

decreased flexibility provides a basis to explain the low affinity of ONC towards nucleotides and, more generally, its lower catalytic activity. The results also suggest the basis of the unusually high thermal stability of the enzyme.[1]

[1] Merlino A., *et al.*, *J. Biol. Chem.*, *accepted for publication.*

**Keywords:** mutations, dynamic properties, crystallography

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#### Structural Basis for Tumor Pyruvate Kinase M2 Allosteric Regulation and Catalysis

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Pyruvate Kinase plays catalyzes the last step of the glycolytic cycle, turning over the substrate phosphoenolpyruvate, PEP, into pyruvate, producing one molecule of ATP per reaction. Four isozymes of this enzyme exist in humans: R, L, M<sub>1</sub>, and M<sub>2</sub>. The R and L isoforms are present in the erythrocytes and liver cells, respectively. Both M<sub>1</sub> and M<sub>2</sub> are encoded by the M gene. The M<sub>1</sub> isoform is found in skeletal muscle and brain tissue. The M<sub>2</sub> isoform is predominately present in fetal tissue and is progressively replaced by the other isoforms after birth. However, the M<sub>2</sub> isoform is again reexpressed in numerous tumor cells.

The overexpression of the M<sub>2</sub> isoform in tumor cells invokes many mechanistic questions regarding the role of hPKM<sub>2</sub> in tumorigenesis, as well as offers an intriguing anti-cancer target. Therefore, our structure may be useful as a template for the discovery of novel compounds that may serve as possible anti-cancer drug leads. We cloned, overexpressed, and purified hPKM<sub>2</sub> from inclusion bodies in *E.coli* through a unique refolding protocol. The enzyme was crystallized and x-ray data were collected at the APS (Argonne National Labs). The human PKM<sub>2</sub> crystal structure was determined to 2.8 Å resolution. Structural analysis and comparison of structural differences among isozymes is presented here.

**Keywords:** pyruvate kinase, allosteric, conformational change

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#### Structural Studies of the Sucrose Isomerase MutB from *Pseudomonas mesoacidophila*

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The sucrose isomerase, MutB, from *Pseudomonas mesoacidophila* belongs to glycoside hydrolase family 13, and catalyzes the isomerization of sucrose into isomaltulose and trehalulose [1]. The 64 kDa enzyme has been crystallized [2] and the three dimensional structure of MutB has been solved to 1.6 Å resolution by the molecular replacement method using the isomaltulose synthase, PalI, from *Klebsiella* sp. LX3 as a search model [3]. The overall structure of MutB is made up of three domains: an N-terminal and catalytic (β/α)<sub>8</sub> domain, a subdomain and a C-terminal domain made up of seven β-strands [4].

The structures of various complexes with inhibitors and/or substrate analogues have been obtained and are currently under refinement. Once the detailed analyses of these structures have been completed, a better understanding of the molecular basis of sucrose decomposition, isomerization as well as the selectivity of this enzyme leading to the formation of different products should be gained.

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**Keywords:** X-ray crystal structure determination, enzymatic structure-activity relationships, sucrose

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#### Recovery of Argininosuccinate Lyase Activity in Duck δ1-Crystallin

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δ-Crystallin is directly related to argininosuccinate lyase (ASL). Two isoforms exist in ducks, δ2 and δ1, which are 94% identical. δ2 is the duck orthologue of ASL, while δ1 is enzymatically inactive. Chimeras of the two isoforms have shown that domain 1 of δ2 is sufficient to recover activity in δ1. Structural comparisons of various δ-crystallin proteins revealed that conformational differences between δ1 and δ2 are localized to residues 23-32 and 74-89 (20's and 70's loops). As the putative catalytic residues are conserved in δ1, the amino acid substitutions in these loops are thought to prevent substrate binding in δ1. However, a δ1 double loop mutant (DLM), with all residues in the 20's and 70's loops replaced with those of δ2, was found to be inactive and binding of the substrate to the DLM could not be detected by ITC. To further investigate this result, crystal structures of the DLM with and without sulfate bound have been determined to 2.2 and 2.5Å resolution, respectively. The conformations of the 20's and 70's loops in the DLM and δ2 are very similar, suggesting the remaining five amino acid differences in domain 1 of the DLM relative to δ2 are important for ASL activity. Mutagenesis experiments reveal that ASL activity can be recovered in the DLM by mutating Met-9 to Trp. Truncation mutants of δ2 demonstrate that although the N-terminal arm is conformationally flexible, this region of the protein is critical for ASL activity. The N-terminal segment is likely involved in stabilizing regions of δ2 involved in substrate binding and catalysis.

**Keywords:** δ-crystallin, argininosuccinate lyase, enzyme mechanism

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#### Crystal Structure of a Native Chitinase from the Fungal Pathogen *Aspergillus fumigatus* YJ-407 (afCHI)

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Chitinase hydrolyzes chitin, which is a structural component of cell walls and coating of many organisms. In fungi, chitinase is thought to contribute to a number of morphogenetic processes in filamentous fungi. Therefore, fungal chitinase is thought to be a putative virulence factor and a promising anti-fungi target molecule. As one of the most ubiquitous of the airborne saprophytic fungi, *Aspergillus fumigatus* has been shown to be an opportunistic pathogen causing pneumonia and other fatal invasive infection. Except for endo- and exo-hydrolytic activities, a transglycosyl activity was observed in the extracellular chitinase (afCHI) from *Aspergillus fumigatus* YJ-407.

This native chitinase from the fungal pathogen *Aspergillus fumigatus* YJ-407 (afCHI) has been crystallized and the X-ray structure has been solved to 2.1Å resolution by molecular replacement. Like other members of the class 18 hydrolase family, this fungal enzyme is of an eight stranded b/a-barrel. And a GlcNAc was observed in the glycosylation site (Asn257-Asp258-Thr259). Structural comparisons revealed that structural features such as substrate binding site, residues in active site and catalytic acid are conserved. Furthermore, the physiological role of saccharide and the structural basis of transglycosyl activity were discussed.

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