crystal structure of human T-protein with folate cofactor and provided the insight into the molecular basis of the disease-causing mutations. Here we present the crystal structure of *Escherichia coli* T-protein in complex with dihydrolipoate-bearing H-protein and 5-methyltetrahydrofolate (5-CH_3-THF): a complex mimicking the ternary complex in the reverse reaction. The structure of the complex shows a highly interacting intermolecular interface limited to a small area and the protein-bound dihydrolipoyllysine arm inserted into the active site cavity of the T-protein. Among the residues contributing to the interface, invariant Arg292 of the T-protein plays a key role in the complex assembly and probably in recruiting the aminomethyllipoyllysine arm to the active site of T-protein. It has been speculated that the aminomethyltransfer reaction from aminomethyllipoate of H-intermediate to THF is initiated by the direct attack of the methylene carbon atom by the nucleophilic N5 or N10 atoms of THF bound to T-protein accompanying the release of ammonia. However, the distances between the tip of the dihydrolipoyllysine arm and the methyl carbon atom of 5-CH_3-THF observed in the complex structure suggests the presence of an intermediary mediating the transfer reaction rather than the direct interaction. The hydrogen bond network surrounding the S8 atom of the dihydrolipoyllysine including invariant Asp96, Asp97, Asn113, and Arg223 of T-protein suggests that the reversible transfer of the methylene group between the lipoate and tetrahydrofolate should proceed through the electron relay-assisted iminium intermediate formation. Based on the structural observations together with mutational analyses, we propose a possible mechanism for T-protein catalysis. The structure also provides novel insights in understanding the disease-causing mutations, in addition to the disease-related impairment in the cofactor-enzyme interactions presented previously.

Keywords: Protein_complex, Catalytic_reaction

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Molybdenum Oxide/Bipyridine hybrid materials: synthesis, structure and catalytic studies

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The chemistry of Mo(VI) is very important in industrial and biological systems [1, 2]. Monomeric complexes of the type $[MoO_2X_mL_n]$ (X = mono/dianionic ligand, L = neutral ligand) have been shown to be active catalysts, or catalyst precursors, for homogeneous epoxidation of non-functionalized olefins. Recently we found that $[MoO_2Cl_2(bipy)]$ (bipy = 2,2'-bipyridine) can produce a molybdenum(VI) oxide-based hybrid material [3] which can be used as an heterogeneous catalyst.

The molybdenum oxide/bipyridine hybrid material $\{[MoO_3(bipy)][MoO_3(H_2O)]\}_n$ can be isolated as a microcrystalline powder, in yields of 72-92%, from the reaction of $[MoO_2Cl_2(bipy)]$ in water using three distinct methods: hydrothermal (100ºC, 19h), reflux (120ºC, 4h) and microwave (120ºC, 4h).

The crystal structure of this hybrid material was determined from synchrotron X-ray powder diffraction data. The material is composed of two distinct neutral one-dimensional polymers: an organic-inorganic polymer, $[MoO_3(bipy)]_n$, and a purely inorganic chain, $[MoO_3(H_2O)]_n$; the two are interconnected by O-H···O hydrogen bonding interactions.

The material is a moderately active, stable, and selective catalyst for the epoxidation of *cis*-cyclooctene at 55 ºC with *tert*-butylhydroperoxide (*t*BuOOH, 5.5 M in decane or 70% aqueous) as the oxidant. Within the process, biphasic solid-liquid or triphasic solid-organic-aqueous mixtures are formed, and 1,2-epoxycyclooctane is the only reaction product. When *n*-hexane is employed as a co-solvent and *t*BuOOH(decane) is the oxidant, the catalytic reaction is heterogeneous in nature, and the solid catalyst can be recycled and reused without loss of activity. For comparison, the catalytic performance of the precursor $[MoO_2Cl_2(bipy)]$ was also investigated. The FT-IR spectra of the solids recovered after catalysis indicate that the discrete complex transforms into the organic-inorganic polymer $[MoO_3(bipy)]$ [4] when the oxidant is *t*BuOOH(decane) and compound $\{[MoO_3(bipy)][MoO_3(H_2O)]\}_n$ when the oxidant is 70% aqueous *t*BuOOH.

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Keywords: dioxomolybdenum(VI), catalysis, olefin-epoxidation

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Structural and functional analysis of rRNA methyltransferase from *Staphylococcus aureus*

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Ribosomal RNAs (rRNAs) are modified post-transcriptionally to generate variety of nucleotides required for fine tuning of ribosomes. In *Escherichia coli*, m2G 2445 of 23S rRNA is produced by *Ec*RlmL (ribosomal large subunit methyltransferase L), which possesses two methyltransferase domains. In *Staphylococcus aureus*, these two domains are separated two proteins, *Sa*RlmL-N and *Sa*RlmL-C. Here we present crystal structure and RNA binding study of *Sa*RlmL-N and *Sa*RlmL-C.

SaRlmL-N and *Sa*RlmL-C were expressed in *E.coli* and purified by Ni-affinity chromatography and size exclusion chromatography. Crystals were obtained by using vapor diffusion method and X-ray diffraction data were collected at BL41XU SPring-8, Harima, Japan and PF BL-17A, Tukuba, Japan. crystals of *Sa*RlmL-N *Sa*RlmL-C belong to space group $P2_1$ with cell dimensions (*Sa*RlmL-N: a = 52.5, b = 107, c = 77.2 Å, β = 100° ; *Sa*RlmL-C: a = 95.8, b = 91.7, c = 103 ∓, β = 93.9−). structures were solved by molecular replacement with the program *Morlep*.

Structure of *Sa*RlmL-N contains methyl donor, *S*-adenosyl-methionine (SAM) in methyltransferase domain. SAM binding pocket is connected to vast cleft which is charged positive and surrounding residues are highly conserved. RNA binding experiments were performed using *in vitro* transcribed RNA fragment including Guanine 2445. Formation of *Sa*RlmL-N and RNA fragment complex was confirmed by size exclusion chromatography. This result indicates that *Sa*RlmL-N may serve as m2G 2445 methyltransferase in *S. aureus*. ased on the structur and RNA binding study, we propose the RNA binding model of *Sa*RlmL-N.

Structure of *Sa*RlmL-C is composed three domains: NTD, EEHEE and MTase, and is similar to *Ec*RlmI with different intermolecular

contact. *Ec*RlmI also a methyltransferase synthesizes m5C 1962 of 23S rRNA. *Ec*RlmI forms dimer in both solution and crystal[1], *Sa*RlmL-C exists as monomer in crystal . In the structure of *Sa*RlmL-C, *S*-adenosylhomocysteine from *E.coli* was found in MTase domain, and located next to deep cleft by three domains. Conserved residues in each domains are concentrated in deep cleft can be considerd as possible RNA binding site. Finally we propose the RNA binding model of *Sa*RlmL-C different from of *Ec*RlmI.

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Keywords: rRNA, methyltransferase

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Structures of engineered phytases

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Phytate (inositol hexaphosphate), that is found in cereals, acts as an anti-nutritive factor in animal feed. Phytase acts on phytate to remove phosphate and eliminate its anti-nutritive effect. Native phytase suffers from insufficient thermal stability for animal feed formulation making an engineered more thermal stable phytatse highly desirable. We have determined the three-dimensional structures of native along with a first and second generation engineered *Buttiaxella* phytase to better understand what structural elements were modified to achieve this thermal stability. The native enzyme has been crystallized in space group $P2_12_12_1$ and determined to 1.8 Å, one variant crystallized in a triclininc form with two molecules in the symmetric unit, was determined at 2.05 Å and a second variant having an additional amino acid substitutions was also crystallized in space group $P2_12_12_1$ and determined to 1.8 Å. These variants were achieved with programs of directed evolution. The analysis of these changes lead to a short set of guidelines that appear to have general applicability for rational engineering thermal stability in these and other enzymes of commercial interest.

Keywords: phytase, protein engineering

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Highlights from the macromolecular x-ray crystallography facility at pennsylvania state university (a) crystal structures of bacterial expansin in complex with cellohexoase and β-glucan (b) solution scattering and crystal structure of sorbitol dehydrogenase with a substrate (c) computation model of human ron receptor and its juxta-membrane domain and (d) model of phospholipase a2 with procyanidins

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This research is in collaboration with various groups at Penn State University **(a)** With the Cosgrove lab, we have solved the crystal structure of an expansin, encoded by the *yoaJ* gene of Bacillus subtilis in complex with polysaccharides, cellohexoase and β-glucan. Although the polysaccharide-binding surface was expected to span the two domains of the protein, it is seen to bind only to the carbohydrate-binding domain. The sugars trigger a dimer formation in the crystal packing with the conserved residues Trp125, Trp126 and Tyr157 from both monomers stacking against the carbohydrate rings. The structures point to a non-enzymatic function for expansin action.

(b) The crystal structure of sheep liver sorbitol dehydrogenase (slSDH) has been determined using the crystal structure of human sorbitol dehydrogenase (hSDH) as a molecular-replacement model. slSDH crystallized in space group I222 with one monomer in the asymmetric unit. A conserved tetramer that superposes well with that seen in hSDH (despite belonging to a different space group) and obeying the 222 crystal symmetry is seen in slSDH. An acetate molecule is bound in the active site, coordinating to the activesite zinc through a water molecule. Glycerol, a substrate of slSDH, also occupies the substrate-binding pocket together with the acetate designed by nature to fit large polyol substrates. The substrate-binding pocket is seen to be in close proximity to the tetramer interface, which explains the need for the structural integrity of the tetramer for enzyme activity. Small-angle X-ray scattering in collaboration with the Gillilan group at CHESS, was used to identify the quaternary structure of the tetramer of slSDH in solution.

(c) Mutation studies done in the Hankey lab have identified residues in the juxta-membrane (JM) segment crucial for human Ron receptor activation and repression. Analysis of conserved residues and electrostatic surface of human Ron kinase domain combined with the crystal structures of other related receptors has enabled building of models for the active and the autoinhibited structural fold of the JM domain. The autoinhibited form of other receptor protein kinases indicates JM approaches the active site from the front end of the nucleotide binding pocket and binds in the substrate binding region. We predict that a short acidic JM-C helix could position itself in the substrate binding region interacting with the activation and P-loops of the active site and a longer JM-D helix could interact with the alpha-C helix of the N-lobe maintaining the human Ron receptor in its inactive conformation. In the active state the JM region could undergo significant conformational changes and move out of the substrate binding region.

(d) Studies in the Lambert lab on cocoa, apple and cinnamon procyanidins have shown them to be potent inhibitors of key digestive enzymes phospholipase A2, lipase and α-amylase. Models of procyanidins of various sizes were built into the active site of phospholipase A2 guided by the dozens of available ligand-bound crystal structures. A 7-mer of procyanidin was the biggest ligand that could fit in the large active site tunnel and have favorable interactions with the protein residues.

Keywords: Expansin, Dehydrogenase, Ron

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Study on Crack of Yb:YAG Laser Crystal

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The 5at.% Yb3+-doped YAG crystal was grown by the Czochraski method, and the crystal growth process was investigated. the optimal process parameters were determined as follows: The crystal growing rate for Shoulder growth and equal-diameter growth were 0.8mm/h and 1mm/h, respectively. The optimal Rotation speed of this crystal was 15rad/min, and the axial temperature gradient was 0.01−0.05ºC/mm. The impact factors to crystal growth were analyzed theoretically, such as growth rate, rotation speed, thermal effect and crystal size

Keywords: Yb:YAG crystal, Czochralski method, Crack analysis