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Thursday, March 19, Session I

L1

CARBORANE-BASED INHIBITORS OF CARBONIC ANHYDRASES

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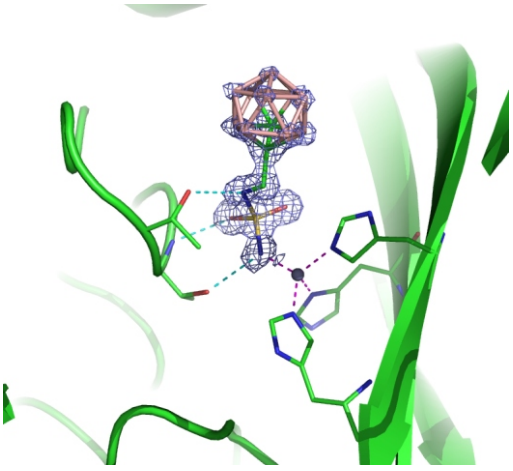
Carbonic anhydrases are ubiquitous zinc metalloenzymes that catalyse interconversion between carbon dioxide and the bicarbonate ion. So far, 15 human carbonic anhydrase (CA) isoforms have been identified displaying significant differences in catalytic activity, subcellular localization and tissues expression. They play key roles in intracellular and extracellular pH homeostasis, in the transport of CO_2 and bicarbonate in respiration, and in several biochemical pathways where either CO_2 or bicarbonate is required. Immense experimental evidence also suggests a role of CAs in various pathological processes and many CA isoenzymes have thus become established diagnostic and therapeutic targets.

The traditional CA inhibitors contain a sulfonamide or sulfamide moiety that coordinates Zn^{2+} cation located in the CA active site. Most of the currently used CA inhibitors lack selectivity, and their use has some problematic side effects. This opens new round of design of inhibitors that can block specific isozymes. Although the conical active-site clefts of different human CA isoezymes are highly homologous, variations exists in the amino acid composition at the entrance to the active site. As a result of their differing in shape and hydrophobicity, these surface pockets can be exploited to design specific inhibitors.

In conclusion, our results suggest that carborane-based compounds are promising lead structures for the development of inhibitors of CA isozymes. Our experiments demonstrated that various types of hydrophobic, space-filling carborane clusters can be accommodated in the CA active site and that substitution with an appropriately attached sulfamide group and other substituents leads to compounds with high selectivity for the cancer-specific CAIX isozyme over the widespread CAII isozyme. Crystal structures confirmed our hypothesis that three-dimensional scaffolds could be efficiently used in CA inhibitors and provided structural information that can be applied to the structure-based design of specific CAIX inhibitors.



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L2

STRUCTURAL BASIS FOR HYPERACTIVITY OF cN-II MUTANTS

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The recent studies demonstrated that 20% of relapsed acute lymphoblastic leukemia are associated with mutations in NT5C2 encoding cytosolic purine 5'-nucleotidase (cN-II). The identified point mutations lead to a biosynthesis of hyperactive enzyme that inactivates drugs used for chemotherapy.

The aim of this project was detailed biochemical and structural characterization of representative hyperactive cN-II mutants. In this study, we analyzed the most common mutant R367Q. Kinetic analysis of nucleotidase reaction with inosine monophosphate as a substrate showed that the K_M value of the R367Q is significantly decreased in the absence of physiological activator of the enzyme - ATP, i.e the K_M value was determined to be 5.82 ± 1.69 mM compared to K_M of 33.55 ± 9.14 mM for the wild-type cN-II. On the contrary, kinetic parameters of the R367Q mutant and wild-type enzyme were not different in the presence of ATP. The analysis of enzyme kinetics indicates that the mutant possessed abolished allosteric regulation being constitutively active.

Biophysical analysis showed that the R367Q cN-II as well as wild-type protein assembled properly into tetramer and thermal stability of the proteins was virtually identical exhibited melting temperature about 55 °C. Subtle changes in conformational properties were been also revealed by circular dichroism spectroscopy and tryptophan-based fluorimetry. The X-ray crystallography together with mass spectrometric studies demonstrated that the overall shape of the wild-type and mutant protein is indistingushable. Nevertheless the integration of the protein crystal structure with mass spectrometric data showed that all of the regions with altered topology are located at the oligomeric interface of the cN-II. Interestingly, the location of the perturbed regions is consistent with the distribution of other identified mutations causing the enzyme hyperactivity. It suggests that oligomeric interface of cN-II might be an important site for pharmacological intervention of relapsed ALL.

L3

THE UNIQUE MECHANICS OF BACTERIAL REPRESSOR DeoR: A STRUCTURAL STUDY

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Deoxyribonucleoside regulator (DeoR) from *Bacillus subtilis* downregulates the expression of enzymes involved in deoxyribonucleosides and deoxyribose catabolism. The DeoR repressor comprises a C-terminal effector-binding domain (C-DeoR) and an N-terminal DNA-binding domain. We present here the crystal structures of the free C-DeoR and of the covalent complex of C-DeoR with the effector molecule deoxyribose-5-phosphate (dR5P). This is the first case of a bacterial transcriptional regulator that binds its effector covalently via a Schiff base linkage to a lysine residue (Lys141). Mutational analysis confirmed the key role of Lys141 in effector binding and the formation of the Schiff base adducts in solution was confirmed by mass spectrometry. Structural analyses of the crystal structures of the free and effector-bound C-DeoR explained the function of DeoR as a molecular switch.

L4

THE CRYSTAL STRUCTURE OF THE PHOSPHATIDYLINOSITOL 4-KINASE II

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Phosphatidylinositol 4-kinase II (PI4K II) is one of the four mammalian lipid kinases that catalyses the conversion of phosphatidylinositol to phosphatidylinositol 4 phosphate, the major precursor of phosphoinositides. PI4K II is a constitutively active membrane bound protein and its catalytic activity is responsive to the membrane composition. It was shown that PI4K II has an important role in intracellular vesicular trafficking, inter-organelle trafficking and signal transduction. Furthermore, PI4K II is indispensable for neuronal survival and regulation of tumor growth. Due to an emerging role of PI4K II in several diseases and in maintaining the phosphoinositide homeostasis in the cell there has been a strong interest in its structural determination. In addition, there are currently no isoform-specific inhibitors of PI4K available to modulate their overlapping enzymatic activity and thereby enable the proper isoform-specific characterisation in different cell compartments. Therefore, here we present the structural domain composition of PI4K II with ATP in the active site. The crystal structure revealed conserved N- and C-terminal lobes with ATP binding site located at their interface and the unique hydrophobic pocket of the C-lobe with second bound ATP. We further used the MD simulation and site-directed mutagenesis to analyze structural features determining substrate specificity and membrane binding mode.

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L5

INSIGHTS INTO THE STRUCTURE OF THE HUMAN RyR2 N-TERMINAL REGION AND ITS MUTATIONS RESPONSIBLE FOR CARDIAC ARRHYTHMIAS

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The human ryanodine receptor 2 (hRyR2), the largest ion channel so far known, mediates calcium release from the sarcoplasmic reticulum into the cytoplasm of cardiac myocytes. It is a transmembrane protein composed of four identical subunits (each of approximately 5000 amino acids), each of which contains 14 domains [1]. hRyR2 is predominantly expressed in heart tissue and is a principal component of cardiac excitation-contraction coupling, which enables regular heart contraction. Several mutations in the gene of this protein are associated with severe cardiac arrhythmias (catecholaminergic polymorphic ventricular tachycardia, CPVT1 and arrhytmogenic right ventricular dysplasia, ARVD2), often causing human death. Here we present a detailed analysis of the N-terminal part of hRyR2 using a 2.38 Å crystal structure (residues 1–606), encompassing 33 mutations which have been linked to these diseases [2]. This part consists of three domains (INS, MIR, RIH) which are held together by a unique interaction network, in which a key role is played by the central helix (the helix preceding the RIH domain). Interestingly, a chloride binding site, reported to be crucial for the stability of the N-terminal region of mouse RyR2 [3], was not observed. The tertiary structure of this fragment also allows us to better understand the physical mechanisms by which several mutations within hRyR2 cause CPVT1 and ARVD2. Finally, we used this structure to help locate the positon of the N-terminal region within the whole ryanodine receptor using previously published electron microscopy data [4].

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Thursday, March 19, Session II

L6

MODULATED PROTEIN CRYSTAL STRUCTURE WITH 28 MOLECULES IN THE ASYMMETRIC UNIT

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In modulated crystals short-range translational order is lost and the atomic structure cannot be defined by the contents of a single small unit cell. The wave of disorder is described by a modulation function, which restores long-range periodicity. If the modulation period divided by the unit cell translation is a rational number, then the modulation is commensurate, and can be described in an expanded unit cell. Otherwise it is incommensurate. The diffraction pattern of a modulated structure contains strong main reflections from the basic unit cell, surrounded by weaker satellites from the modulation wave. Modulated structures are rare in protein crystallography.

Stress factors induce in plants the expression of Pathogenesis-Related (PR) proteins, divided into 17 classes. PR proteins of class 10 (PR-10) are well studied structurally but their biological function is unclear with an implication in phytohormone binding. PR-10/hormone complexes are studied using fluorescent probes such as ANS (8-anilino-1-naphthalene sulfonate). We crystallized Hyp-1, a PR-10 protein from St John's wort, in complex with ANS. Solution of the apparent $P4_122$ crystal structure was impossible by standard molecular replacement because of evident tetartohedral twinning and a bizarre modulation of reflection intensities with l periodicity of 7. The structure was solved using Phaser and data expanded to $P1$ symmetry. Ultimately, the structure turned out to have $C2$ symmetry with 28 independent protein molecules, arranged in dimers around a non-crystallographic (NCS) 2_1 screw along c with a pitch of ~1/7. The seven-fold repetition along c is indicative of a commensurate modulated structure: the NCS copies are similar but not identical. For instance, the consecutive Hyp-1 molecules bind a varying number (0-3) of the ligand molecules. The structure has been successfully refined to R=22.2% using conventional methods, i.e. with unit cell expanded to encompass the entire commensurate modulation period.