MS10-O5

Joint X-ray and Neutron protein crystallography for the study of enzyme-isoform selectivity by small molecule inhibitors

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Human carbonic anhydrase IX (CA IX) expression is actHuman carbonic anhydrase IX (CA IX) expression is activated by hypoxic condition in aggressive, metastatic tumors. Cancer patietns positive for CA IX have generally a poor prognosis. CA IX has emerged as an important cancer target, but efforts to develop isoform selective inhibitors are complicated by the presence of 14 other CA isoforms that share high sequence and structural similarity. This leads to off-target inhibitor binding and side effects. Recent studies showed that saccharin (SAC) already shows some isoform discrimination, and that conjugating SAC to a glucose molecule (Saccharin-Glucose Conjugate, SGC) further improves the K_i against CA IX by 2-fold. Ligand binding to proteins are mediated through numerous interactions, including: H-bonding directly and/or through intervening waters, electrostatic interactions with charged or polar amino acid side chains, metal coordination, energetic changes through water displacement, aromatic ring stacking, or other hydrophobic interactions. As neutrons scatter strongly from atomic nuclei of light atoms ^{1}H (Hydrogen), and its isotope ^{2}H (Deuterium), it is possible to use neutron protein crystallography (NPX) to "see" the light atoms and any interactions they are involved with. (e.g. H-bonds). We used joint X-ray and neutron crystallography methods to determine the crystal structures of a CA IX mimic alone and in complex with SAC and SGC, respectively. Our analyses reveal the molecular details of solvent displacement upon ligand binding, the H-bonding between the ligands and the proteins, involvement of water-mediated H-bonds, and the remodeling of H-bonds to accommodate ligand binding. The structures and analysis also provide an explanation for the observed CA isoform selectivity of the ligand under study.

Keywords: joint neutron and x-ray crystallography, hydrogen bonding

MS11 Hot structures in biology

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MS11-O1

Structural basis of human mitochondrial transcription

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Human carbonic anhydrase IX (CA IX) expression is activated by hypoxic condition in aggressive, metastatic tumors. Cancer patietns positive for CA IX have generally a poor prognosis. CA IX has emerged as an important cancer target, but efforts to develop isoform selective inhibitors are complicated by the presence of 14 other CA isoforms that share high sequence and structural similarity. This leads to off-target inhibitor binding and side effects. Recent studies showed that saccharin (SAC) already shows some isoform discrimination, and that conjugating SAC to a glucose molecule (Saccharin-Glucose Conjugate, SGC) further improves the K_i against CA IX by 2-fold. Ligand binding to proteins are mediated through numerous interactions, including: H-bonding directly and/or through intervening waters, electrostatic interactions with charged or polar amino acid side chains, metal coordination, energetic changes through water displacement, aromatic ring stacking, or other hydrophobic interactions. As neutrons scatter strongly from atomic nuclei of light atoms ^{1}H (Hydrogen), and its isotope ^{2}H (Deuterium), it is possible to use neutron protein crystallography (NPX) to "see" the light atoms and any interactions they are involved with. (e.g. H-bonds). We used joint X-ray and neutron crystallography methods to determine the crystal structures of a CA IX mimic alone and in complex with SAC and SGC, respectively. Our analyses reveal the molecular details of solvent displacement upon ligand binding, the H-bonding between the ligands and the proteins, involvement of water-mediated H-bonds, and the remodeling of H-bonds to accommodate ligand binding. The structures and analysis also provide an explanation for the observed CA isoform selectivity of the ligand under study.

The mitochondrial genome is transcribed by a dedicated mitochondrial RNA polymerase (mtRNAP), which also generates the RNA primers required for DNA replication. Unlike the distantly related bacteriophage RNA polymerases, mtRNAP requires auxiliary protein factors for each step of the transcription cycle. However, the molecular mechanisms underlying mitochondrial transcription are poorly understood. While the structures of the mitochondrial polymerase and of some mitochondrial transcription factors have been reported, structural data on the interplay between these factors and the polymerase in functional complexes has been lacking. We have determined the structure of the human mitochondrial transcription initiation complex, which reveals how the initiation factors TFAM and TFB2M facilitate promoter binding and DNA opening, respectively. Furthermore, we have solved the structure of the mitochondrial

transcription elongation factor TEFM and of an anti-termination complex consisting of TEFM bound to the transcribing polymerase. These structures illustrate how TEFM interacts with both the nucleic acid and the polymerase in the elongation complex to facilitate processive transcription and drive gene expression over primer formation for DNA replication. Together, these results elucidate the mechanistic basis of transcription initiation and processive elongation in human mitochondria and provide the framework for studying the regulation of mitochondrial gene transcription.

Keywords: Mitochondria, Transcription, RNA

MS11-O2

Structural and functional insight into human O-GlcNAcase

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O-GlcNAc hydrolase, OGA, removes O-linked N-acetylglucosamine (O-GlcNAc) from myriad nucleocytoplasmic proteins. O-GlcNAcylatation plays a vital role in a range of cellular processes including, for example, transcriptional regulation and the stress response (reviewed in Ref [1,2]). Dysregulation of O-GlcNAcylation has been implicated in diseases including cancer[3], and neurodegenerative diseases.[4,5] Notably, therapeutic agents targeting the O-GlcNAc modification have entered phase I clinical trials against neurodegenerative disorders, stimulating interest in the molecular and chemical basis of *O*-GlcNAcylation and its manipulation with small molecules.[6] We aimed to shed light on the multi-domain architecture of OGA and the structural basis of activity. Through co-expression and assembly of OGA fragments we determined the 3-D structure of human OGA, revealing an unusual helix exchanged dimer that lays a structural foundation for an improved understanding of substrate recognition and regulation of OGA.[7] Structures of OGA in complex with a series of different inhibitors define a precise blueprint for the design of lead structures having potential clinical value.

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Keywords: glycoside hydrolase, neurodegenerative disease, inhibitor design