3. Protein Structures and Dynamics

3A. Protein Structure, Modeling and Drug Design

PP3A-1

Studying the binding properties of BclA lectin - experiment and modeling

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Introduction: Burkholderia cenocepacia is a ubiquitous bacterium that can act as opportunistic human pathogen, responsible for lethal complications in cystic fibrosis patients. The genome of the bacterium contains several lectin-like sequences that are related to previously characterized fucose-preferring lectin PA-IIL from Pseudomonas aeruginosa (Mitchell et al. Nature Struct. Biol.2002; 12: 918–921). BclA is a lectin from B.cenocepacia that shares the unique sugar binding mode common to PA-IIL family lectins.
Methods: Molecular docking was performed using the AUTODOCK and DOCK software. The AMBER package was used for molecular dynamics simulations. Enzyme-linked lectin assay, surface plasmon resonance and isothermal titration calorimetry was used to determine the binding properties experimentally.
Results: Experiments determined that BclA prefers mannose to fucose as the binding partner. Despite the sequence similarity, BclA adopts dimeric form, as opposed to tetrameric PA-IIL – possible consequence of the presence of an extra loop. Docking experiments combined with molecular dynamics were performed to help with the structural reasoning.
Conclusions: Despite the homology, the PA-IIL family lectins differ in their binding preferences that are closely dependent even on slight changes in the binding site. Further protein engineering both in silico and *in vitro* will improve the understanding of rules of this pathogen lectin behavior.
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PP3A-2

Differential estrogen receptor modulators: assessment of estrogen receptor binding selectivity and transcriptional modulation bias

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Introduction: Estrogens play important roles in the regulation of physiology and pathophysiology, mostly via regulating the transcription of estrogen target genes. The two subtypes of estrogen receptor (ER), ERα and ERβ, are known to exhibit different ligand-binding selectivity and cell and promoter context-specific transcriptional regulation. Here we describe a series of novel cycloalkyl pyrazoles exhibiting ER subtype binding selectivity and differential regulation of transcription.
Methods: The binding affinity of the pyrazoles to isolated human ERα and ERβ was assessed using fluorescence polarisation. Molecular modelling revealed the mode of interaction of selected pyrazoles with the two ER subtypes. The ability of the compounds to modulate ERα- and ERβ-dependent transcription was assessed using ER subtype-specific reporter cell lines stably transfected with estrogen responsive element (ERE)-dependent luciferase gene.
Results: While all pyrazoles could bind to both ER subtypes, certain showed a bias towards ERβ. A subset of these pyrazoles was also shown to behave as full ERβ antagonists while exhibiting marginal ERα antagonist properties. The ER subtype selective agonist/antagonist behaviour was modulated by the cycloalkyl ring fused to the pyrazole core. The ERβ antagonistic pyrazoles inhibited estrogen and androgen stimulation of growth of LNCaP prostate cancer cells.
Conclusions: Development of subtype selective ERβ antagonists is a useful tool for deciphering the ligand structure requirements of ERβ-selective antagonism as well as for studying the role of ERβ in prostate cancer chemoprevention.

PP3A-3

SmCI, a bifunctional inhibitor of metallo carboxypeptidase and serine proteinase isolated from the marine annelid *Sabellastarte magnifica*. Isolation, characterization, cDNA cloning and recombinant expression

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Introduction: Metallocarboxypeptidases (CP) are an important and diverse class of enzymes with different digestive and regulatory functions. Consequently, their inhibitors are molecules with potential effectiveness in Biotechnology and Biomedicine. The variety of inhibitors (PI) of CP found in nature is somewhat limited, as compared to those of other proteases. The availability of new inhibitors will contribute to CP structure-function advances and to develop new potential drugs.
Methods: The purification procedure combines heat treatment, affinity chromatography using Carboxypeptidase A (CPA) immobilized on glyoxil Agarose and RP-HPLC on a C-8 column. CPA inhibition assay was carried out using AAFP as substrate [1] K_i values of the inhibitor against CPA and serine proteases were determined using tight binding inhibition strategy [2]. SmCI sequence was determined combining Edman degradation, ESI-MS-MS and cDNA cloning. Heterologous expression of SmCI was carried out into Pichia pastoris expression system.
Results: A new inhibitor was isolated from the tentacles crown of the annelid marine *S. magnifica* termed SmCI. It is a glycoprotein formed by 165 amino acids with 18 cisteine residues and 9 disulfide bonds (UniProt ID: PCPI_SABMA, AC: P84875). The full sequence information, revealed the presence of three BPTI Kunitz domains in SmCI structure, and a high homology with other Kunitz serine inhibitors. Kinetic and structural results have demonstrated that SmCI is a novel serine/carboxy bifunctional inhibitor, active against both, pancreatic CPA and serine proteinases, such as trypsin, chymotrypsin and pancreatic elastase. The inhibitor was successfully expressed into Pichia pastoris expression system.
Conclusions: SmCI is a novel bifuntional and multidomain inhibitor, and constitutes the first BPTI/Kunitz protein able to inhibit metallocarboxypeptidase A. Recombinant inhibitor showed similar molecular and bifunctional kinetics properties to the natural one.
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PP3A-4

Comparison of hydratase, esterase activities and inhibition manner of mutant Asn67/Ile and Leu204/Ser of human CAII

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Carbonic anhydrases (CAs; also known as carbonate dehydratases EC 4.2.1.1) are a family of enzymes which catalyze the reversible reaction from H_2O and CO_2 to HCO_3^- ions. Carbonic anhydrases are ubiquitous metalloenzymes present in prokaryotes and eukaryotes that are encoded by five evolutionarily unrelated gene families, namely α- CAs, β-CAs, γ-CAs, δ-CAs and ε-CAs. In mammals, 15 α-CA isozymes or CA-related proteins have been described with different catalytic activity, subcellular localization and tissue distribution.
The hCAII enzyme is the target for drugs, such as acetazolamide, methazolamide and dichlorphenamide for the treatment of glaucoma. However, since the specificity of sulphonamides against CA enzymes is low, the adverse effect could be encountered during the treatment of the related diseases. Therefore, it is important to elucidate the inhibition mechanism of the enzyme in order to develop more specific novel inhibitors.
In this work, we plan to identify some amino acids that may be involved in catalytic centre of hCAII enzyme. PCR based site-directed mutagenesis strategy has been performed replacing Asn67 to a hydrophobic amino acid, Isoleucin and Leu204 to a hydrophilic amino acid, Serin. The related mutations have been confirmed by sequence analysis. The mutant proteins were expressed in E coli by inducing IPTG. The expressed proteins were purified by sepharose 4B-Ltyrosine-sulphonamide affinity chromatography. The hydratase, esterase activity and inhibition manner of the mutant enzymes were compared to the wild type enzyme.