHESIS

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DETERMINATION OF THE STRUCTURE OF KDO8P SYNTASE WITH THE NATURAL SUBSTRATE A5P, Z-FPEP AND E-FPEP

R. Vainer N. Adir E. Rabkin V. Belakhov T. Baasov
Technion, Technion City, Haifa 32000 Israel Derch Jad L Banim 79/10, Haifa HAIFA 32000 ISRAEL

The enzyme KDO8PS (3-Deoxy-D-Manno-Octulosonate 8-Phosphate Synthase) catalyzes the condensation reaction between the PEP (phosphoenolpyruvate) and A5P (arabinose 5-phosphate) to form KDO8P and inorganic phosphate. This important enzymatic reaction controls the carbon flow in the biosynthetic formation of a phoshorylated precursor of the unique monosaccharide KDO, which is an essential constituent of the lipopolysaccharide of all Gram-negative bacteria and plays a crucial role in their assembly process. The knowledge of the three-dimensional structure with substrates will aid in the design of drugs to treat infection caused by antibiotic resistant Gram-negative bacteria . The aim of this study is to determine the three-dimensional structure of the enzyme KDO8PS from *E. coli* binary complexes with A5P, E and Z fluorophosphoenolpyruvate (Z-FPEP and E-FPEP). The crystal structures of KDO8PS with different substrates have been previously determined. In the course of this work we obtained crystals of the following mentioned above enzyme complexes: KDO8P:Z-FPEP, KDO8P:E-FPEP and KDO8P:A5P, which crystallized in the cubic space group I23 with cell constants of =119.4 Å, 118.02 Å and 117.44 Å, respectively.
Crystallographic phases were determined using molecule replacement. These protein structures are in the process of refinement. The structural information achieved in this study confirms in many aspects the recently reported substrate-free structures of KDOPS. However, major differences are observed in the conformations of the active site residues responsible for the binding of both the PEP and A5P substrates. Visualization of the A5P, Z-FPEP and E-FPEP in our structures allows us to rationalize the chemically determined stereochemical course of the enzyme-catalyzed reaction.

Keywords: **LIPOPOLYSACCHARIDE, GRAM-NEGATIVE BACTERIA, CATALYZES**

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OROTIDINE 5'-MONOPHOSPHATE DECARBOXYLASE: REACTION MECHANISM ELUCIDATED BY MUTATION STUDIES

N. Wu[1] E. F. Pai[1,2,3,4,5]
[1]Dept. of Biochemistry, University of Toronto [2]Dept. of Medical Biophysics, University of Toronto [3]Ontario Cancer Institute 610 University Ave. Room 7-611 TORONTO ONTARIO M5G 2M9 CANADA [4]Dept. of Molecular and Medical Genetics, University of Toronto [5]Protein Engineering Network, Centres of Excellence, University of Toronto

All decarboxylation reactions require stabilization of the carbanion intermediate. This is usually accomplished with the help of metal ions or the formation of Schiff base with either a lysine residue or cofactors such as pyrodoxal monophosphate (PLP). However, orotidine 5'-monophosphate decarboxylase (ODCase) accomplishes the decarboxylation of OMP to UMP in the absence of any cofactors, yet with the highest proficiency known. It was proposed that the substrate, OMP, is protonated at the O2 position by a lysine residue to form a zwitterion first, putting a positive charge on N1 which subsequently acts as an electron sink. The recent crystal structure of an inhibitor-ODCase complex negates this zwitterion mechanism since the catalytic lysine is located on the wrong side of the base. Instead, the structure reveals a tight hydrophobic active site with an alternating charge network K42-D70-K72-D75B (M. thermoautotrophicum designation) lining the reaction side of orotidine base; the phosphoribose is interacting very tightly with the protein. Modeling indicates that orotate carboxylate is in direct spatial and charge conflict with D70. We have proposed a new mechanism based on Jencks' "Circe effect": the binding energy from phosphoribose is used to push the negative orotidine head group into a sterically and electrostatically unfavorable environment where decarboxylation readily occurs to relieve the stress. To verify our hypothesis, we mutated the residues of the charged network individually and in combination, then complexed the mutants with either the substrate or inhibitors for structural analysis. Our results support the importance of D70 in addition to K72, the catalytic lysine. Only when both residues are mutated away is the enzyme inactivated.

Keywords: DECARBOXYLATION, REACTION MECHANISM, MUTATION

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CRYSTAL STRUCTURE OF CLPA, AN HSP100 CHAPERONE AND REGULATOR OF CLPAP PROTEASE: STRUCTURAL BASIS OF DIFFERENCES IN FUNCTION OF THE TWO AAA+ ATPase DOMAINS

D. Xia F. Guo M. Maurizi L. Esser
National Cancer Institute, NIH Laboratory of Cell Biology 37 Convent Dr., Buidling 37, Room 1B22 BETHESDA MD 20892 USA

The crystal structure of E. coli ClpA, an hsp100/Clp chaperone and integral component of the ATP-dependent ClpAP protease, has been determined to 2.6 **Å** resolution. ClpA consists of five tandemly connected structural domains corresponding to three functional groups. The N-terminal domain represents a novel fold of a repeating motif with a pseudo two-fold symmetry. The two AAA+ modules (D1 and D2), with bound ADP, are connected head-to-tail with a relative rotation of 90 degree. In the crystal, ClpA subunits form a hexameric spiral in which the D1-D1 interface has considerablely more electrostatic nature than the D2-D2 interface, providing a structural basis for difference in function of the two AAA+ modules. A hexameric ring model of ClpA locates potential ClpP-interaction loops on the ring surface of D2 and reveals a large central cavity with bands of negative charge and a narrow constriction that may serve as a pore for ClpA chaperone activity.

Keywords: **AAA, ATP-DEPENDENT PROTEase, CHAPERONE**