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Novel thiadiazole inhibitors of human carbonic anhydrases

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Human carbonic anhydrases are potential drug targets for a number of diseases. One of the novel applications is to use some of their isozymes as anti-cancer drug targets. Structure-thermodynamic property relations of novel hCA thiadiazole class inhibitors with a triple-ring system bound to hCAII will be discussed. Structures of several inhibitors are solved to atomic resolution using X-ray diffraction of hCAII-inhibitor complex crystals. The structural data are correlated with the isothermal titration calorimetry measurements. The calorimetric data together with the structures provide insight into the structural base of the tight and selective hCA inhibitor binding.

Keywords: carbonic anhydrase, isothermal titration calorimetry, structure-activity relation

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Molecular basis for inhibition and resistance of glutamate racemase to a family of D-glu analogues

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Proteins involved in the synthesis of compounds of the bacterial cell wall have been long exploited in the development of effective antibiotics. D-glutamate is an essential component of the peptidoglycan layer, and is synthesized from L-glutamate via a co-factor independent reaction catalysed by glutamate racemase. Co-crystals of Streptococcus pneumoniae glutamate racemase, in complex with a potent inhibitor, (2R, 4S)-2-Amino-4-(2-naphthyl) methyl Pentanedioic Acid, have been grown and the structure solved using X-ray crystallographic techniques. The structure reveals that, in contrast to previously published data on the location of the inhibitor binding site by crystal soaks, the inhibitor is bound in a very similar fashion to the D-glutamate substrate. The structure has provided new insight into the narrow spectrum activity of the family of compounds to which this inhibitor belongs, and has provided clues as to how a broad spectrum antibiotic might be developed.

Keywords: antibiotic resistance, Inhibitor interactions, racemases

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The LIM code for motor neuron specificity

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LIM-HD (LIM homeodomain) proteins are essential for defining cell fate, especially in the developing central nervous system. Isl-1 and Lhx-3 are two LIM-HD proteins implicated in neuronal development that form the basis of regulatory complexes in two adjacent cell types in the ventral spinal chord, V2 interneurons and motor neurons. Both cell types express Lhx-3 and the nuclear adaptor protein Ldb1, however, Isl-1 is only expressed in postmitotic motor neurons. In the two complexes, the two LIM domains of Lhx-3 mediate different protein:protein interactions that appear to be critical for the regulation of the two different cell types. In V2 interneurons, this involves a direct interaction with the LIM interaction domain (LID) of Ldb1, whereas in motorneurons Isl-1 interacts directly with Ldb1-LID and Lhx-3 binds instead to Isl-1. We are interested in characterising these interactions with the overall goal of understanding their role in neuronal development. Deletion mutagenesis analyses have revealed a 30-residue region of Isl-1 that binds the Lhx-3 LIM domains (LIMs). We have solved a crystal structure of a protein complex between Lhx3 and Isl1 to show that, despite low sequence homology, the LIDs from Ldb1 and Isl1 bind Lhx3 in an essentially identical manner. Binding and stability studies of these different complexes suggest that a ternary Lhx3:Isl1:Ldb1 complex can only form if the complex binds to DNA that contains Lhx3 and Isl1 recognition sequences. This work highlights a general mode of interaction between different LIM-HD proteins involved with LIM codes.

Keywords: protein crystallography, protein interactions, zinc finger proteins

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Crystal structure determination of sheep *(Ovis aries)* methemoglobin at 2.7Å resolution

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Hemoglobin is a tetrameric protein, which is in equilibrium between low affinity tense (T) state and high affinity relax (R) state. Mammalian hemoglobin can be broadly classified into two groups: those with intrinsically high oxygen affinity and another with low oxygen affinity. Human, rodent, dogs, pigs, horses, camels, marsupials and most primates belong to the high oxygen affinity category in contrast to cows, sheep, goats, deer, cats and lemur belong to the low oxygen affinity. In order to unravel the structure-function relationship of low affinity mammalian species, the sheep hemoglobin structure has been determined at 2.7Å resolution. The oxygen affinity of sheep hemoglobin is about 10 times lesser than the human hemoglobin and the 2,3-diphosphoglycerate, the potential allosteric effector of mammalian hemoglobin, does not alter its oxygen affinity. Sheep hemoglobin is purified from the blood plasma and crystallized in orthorhombic space group $P2_12_12_1$ with one whole biological molecule ($\alpha 2\beta 2$) in the asymmetric unit with cell dimensions a=60.231Å, b=70.695Å, c=131.479Å. The structure was solved by molecular replacement method and the final refinement converges to R=19% and R_{free}=25%. Obviously the overall structural features of sheep hemoglobin is found to be similar to human oxyhemoglobin, in contrast significant tertiary structural differences are observed. Comparing with human model, the shift of 2.1Å in

A-helices of the β-subunits, differences in the interactions of dimer-dimer interface hinge region and the constrained heme environment associated with β_1-subunit are the most significant structural differences which presumably play a remarkable hampering effect on oxygen affinity of sheep hemoglobin.

Keywords: methemoglobin, allosteric mechanism, low-oxygen affinity

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Purification, crytallization and X-ray structure determination of cocosin from *Cocos nucifera*

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Plant proteins are the cheapest available sources of nutrition in many countries; hence form an important part of human diet. Large quantities of storage proteins are accumulated in developing seeds of leguminous plants that function as a reserve of carbon and nitrogen used during germination and early growth. These proteins are deposited in an aggregated form within specialized organelles of the seed called protein bodies. There are two major types of storage proteins in legume seeds, known as vicilins and legumins, which are distinguishable by their sedimentation coefficients (7S/11S), oligomeric organization (trimeric/hexameric) and polypeptide chain structure (single chain/disulfide linked pair of chains). 11S globulins are non-glycosylated proteins, each of the subunits in the hexamer itself is composed of an acidic and a basic chain derived from a single precursor and linked by a disulphide bond. Globulin (11S) is one of the major storage proteins of many legume and nonlegume seeds. Coconut *(Cocus nucifera)*, is a major source of plant protein in most tropical and subtropical regions of the world. The globulin cocosin is a legume class reserve protein in coconut. It is important to understand the biosynthesis, targeting and biological functions of seed proteins as a prerequisite to their rational manipulation in improving nutritional value. However, such information is still in paucity for seed storage proteins of coconut. Therefore, the cocosin is purified from the coconut endosperm and the crystal structure was determined at 2Å resolution. The detailed structural description is to be presented.

Keywords: seed storage protein, cocosin, crystal structure determination

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Structural analysis of a giant cell wall-associated adhesion protein Ebh from *Staphylococcus aureus*

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Staphylococcus aureus is a major cause of hospital- and community-acquired infections. *S. aureus* causes serious and fatal diseases, such as toxic shock syndrome or septicemia. Genome analyses of several strains of *S. aureus* revealed the presence of a giant gene 31494 bp in length. The putative protein of ca. 1.1 MDa encoded by this gene shows homology to the major adhesion protein of Streptococcus defectivus, Emb, a protein that binds to the extracellular matrix (ECM) of host cells, and therefore the giant protein was named Ebh (ECM binding protein homologue). Ebh consists of several distinct regions, including a large central region with 52 imperfect repeats of 126 amino acid residues. In the present study, we investigated the structure of this giant molecule by X-ray crystallography, CD spectrometry, and small-angle X-ray scattering (SAXS). The crystal structure of two repeats showed that each repeat consists of two distinct three-helix bundles, and two such repeats are connected along the long axis resulting in a rod-like structure 120 Å in length. CD and SAXS analyses of the samples with longer repeats suggested that each repeat has an identical structure and such repeats are connected tandemly to form a rod-like structure in solution the length of which increased proportionately to the number of repeating units. On the basis of these results, it was proposed that Ebh is a rod-like molecule 320 nm in length with some plasticity at module junctions.

Keywords: small-angle X-ray scattering, *ab-initio structure* determination, circular dichroism

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Crystal structures of the 70-kDa heat shock proteins in domain disjoining conformation

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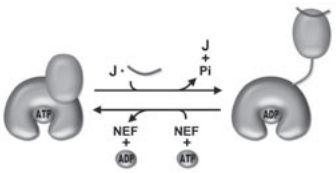
The 70-kDa heat shock proteins (Hsp70s) are highly conserved ATP-dependent molecular chaperones composed of an N-terminal nucleotide binding domain (NBD) and a C-terminal protein substrate binding domain (SBD) in a bilobate structure. Interdomain communication and nucleotide-dependent structural motions are critical for Hsp70 chaperone functions. Our understanding of these functions remains elusive due to insufficient structural information of functionally intact Hsp70s in different chaperone cycle states. We report here the crystal structures of DnaK from Geobacillus kaustophilus HTA426 bound with ADP-Mg^{2+}-Pi at 2.37 Å and 70-kDa heat shock cognate protein from Rattus norvegicus bound with ADP-Pi at 3.5 Å. The NBD and SBD in these structures are significantly separated from each other and they may be corresponding to the ADP-bound conformation. Moreover, a Trp reporter was introduced at the potential interface region between NBD and interdomain linker of GkDnaK to probe the environmental changes. The result of fluorescence measurement further supports that the substrate binding enhanced domain disjoining behavior for Hsp70 chaperone family.



Keywords: Hsp70, chaperone, heat shock protein