A01

Polymorphic Variation of CYP2D6 and GSTT1 Genes and its Association with Susceptibility to Chronic Myeloid Leukemia (CML)

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Abstract

BACKGROUND

Polymorphism in genes encoding carcinogen metabolizing enzymes may have relevance in determining susceptibility to cancer. Individuals carrying the more active form of an enzyme involved in the activation of carcinogens and less active form of detoxifying enzymes will be at greater risk of cancer. The mutant of CYP2D6*4 allele is among the most common polymorphic alleles of CYP2D6 gene, resulting in a decreased or no activity of CYP isoenzyme. GSTT1 null genotypes may result in less effective or absent enzymatic detoxification and thus increased susceptibility to CML. This study was aimed to determine the association of *CYP2D6* allelic variants and GSTT1 null genotypes if any, as risk factor to develop CML.

METHODS

DNA was isolated by standard phenol-chloroform method. PCR was carried out to determine GSTT1 null genotype CYP2D6*4 alleles. BstN1 digestion was carried out to detect allelic variants in CYP2D6*4 PCR products were separated using 2% agarose gel. The relationship between CYP2D6*4 alleles and GSTT1 null genotype and risk of CML was assessed by means of chi square test.

RESULTS & CONCLUSIONS

In CML patients CYP2D6*4 77% (35/45) were homozygous wild, 13% (6/45) heterozygous and 8% (4/45) homozygous mutant alleles while in controls the frequency was 75% (42/56), 14% (8/56) and 10% (6/56) respectively. However, results were statistically significant with GSTT1 null genotype frequency in CML patients as compared to controls 16/80 (20%) vs 9/105 (8.5%); it projects a 2.67-fold increased risk for CML in individuals with GSTT1 null genotype as compared to those possessing both alleles of the gene.

Functional Characterization of EZH2 Downstream Signaling Leading to Cell Proliferation by Proteomics Strategies

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Abstract

Enhancer of zeste homolog 2 (EZH2) is suggested to be a potential therapeutic target and a diagnostic marker for cancer. Our previous study also showed the critical role of EZH2 in hepatocellular carcinoma (HCC) tumorigenesis. The present study is aimed at revealing the comprehensive downstream pathways of EZH2 by functional proteomic profiling. Lentivirus mediated RNA interference (RNAi) was employed to knockdown EZH2 in HCC cells. The 2-DE was employed to compare the expression profile difference between parental and EZH2-knockdown HCC cells. In total, 28 spots were differentially expressed during EZH2 inhibition. Among all, 18 proteins were identified by PMF with MALDI-TOF MS. Western blotting further validated upregulation of 60S acidic ribosomal protein P0 (L10E), and downregulation of two proteins with EZH2 inhibition: stathmin1 and probable protein disulfide isomerase (PDI) ER-60 precursor (ERp57). Moreover, L10E was downregulated with overexpression of EZH2 in hepatocytes, and L10E reversed the effect of EZH2 on cell proliferation, suggesting it a downstream target of EZH2. The comprehensive and comparative analyses of proteins associated with EZH2 could further our understanding on the downstream signal cascade of EZH2 leading to tumorigenesis.

A03

The Crystal Structure of Seabream Antiquitin Reveals the Structural Basis of its Substrate Specificity

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Abstract

Antiquitin, an evolutionarily conserved protein sharing high amino acid sequence identity (~60%) among homologs from plants to human, is a member of the aldehyde dehydrogenase superfamily. We have previously purified this enzyme from black seabream (*Acanthopagrus schlegeli*), cloned its full-length cDNA sequence, and expressed the biologically active recombinant enzyme in *E. coli*. Herein we solved the crystal structure of seabream antiquitin in complex with the cofactor NAD^+ at 2.8 Å resolution. The tetrameric antiquitin, similar to other aldehyde dehydrogenases, is comprised of catalytic, NAD^+-binding and oligomerization domains. We have shown that α-aminoadipic semialdehyde (α-AASA) is a good substrate of antiquitin, with Km and *k*cat values of 67 μM and 6.5 s^{-1} respectively. The crystal structure of antiquitin reveals the structural basis of its specificity towards α-AASA. The mouth of the substrate binding pocket is guarded by two conserved residues, Glu120 and Arg300, and the lower part of the pocket is surrounded by the hydrophobic residues Phe167, Ala170, Trp174 and Phe467 that can interact with the aliphatic chain of α-AASA. To test the role of Glu120 and Arg300, we have prepared two mutants, E120A and R300A, of antiquitin. Our model and kinetics data suggest that substrate specificity towards α-AASA is contributed by Glu120 and Arg300 forming stabilizing interactions with the α-amino and α-carboxylate groups of the substrate. The crystal structure also provides a molecular explanation for the inefficient oxidation of α-AASA in pyridoxine-dependent epilepsy patients carrying mutations in their antiquitin gene.

Novel Motifs in NAD^+ Dependent DNA Ligases: Functional Implications about Ligases Binding to Nicked DNA

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Abstract

DNA ligases catalyze the joining of nicked DNA, which is necessary in DNA replication and repair pathways where the re-synthesis of DNA is required. Most organisms use ATP powered DNA ligases, but eubacteria appear to be uniquely use ligases driven by NAD^+. The sequence comparison between NAD^+ dependent DNA ligases led us to identify two novel conserved motifs, designated motif A and B, present in all NAD^+ dependent DNA ligases. Compared to motif I (KxDG), which essential for catalyze identified previously, the result of searching against NR database show highly identity. In order to realize how their function works, we carried out structure comparison between NAD^+ and ATP dependent DNA ligases, the result shows that the regions of novel motifs are also similar at structure levels, and correspond to DNA binding regions of ATP dependent DNA ligases. The region of motif A nearby 3'-OH DNA nick and opposite one strand of the minor groove. Motif B opposite the minor groove of the 3'-OH DNA nick and between the two strands of the minor groove, its axis is parallel to minor groove orientation. These finding suggest that except for the five motifs which essential for catalyze identified previously, there are two conserved motifs located at the adenylation domain among NAD^+ dependent DNA ligases. Homology-model-guided structural analysis show functional implications: the novel motifs possibly interact with minor groove of 3'-OH of DNA nick and stabilized the DNA nick. Recently, the structure of *E.coli* DNA ligase supports our view strongly.

A05

Crystal Structure of Human Pyridoxal Kinase.

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Abstract

Pyridoxal kinase, a member of the ribokinase superfamily, catalyzes the ATP-dependent phosphorylation reaction of vitamin B6 and is an essential enzyme in the formation of pyridoxal-5'-phosphate, a key cofactor for over 100 enzymes. Pyridoxal kinase is thus regarded as a potential target for pharmacological agents. In this paper, we report the 2.8 Å crystal structure of human pyridoxal kinase (HPLK) expressed in Escherichia coli. The diffraction data revealed unexpected merohedral perfect twinning along the crystallographic c axis. Taking perfect twinning into account, the structure in dimeric form was well refined according to the CNS program. Structure comparison reveals that the key 12-residue peptide over the active site in HPLK is a ß-strand/loop/ß-strand flap, while the corresponding peptide in sheep brain enzyme adopts a loop conformation. Moreover, HPLK possesses a more hydrophobic ATP-binding pocket. This structure will facilitate further biochemical studies and structure-based design of drugsrelated to pyridoxal kinase.

Dynamics Study of a Thermophilic Acylphosphatase and its Mutants from *Pyrococcus Horikoshii* by NMR Spectroscopy

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Abstract

Thermophilic enzymes adapted to perform catalysis at elevated temperatures are often sluggish enzymes at lower temperatures, when comparing to mesophilic homologues. Reduced flexibility is often regarded as the culprit behind the reduced catalytic efficiency of thermophilic enzymes. We have cloned the coding sequence of acylphosphatase from *Pyrococcus horikoshii* (PhAcP) and expressed the protein in *E. coli*. We propose to use an acylphosphatase from *Pyrococcus horikoshii* as a model system to study the stability, activity, and flexibility relationships of enzymes. Acylphosphatase (AcP, ~90-100 residues) is one of the smallest enzymes known. We have recently solved the structure of PhAcP, and measured its stability and kinetics parameters. High-resolution structure of the thermophilic AcP is compared to the structures of mesophilic AcPs to understand the structural adaptation of *P. horikoshii* AcP in coping with high temperatures. We have found that while PhAcP is extremely stable, with a melting temperature of ~112^{o}C, and a free energy of unfolding of ~54 kJ/mol. Kinetics studies showed that PhAcP is a less efficient enzyme than other mesophilic AcP, for its k_{cat} value of ~95 s^{-1} is much lower than the value of ~1500 s^{-1} reported for mesophilic AcP. As there is no structural difference between the active site of PhAcP and mesophilic AcP, we hypothesize that the reduced activity of PhAcP is due to reduced flexibility of the active site. To investigate the structural and dynamical change upon binding of phosphate to the active site, we are studying the backbone dynamics of free and bound forms of AcP by NMR spectroscopy. At the same time, we have created mutants designed to perturb flexibility or stability of PhAcP. The protein dynamics of PhAcP and its mutants characterized by NMR relaxation experiments will also be compared.

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A07

Crystal Structure of CaiE, a Protein Involved in L-Carnitine Metabolism

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Abstract

L-(−)-Carnitine is an important molecule in human metabolism, mainly because of its role in the transport of activated fatty acid across the inner mitochondrial membrane. In prokaryotes, L-carnitine can be used as carbon and nitrogen source for aerobic growth, or as electron acceptor under anaerobic conditions. In *Shigella flexneri*, the proteins involved in L-carnitine metabolism are encoded by the *caiTABCDE* operon. While the other proteins encoded by the *caiTABCDE* operon have been characterized, the function of CaiE is still unknown. Here we report the 1.6Å resolution crystal structures of CaiE. CaiE adopts a typical left-handed parallel β helix (LβH) fold. The N-terminal domain is composed of 18 tandem copies of the hexapeptide sequence motif folded as a left-handed triangular helix. The overall shape of this domain is that of an equilateral prism. CaiE is a homotrimer in solution and in crystallized form. Three zinc ions were identified in the trimer at the intermolecular interface, each coordinated by three histidine side chains from two different CaiE molecules. The overall folding and the structure at the zinc site of CaiE are very similar to those of the carbonic anhydrase from the archaeon *methanosarcina thermophila*. Based on the crystal structures of CaiE and its complex with bicarbonate, we propose this protein is a carbonic anhydrase.

A08

Three-dimensional Structure of *Chlamydia trachomatis* Heat Shock Protein 60 Reveals Cross Presentation of Epitopes in Infected Women Causing Infertility

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Abstract

The purpose of the study is an attempt to unravel the pathological mechanism associated with chlamydial heat shock protein 60 (cHSP60) using *in silico* tools followed by wet lab experiments. *Chlamydia trachomatis* (CT) infections are most prevalent sexually transmitted infection in the world and are often associated with various fertility disorders. We found a significant high seropositivity of anti-cHSP60 antibodies in women who had developed infertility due to prolonged CT infection. Heat shock proteins are major bacterial antigens that are conserved across the species and thereby mimic the response generated after host immune system. The cross presentation of human HSP60 (hHSP60) in place of cHSP60 to the antibodies of cHSP60 increases the propensity of pathological sequelae in CT infected women. The mechanistic explanation underlying this pathology remains undefined. We therefore undertook detailed bioinformatic and serological study to determine whether this knowledge could shed light on their role in progression of CT infection to infertility. In order to get three-dimensional structure of cHSP60, we have used different *in silico* tools e.g. mGenTHREADER, Swiss-PdbViewer package, MODELLER and PROCHECK. Moreover, active site mapping and motif scan reveals some common epitopes between cHSP60 and hHSP60, which was further validated in wet lab experiment using ELISA. In conclusion, cross presentation of specific antibody binding epitopes from cHSP60 and hHSP60 is responsible for serious clinical complication like infertility in persistent CT infected women.

A09

Development of Novel Fluorescence-Detection Method for Uracil and Cytosine

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Abstract

Pyrimidine-based compounds are of a great importance in biomedical and clinical chemistry. For example, uracil analogues have been used for therapeutic purposes e.g. anticancer drugs, and cytosine derivatives serve many important functions in cell replication and metabolism in mammals. Therefore, a highly sensitive and specific detection method of pyrimidine-based compound in biological samples, such as blood, serum, tissue and urine, is highly desirable. Fluorescence derivatization of a specific substance is one of the best methods to detect the objective compound in a biological sample containing numerous UV-active materials. However, only few methods for the fluorescence derivatization of pyrimidine analogues have been reported. We have found that uracil and cytosine react with benzamidoxime in the presence of potassium hexacyanoferrate in aqueous potassium hydroxide solution to give fluorescent derivatives. The reaction is highly specific to uracil and cytosine, and their nucleosides/nucleotides gave no fluorescence. Interestingly, thymine did not produce the fluorescence under the same condition, indicating that the substitution at 5 position of pyrimidine ring inhibits the reaction. The reaction is sensitive to the concentration of each reactant. However, reverse-phase liquid chromatographic analysis showed that the reaction gives a single fluorescent product under the optimized condition. We hope that our novel fluorescence derivatization is a promising method for micro detection of cytosine and uracil analogues in biological materials. Structure determination of the fluorescent derivatives of uracil and cytosine will also be presented along with the detailed reaction condition of the fluorescence derivatization.

Expression of Mouse Prion Protein in *E. coli* to Develop Aptamer

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Abstract

Prion protein has a key role in the occurrence of transmissible spongiform encephalophathies (TSE), such as Creutzfeldt-Jakob disease in human and bovine spongiform encephalophathy. A hallmark of these diseases is the transformation of nomal cellular prion protein (PrPc) into an infectious disease associated isoform (PrPsc). Therefore, a reagent, which can distinguish between PrPc and PrPsc, will be a useful tool for the TSE research. For example, PrP-specific aptamers will play an important role in the development of a diagnostic assay for the detection of TSE. Thus, we are trying to identify a series of PrP-specific aptamers, and characterize their binding properties in this study. To construct the PrP-specific aptamers, we selected mouse PrP (mPrP) as a target molecule. First, the cDNA of mPrP from mouse brain cDNA library was amplified by PCR, and its nucleotide sequence was confirmed by DNA sequencer. The cDNA of mPrP was inserted to pMal c2x vector, and expressed as a fusion protein with maltose-binding protein (MBP) in *E. coli*. Expressed fusion protein (mPrP-MBP) was purified with maltose resin column chromatography. From cultured *E. coli* (50 ml), we could obtain approximately 0.8 mg fusion protein. The purified fusion protein was digested by factor Xa to remove the MBP-tag. Interestingly, mPrP became insoluble after digestion of mPrP-MBP with factor Xa. This reason is unclear, however, it might be the conformational change due to the removal of MBP-tag. Now we are searching aptamer against purified mPrP.

A11

Structural and functional characterization of CheY from *Helicobacter pylori*

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Abstract

Helicobacter pylori is the human pathogen that causes gastritis, duodenal ulcer and gastric cancer. About 50% of the world population has been infected with *H. pylori*. Chemotaxis is an important virulence trait for *H. pylori* infection. From animal model in previous studies, *cheA*, *cheW* and *cheY* mutants are non-chemotactic and show attenuated phenotype, suggesting that chemotaxis is essential in colonization. CheY belongs to response regulator superfamily that controls the clockwise or counter-clockwise movement of flagella. The process is determined by phosphorylation and dephosphorylation of CheY. Phosphorylation increases the affinity of CheY to FliM, a component of the switch protein complex, altering the flagella rotation to opposite direction. To study the underlying mechanism of chemotactic regulation in *H. pylori*, we have expressed and purified recombinant CheY. Here we report a 1.8^{o}A crystal structure of activated CheY. Comparison of the structure with inactive and activated CheY from *E. coli* will be discussed.

Structural Analysis of FliS, Flagellar Protein of *Helicobacter pylori*

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Abstract

Helicobacter pylori is a gram negative microaerophilic bacterium which colonizes in gastric mucosal cells of nearly half of total population in the world especially in developing countries. It is the causative agent of gastritis, peptic ulcer and gastric cancer. Motility of bacterium in acidic and viscous environment depends on the formation of functional flagella. Recent studies show that mutation of a 14-kDa flagellar protein, FliS would reduce the adhesion ability to mucosal cells and biosynthesis of flagella. Thus, FliS is important in pathogencity of *H. pylori*.

To understand the molecular structure of FliS, we over-expressed recombinant FliS as a glutathione S-transferase (GST)-tagged fusion protein in *Escherichia coli* expression system. FliS was purified with affinity chromatography using GST-sepharose and Superdex-75 gel-filtration chromatography. Crystals of FliS were obtained by hanging-drop vapour-diffusion method. Diffraction data was collected at 105K to 2.8 Å with an in-house X-ray crystallography set-up. The crystal was in rod-like structure, belonging to space group of $I4_122$, with unit-cell parameters a = b = 92.54, c = 144.29 Å. Two molecules are found in an asymmetric unit. The structure phase was solved by molecular replacement using FliS of *Aquifex aeolicus* as the search model. Structure details will be investigated.

A13

A Single Salt Bridge Accounts for the Large Reduction in Activation Energy for Thermophilic and Mesophilic Acylphosphatases

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Abstract

It has been observed that thermophilic enzymes tend to be less active than their mesophilic counterparts at lower temperatures. On the other hand, these thermophilic enzymes exhibit extraordinary thermostability. Despite of the high structural similarity, thermophilic acylphosphatase from *Pyrococcus horikoshii* (PhAcP) is more sluggish than the mesophilic human acylphosphatase (HuAcP) at lower temperatures. According to the structural study, the reduced activity of PhAcP at lower temperatures may be attributed by an extra salt bridge formed in the active site of the protein, consequently to increase the activation enthalpy of PhAcP. To examine the role of this salt bridge, mutant (G91A) was created to disrupt the salt bridge. Temperature-dependency kinetics had revealed that the G91A without salt bridge showed significantly decrease in activation energy (E_a) but increase in change of entropy ($\triangle S^{\#}$). On the other hand, mutant of HuAcP (A99G) with salt bridge engineered in showed remarkably increase in E_a but decrease in $\triangle S^{\#}$. Taken these together, our data strongly suggest that a specific salt bridge is directly associated with the temperature adaptation of AcP.

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A14

Interaction between Trichosanthin, a Ribosome-inactivating Protein, and the Ribosomal Stalk Protein P2 by Chemical Shift Perturbation and Mutagenesis Analyses

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Abstract

Trichosanthin (TCS) is a type I ribosome-inactivating protein that inactivates ribosome by enzymatically depurinating the A(4324) at the alpha-sarcin/ricin loop of 28S rRNA. TCS was found to interact with human acidic ribosomal proteins P0, P1 and P2, which are protein components of the lateral stalk of eukaryotic ribosome. Deletion mutagenesis showed that TCS interacts with the C-terminal tail of P2, which is well-conserved among P0, P1 and P2. The P2-binding site on TCS was found to be located in the C-terminal domain by chemical shift perturbation experiments. By scanning charge-to-alanine mutagenesis, it has been shown that K173, R174 and K177 in the C-terminal domain of TCS are involved in TCS-P2 interactions, which is suggested to form charge-charge interactions to the conserved DDD motif at the C-terminal tail of P2. Therefore, a triple-alanine variant K173A/R174A/K177A of TCS was produced. This variant fails to bind P2 and ribosomal stalk in vitro and was found to be 18-fold less active in inhibiting translation in rabbit reticulocyte lysate, suggesting that interaction with P-proteins is required for full activity of TCS. The fact that stalk proteins interact with elongation factor suggested that interaction with acidic ribosomal stalk proteins help TCS to locate its RNA substrate.

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A15

Crystal structure of ptcA(IIA^{cellobiose}) and ptxB (IIB^{galactitol}) from *Streptococcus mutans*

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Abstract

Streptococcus mutans (S.mutans), a dental pathogen, is the leading cause of tooth decay worldwide. *S.mutans* can metabolize carbohydrate actively and thrive at quite low pH. It is important to study the sugar metabasis related topics for understanding *S.mutans'*pathogenicity. Four genes ptcA (IIA^{cellobiose}), ptcB (IIB^{cellobiose}), ptxA (IIA^{galactitol}), and ptxB (IIB^{galactitol}) of *S.mutans* belong to sugar phosphotransferase system (PTS), which is responsible for the binding, transmembrane transport and phosphorylation of numerous sugar substrates, and also involved in the regulation of a variety of metabolic and transcriptional processes. The PTS is composed of two non-specific components, enzyme I and a heat stable phosphocarrier protein (HPr), as well as sugar specific enzymes II complexes. The Enzymes II complexes commonly consist of three independent domains, IIA and IIB, which are hydrophilic and located in the cytoplasm, IIC that is an integral membrane protein. In order to understand the protein-protein interactions of Enzymes II complexes, ptcA, ptcB, ptxA and ptxB were cloned into pET28a and expressed at high level in the *Escherichia coli* strain BL21(DE3). These proteins were purified to homogeneity using Ni^{2+}-chelating chromatography followed by gel filtration. The crystals of ptcA and ptxB were obtained by the sitting-drop vapour-diffusion. The structure of ptcA has been solved at 2.2 Å. It's a homotrimer, each subunit comprise three helices and pack against each other forming a nine-helix bundle. The ptxB was diffracted to 1.8 Å, the crystal belongs to space group p212121 and the structure are determining.

A16

Crystal Structure of Mabinlin II: a Novel Structural Type of Sweet Proteins and the Main Structural Basis for its Sweetness

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Abstract

Mabinlin II is a sweet protein unique in synthetic sweet perception and high thermostability, which is isolated from the mature seeds of *Capparis masaikai Levl.* grown in Southern China. The crystal structure of Mabinlin II has been determined at 1.7Å resolution by the SIRAS method, which features in an "all alpha" structure consisting of A and B chains crosslinked by four disulfide bridges, distinct from all known sweet protein structures. The experiments for testing the possible interactions of separated A-Chain and B-Chain and the native Mabinlin II were performed through the calcium imaging experiments with the HEK293E cells coexpressed hT1R2/T1R3. The result shows that hT1R2/T1R3 responds to both the integrated Mabinlin II and the individual B-Chain in the same scale, but not to A-Chain. The sweetness evaluation further identified that the separated B-Chain can elicit the sweetness alone, but A-Chain does not. All data in combination revealed that the sweet protein Mabinlin II can interact with the sweet taste receptor hT1R2/T1R3 to elicit its sweet taste, while the B-Chain with a unique [NL/I] tetralet motif is the essential structural element for both the interaction with sweet taste receptor and the eliciting the sweetness, and the A-Chain may play a role in assisting the integrate Mabinlin II to gain the synthetic sweet perception with a long aftertest. The findings reported in this paper will be advantage for understanding the diversity of sweet proteins and engineering research for development of a unique sweetener enable the practical use of Mabinlin II.

Correlation between the Protein Local Structural Differences and the Specificity and the Applications for Drug Design

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Abstract

Homologous proteins usually hold the similar overall structure, but the differences among the local structures nearby the active site also can be found. Whether these small structural differences determine the specificity in a superfamily or not is not very clear. Here, we analyzed the relationship between the structure and function in the DNase1-like superfamily, which contains DNase1 family and IPP5C family. We found that three regions according to the DNA binding are great different in different members of DNase1 family. The later sequence analysis shows that the length of the three regions in each member is conserved. We also found that one of the three regions block the DNA binding activity in all of the members of the IPP5C family. These results suggested that these local structural differences nearby the active site in a superfamily determine the protein specificity. Based on this theory, series methods developed to design the short peptide or compound to inhibit the protein function. Recently, we cooperate with other laboratories designed a short anti-HIV peptide base on GP41 (Envelope transmembrane glycoprotein 41), and anti-drug addiction peptide base on CB1 (Cannabinoid receptor 1) and D2 (Dopamine receptor 2). These systematic researches will be help for deeply understanding the protein structure-function relationship and then directly guide the drug design.

Validation of Inter-helical Orientation of the Sterile-α-motif Domain of the Deleted in Liver Cancer 2 (DLC2-SAM) by Residual Dipolar Couplings

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Abstract

The *deleted in liver cancer 2 (DLC2)*, a tumor suppression gene which is frequently found to be underexpressed in hepatocellular carcinoma, encodes a multi-domain proteins including a sterile α-motif proteins (DLC2-SAM) [1]. Previous NMR structural studies of the SAM domain proteins (DLC2-SAM) revealed a unique four helical bundle structure, distinct from any other known SAM domain structures [2]. Residual dipolar couplings measured have been demonstrated to be able to provide important information of long range order in proteins, which complement nuclear Overhauser effects and J-couplings for the determination of the three-dimensional structure of proteins in solution [3, 4]. In the present study, we have applied ^{15}N-^{1}H residual dipolar couplings as additional constraints to refine the solution structures of the DLC2-SAM together with nuclear Overhauser enhancement and TALOS data. The resulting structures show improved quality factors comparing with the structures without RDC constraints and have low Q factors. The orientations of helices, in particular the helix 4 are validated by residual dipolar coupling data.

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The Particular Structural Changes between PRS1 and 7 Pathogenesis Superactivity Mutants

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Abstract

PRPP (phosphoribosylpyrophosphate) is both the pivotal substrate and the activator in human purine synthesis pathway, which can feed-forward activate GPATase to synthesize 5-phosphoribosyl-1-amine by PRPP itself and glutamine, then finally influence purine concentration in human body. Phosphoribosylpyrophosphate synthetase, especially its isoform 1 (PRS1), decides the PRPP content in human body; 7 different single-base substitutions in the translated region of PRS1 have been identified overactivity and regarded as etiological factor for some cases of gout. We have recombinantly expressed PRS1 and its 7 kinds of superactivity mutant proteins, then achieved 2.6 Å resolution apo PRS1 3D structure and 5 kinds of protein crystals for PRS1 superactivity mutants. The rest crystallography work is under way and the further particular structure analysis will reveal structural changes between PRS1 and its 7 pathogenesis superactivity mutants, and provide more information to gout clinic therapy.

A20

Comparison of protein interaction networks and its applications

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Abstract

The activity of cellular life relies on properly functioning of the extremely complex interaction networks among numerous intracellular constituents. Recent progresses in high-throughput proteomics have provided us with a first chance to characterize protein interaction networks (PINs), but also raised new challenges in interpreting the accumulating data. As with sequence analysis, a comparative apporoach often provides insights into the underlying laws behind complex phenomena. Motivated by this, we propose a computational strategy NetAlign to enable comparison of two protein interaction networks. NetAlign searches for conserved network substructures (CoNSs) that can pair in two PINs by combining interaction topology and sequence similarity. Using this approach we perform twenty-one pairwise comparisons among the seven recently available PINs of *E.coli*, *H.pylori*, *S.cerevisiae*, *C.elegans*, *D.melanogaster*, *M.musculus* and *H.sapiens*. We show that beyond what is gleaned from the genome, PIN comparison not only reveals species conservation but also indicates potential species divergence at the PIN level. And the identified CoNSs are known or candidate conserved complexes and can be used to predict PPIs, protein functions and orthologs. To meet the need for comparative tools at the PIN level, we implemented a web-interfaced version of NetAlign. The NetAlign server enables on-line comparison of two large-scale PINs. Furthermore, to facilitate the comparison of multiple PINs, we introduce mNetAlign, a NetAlign-based database designed to enable comparison of PINs across multiple species.

Structural Characterization of Rat Lipocalins by NMR Spectroscopy

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Abstract

Lipocalins play important roles in regulation of immunological and developmental processes. Several lipocalins were identified to involve in sperm maturation, including mouse Lcn2, Lcn5, Lcn6, Lcn8, and bull prostaglandin D synthase (L-PGDS), etc. We cloned, expressed and purified rat lipocalins from epididymis including Lcn2, Lcn6, L-PGDS, etc. Solution structures and dynamics of these lipocalins were studied by NMR spectroscopy. Fig.1 shows 2D ^{15}N-^{1}H HSQC spectra recorded on rat Lcn2 and L-PGDS samples using Varian Unity Inova 600 MHz spectrometer at 25 ^{o}C. A suite of 3D heteronuclear NMR experiments were performed for resonance assignments. Almost complete backbone resonance assignments were obtained (Fig. 1). More than 90% of side chain resonances were assigned. Secondary structures were identified by the Chemical Shift Index approach based on chemical shifts of H_α, $^{13}C_\alpha$, $^{13}C_\beta$, $^{13}C'$, indicating that both Lcn2 and L-PGDS contain eight anti-parallel β-strands. The 3D structures were calculated and evaluated using programs CNS and PROCHECK, respectively.

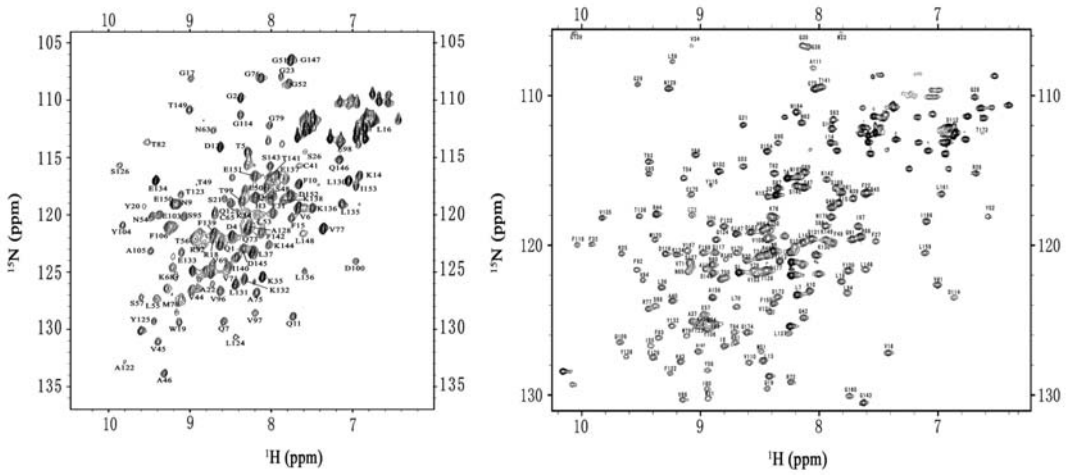

Fig.1 2D ^{15}N-^{1}H HSQC spectra of rat Lcn2 (left) and L-PGDS (right)

A22

Trypsin Inhibitors from Leguminous and Non-leguminous Plant

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Abstract

Trypsin inhibitors have been isolated from seeds of a number of leguminous plants including Chinese black soybean, cv. 'Dull Black' and cv. 'Small Glossy Black', Hokkaido black soybean, green lentil, black gram and fresh lily bulbs. The sequence of lily bulb trypsin inhibitor is similar to Kunitz type trypsin inhibitor from *Populus tremula*. Trypsin inhibitors from the two cultivars of Chinese black soybean belong to the Kunitz-type as reflected by the N-terminal sequence whereas the remaining leguminous trypsin inhibitors belong to the Bowman-Birk type.

All of the aforementioned trypsin inhibitors are devoid of antifungal activity. The above leguminous trypsin inhibitors lack antiproliferative activity toward tumor cells and substantial inhibitory activity toward HIV-1 reverse transcriptase with the exception of these from Hokkaido black soybean and Chinese black soybean cv. 'Dull Black'.

A23

The Structure of Gelsolin Bound to ATP

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Abstract

Calcium activation of the actin-modifying properties of gelsolin is sensitive to ATP. Here, we show that soaking calcium-free gelsolin crystals in ATP-containing media results in ATP occupying a site that spans the two pseudosymmetrical halves of the protein. ATP binding involves numerous polar and hydrophobic contacts and is identical for the two copies of gelsolin related by non-crystallorgraphic symmetry within the crystal. The γ-phosphate of ATP participates in several charge-charge interactions consistent with the preference of gelsolin for ATP, as a binding partner, over ADP. In addition, disruption of the ATP-binding site through Ca^{2+} activation of gelsolin reveals why ATP binds more tightly to the inactive molecule, and suggests how the binding of ATP may modulate the sensitivity of gelsolin to calcium ions. Similarities between the ATP and PIP_2 interactions with the C-terminal half of gelsolin are evident form their overlapping binding sites and in that both molecules bind more tightly in the absence of calcium ions. We propose a model for how PIP_2 may bind to calcium-free gelsolin based on the ATP-binding site.

A24

Structure-function Study of Maize Ribosome-inactivating Protein: Implications for the Internal Inactivation Region and the Sole Glutamate in the Active Site

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Abstract

Maize ribosome-inactivating protein (RIP) inactivates ribosome by N-glycosidase activity. It belongs to a unique class of RIP with an internal inactivation loop (Pro-RIP), which is removed in the mature form (MOD). This 25-amino acid sequence between A163 and D189 is the major element to suppress in vitro protein synthesis and cytotoxicity. Crystals of Pro-RIP and MOD were diffracted to 2.3 and 2.5 Å, respectively. Their structures are similar, with main chain RMSD of 0.519. In Pro-RIP, the internal inactivation fragment is found on the protein surface and it consists of a flexible loop followed by a long alpha helix. The presence of this fragment diminished the interaction of Pro-RIP with ribosome, but not stability and cellular up-take. Unlike RIPs from dicotyledonous plants, maize RIP does not have a back-up glutamate residue in the active site, which helps the protein to retain some activity if the catalytic glutamate is mutated. Recreating the back-up residue by changing Val238 to Glu238 destroyed the structure stability. On the other hand, the V238E mutation could only be created in the E207A variant. Compared to trichosanthin and ricin, the main chains of I134-M144 and Q225-T231 in maize RIP are found to shift 3.38 Å and 3.74 Å respectively towards the active site. As a result, the active site has become too small to accommodate an extra glutamate residue. In this regard, maize RIP is more primitive, as bacterial RIPs such as Shiga toxin and verotoxin do not possess the backup glutamate residue.

A25

The Crystal Structure of Human Chloride Intracellular Channel Protein 2: a Disulfide Bond with Functional Implication

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Abstract

Human chloride intracellular channel proteins (CLICs) are recently discovered as intracellular ion channel proteins based on sequence similarity. Human CLICs include 6 members (CLIC1-6) and the most distinguished feature of them is that they can exist in two states: the water-soluble state and the integral membrane state. Here we present the crystal structure of CLIC2 in the water-soluble state. Similar to CLIC1 and CLIC4, CLIC2 has a glutathione S-transferase (GST) fold. However, a unique feature discovered in the CLIC2 structure is that Cys^{30} and Cys^{33} in the sequence of Cys-Pro-Phe-Cys formed an intramolecular disulfide bond. The N-terminal domain of CLIC2 adopts a glutaredoxin fold and the disulfide bond between Cys^{30} and Cys^{33} is very similar to that of the oxidized glutaredoxin. Furthermore, the unique long loop between the helices α5 and α6 has different conformation compared with other CLICs. It inserts into a cavity between the N- and C-terminal domains of a crystal packing related molecule and is very close to the disulfide bond. The structure indicates that CLIC2 might play important role in redox-regulated cellular events.

A26

Human Milk Protein, Inducing Apoptosis of Adenocarcinoma MCF-7 Cells

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Abstract

It is well known human milk contains proteins and lipids able to induce apoptosis of mammalian cells in culture. Besides, there are delitescence factors in milk, by the activation and under the influence of which apoptosis of secretory epithelium cells takes place.

In this study out of human milk plasma a protein inducing apoptosis of MCF-7 cells (human mammary gland adenocarcinoma epithelium cells) was isolated. For isolation of protein human milk was fractionated by using following chromatographies: two ion-exchange chromatographies using DEAE-650 (S) and Fractogel CM 650 (M) sorbents, blue Sepharose (blue dye) affinity chromatography, reverse-phase HPLC on a Protein C (4) column. This strategy of isolation allows obtaining protein fractions, incubation of which with adenocarcinoma MCF-7 cells leads to apoptotic changes.

The influence of milk components to MCF-7 cell viability on each isolation step was examined by MTT assay and trypan blue dye exclusion test. Morphological changes of the cells, induced by compounds of human milk, were estimated by light-microscopy.

Cytotoxic fractions obtained by series of following chromatographies were analyzed by polyacrylamide (PAGE) SDS-gel electrophoresis, MALDI-TOF spectroscopy and MALDI MS/MS microscopy methods. Analysis showed that fraction represented with one peak showed a molecular mass 8606.5 ± 0.5 Da and this peak is corresponding to a fragment of human κ-casein (fragment 66-123).

Crystal Structure of Influenza A Virus H5N1 Nucleoprotein: Insights towards its RNA Binding and Oligomerization Mechanisms

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Abstract

Since the first human case of influenza A virus H5N1, pandemic threat has become a major concern worldwide. Universal vaccine targeting all subtypes and effective anti-viral agents would be beneficial to combat the disease. NP confers both structural and functional roles in the virus; its essentiality and conservation make it an attractive candidate for disease control. Here we present the 3.3 Å crystal structure of nucleoprotein (NP) of H5N1 subtype from avian origin. NP consists of a head domain, a body domain and a long tail loop. In the structure, the linker residues (aa. 397-401, 429-437) which connect the tail loop with the main molecule were resolved. Based on the flexibility of the linkers, a ring-like 9-mer NP model is proposed, which mimics a physiological state in virus-infected cells and indicates how NP shuttles between cytoplasm and nucleus. We also obtained electron densities for part of the aa. 73-91 flexible loop. This basic loop extends into the RNA-binding groove, suggesting an induced-fit mechanism for RNA-binding. The H5N1 NP structure provides insight into the possible functional surface area, including the oligomerization interface, the RNA-binding groove and the PB2-interaction site, where small molecules can disrupt the proper ribonucleoprotein (RNP) function.

A28

A New Ribosome Inactivating Proteins from the Mushroom *Hypsizigus marmoreus*

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Abstract

A ribosome inactivating protein (RIP) with an N-terminal sequence dissimilar to those of known proteins has been isolated from fresh fruiting bodies of the mushroom *Hypsizigus marmoreus*. The isolation procedure entailed ion exchange chromatography on DEAE-cellulose, affinity chromatography on Affi-gel blue gel, and gel filtration on Superdex 75. The RIP was unadsorbed on DEAE-cellulose and adsorbed on Affi-gel blue gel. The RIP inhibited translation in a cell-free rabbit reticulocyte lysate system with an IC_{50} of 45 μg/mL. It exerted an antiproliferative action on tumor cell lines with IC_{50} of 15 μg/mL for HepG2 and 52 μg/mL for MCF-7, and inhibited HIV-1 reverse transcriptase with an IC_{50} of 250 μg/mL. It also stimulated mitogenic response in murine splenocytes.

This research is supported by an earmarked grant CUHK4272/04M from the Research Grants Council of Hong Kong.

A29

Hemolysins and ribonucleases from fungi and plants

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Abstract

Hemolysins were purified from fresh fruiting bodies of the fungi *Pleurotus eryngii* and *Flammulina velutipes*. Ribonucleases were isolated from the fungus *Hypsizigus marmoreus* and roots of the plant *Panax ginseng*. The thermostability and acid/base stability of the proteins were examined. The first three proteins are monomeric while the last one is dimeric. All four proteins exhibit an antiproliferative action on tumor cells, but only the last protein demonstrates antifungal activity. *P. eryngii* hemolysin shows anti-mitogenic activity. In contrast, *F. velutipes* hemolysin exhibits anti-mitogenic activity. Hemolysin-induced hemolysis is osmotically protected by polyethylene glycol 10000 with a specific mean hydrated diameter. The data suggest that the two fungal hemolysins do not lyse every type of cells although they lyse red blood cells.

Preliminary Crystallography Study on Cardif, a Newly Found Adaptor Protein in the Host Antivirus Signal Pathway

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Abstract

Cardif is a newly identified adaptor protein which is essential in the host antiviral response. It contains an amino terminal CARD domain and a carboxyl terminal transmembrane sequence which helps it localizes to the mitochondrial membrane. Cardif plays its role downstream of the cytoplasmic RNA helicase RIG-I which detects intracellular RNA virus infection. Furthermore, Cardif is also a target of the hepatitis C virus NS3-4A protease and can be released from the mitochondrial membrane by its cleavage reaction on cysteine 508, which leads to the disruption of the host antiviral signal pathway. We purified and crystallized the cytodomain of Cardif and collected data of native crystal in KEK, Japan. Heave atom derivates have also been prepared and structure determination is in progress.

Conformational Changes of *Saccharomyces cerevesiae* Mitochondrial $NADP^+$-dependent Isocitrate Dehydrogenase 1 during the Catalytic Reaction

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Abstract

Isocitrate dehydrogenases (IDHs) catalyze oxidative decarboxylation of isocitrate (ICT) into α-ketoglutarate (AKG). Apart from prokaryotes, eukaryotes have several $NADP^+$-dependent IDH (NADP-IDH) isoenzymes localized in different subcellular compartments which presumably exert divergent functions. We report here a series of crystal structures of a mature form of *Saccharomyces cerevesiae* mitochondrial NADP-IDH Idp1p in complexes with different ligands representing different states of the enzyme during the catalytic reaction: in binary complexes with cofactor $NADP^+$, or substrate ICT, or product AKG, and in quaternary complex with NADPH, AKG, and Ca^{2+}. Comparisons among these structures and with the previously reported structures of other NADP-IDHs reveal that the eukaryotic NADP-IDH undergoes substantial conformational changes during the catalytic reaction. It adopts an open conformation when bound with $NADP^+$ or a semi-closed conformation when bound with ICT, but a fully closed conformation both before and after turnover when the ligands and the metal ion are bound. With the releasing of the products, the enzyme changes back to an open or a semi-closed conformation. The observed conformational changes are quite different from those of *Escherichia coli* NADP-IDH for which substantial domain shift occurs only between two crystal forms of the apo enzyme, suggesting that eukaryotic and prokaryotic NADP-IDHs might have different catalytic and regulatory mechanisms. Our results also indicate that the self-regulatory mechanism proposed for human cytosolic NADP-IDH does not apply to mitochondrial Idp1p. Based on the structural and biochemical data, we propose that eukaryotic NADP-IDHs might mimic their bacterial homologs by using post-translational modifications to regulate their activities.

A32

Expression of Mutated Hpn - a Nickel Binding Protein from *Helicobacter pylori*

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Abstract

Helicobacter pylori (H. pylori) is a bacterium that colonize in human gastric mucosa and can cause gastric ulcers and cancers. Nickel plays a major role in the bacterium colonization.

Hpn is a small nickel-binding cytoplasmic protein, which is abundant in *H. pylori* and proven to be important in nickel metabolism. It consists of 60 amid acids, 28 of which are histidines which constitute the binding site of nickel. The molecular weight of hpn monomer is 7KD; however, hpn can form a high molecular weight aggregate of >500kDa, and other major species at 55, 34, 26, 20, 14 and 7kDa. This suggests that complicated intermolecular and intra molecular disulfide bonds can be formed.

In order to study the structure of hpn by X-ray crystallography and NMR, we decide to remove the disulfide bond. The four cysteines in hpn are mutated to alanines which has similar molecular size with cysteine. The mutated gene is synthesized by two-step PCR and protein has been expressed in E. coli. We expect to obtain hpn monomer to for NMR and X-ray crystallography studies.

Sensitive Fluorescence-Detection Method of Amino Acids for Protein Sequencing

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Abstract

Edman degradation is a common sequencing method consisted of three conventional steps; phenylisothiocyanate (PITC) coupling, cyclization to anilinothiazolinone (ATZ) and isomerization to phenylthiohydantoin (PTH). Although UV-active PTH derivatives are separated and characterized by HPLC at the end of the degradation, a large amount of sample is required due to the low sensitivity for UV detection of the PTH derivatives. In order to improve the detection sensitivity, several Edman-type fluorescent reagents instead of PITC have been developed for generating fluorescent amino acid derivatives. However, these reagents are not routinely used since the coupling yield is poorer than PITC and their by-products interfere with the determination of amino acid residues. Recently, we have developed a novel fluorescent and chemiluminescent molecule, 4-(1'-cyanoisoindolyl) aniline (CIA). CIA contains a primary amino group and can react with ATZ form of amino acids in a small disk (4-mm i.d.) filled with two glass fiber membranes and one PTFE membrane, and then its corresponding fluorescent derivative is formed in organic solvent with heating. According to HPLC analysis, the detection sensitivity for the fluorescent products was approximately 10 times higher than UV detection of PTH products by the conventional Edman degradation. The results of manual sequencing of several peptides with CIA showed that more than 20 amino acid residues can be analyzed directly and consecutively. Our newly developed fluorescence-derivatization system with CIA can be a promising technique for the manual sequence analysis of a minimal protein sample.

Expression of Carbohydrate-Response Element-Binding Protein Fused with GFP in Two Cell Lines

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Abstract

In mammals, the liver is the principal organ responsible for conversion of excess dietary carbohydrates into triglycerides, the predominant form for energy storage. Carbohydrate-response element-binding protein (ChREBP), a transcription factor that is activated in responds to high concentration of glucose. There are three phosphorylation sites for cAMP-dependent protein kinase in ChREBP, and ChREBP forms a heterocomplex with Max-like factor X (Mlx). By glutathione *S*-transferase pull-down assay and electrophoretic mobility shift assay, we confirmed that the stable complex between the basic helix-loop-helix/leucine zipper (bHLH/LZ) of ChREBP and Mlx could form in vitro. This complex was able to bind to the ChRE sequence consisting of two E-box motifs separated by 5 bp, although the bHLH/LZ of ChREBP was not able to bind alone. From mutational experiment in bHLH/LZ, we found that the Glu671 and Arg675 in basic region were important residues for DNA binding ability. Furthermore, we observed that the mutation of the phosphorylation site in bHLH/LZ of ChREBP influenced neither the protein interaction nor the DNA binding ability. These results indicate the possibility of the existence of an unknown regulation mechanism of ChREBP. To examine this possibility, we constructed a eukaryotic expression vector, fusion expressing ChREBP and GFP both in HeLa and HepG2 cells. Now, we are analyzing the function of this GFP-fusion protein, and constructing the plasmid to express the mutant ChREBP in mammalian cells.

Evolution of Aminoacyl-tRNA Synthetase (aaRS)

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Abstract

A lot of studies show that many aminoacyl-tRNA synthetase (aaRS) gene evolutionary mechanisms and modes, aminoacylation routes, and the evolutionary mechanisms in structure and function, are diverse in some bacteria and eukaryotes. Thus, the further study on the diversities will be very helpful for understanding the protein structural and functional evolution. Although the essential mechanism responsible for these diversities has not been understood, these diversities may suggest that some major differences in certain aspects of biological processes between bacteria and eukaryotes are required for further study. The glycyl-tRNA synthetase (GlyRS) is a special enzyme among aaRS-II, which includes two oligomeric types in genome, the $\alpha_2\beta_2$ tetramer and α_2 dimer. The anticodon-binding domains (ABDs) of dimeric and tetrameric GlyRSs are non-homologous, although their catalytic central domains (CCDs) are homologous. The results suggest that the same function and specificity in some proteins can be kept by changes in structure and recognition mechanism during evolution, which also implies a special evolutionary way in recognition mechanism between GlyRS and tRNA(Gly). Domain shuffling plays an important role in the evolution of PheRS. The various combinations of aaRS-I and aaRS-II domains have resulted in the striking different structures of PheRSs in bacteria and archaebacteria/eukaryotes, which could lead to different recognition mechanisms between PheRS and $tRNA^{Phe}$ during evolution from bacteria, archaebacteria to eukaryotes.

Preliminary Crystallographic Studies of Fructosyl Amine Oxidase in *Aspergillus fumigatus*

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Abstract

Fructosyl amine oxidase (FAO), which is also called amadoriase, catalyzes the Amadori product deglycation and generates free amine groups. The Amadori compound is amino acid or protein which is glycated in the α- or ε-amino groups. It is the major single modification of the extracellular matrix by the Maillard reaction *in vivo*. It is also a source of biologically active glycoxidation products and the formation of glucosepane, the major protein cross-link found in biological tissues so far. However, there is growing evidence to suggest that accumulation of glycation product is causative or correlative with aging and disease progress, such as atherosclerosis, diabete, arthritis, and neurodegenerative diseases. Researchers studied a lot on the biochemical properties, kinetic mechanism, and substrate specificity of FAO, but got a little insight into its' structure characters. FAO from *Aspergillus fumigatus* is expressed in *E. coli* and purified by affinity chromatography and gel filtration. The purified FAO is crystallized by the hanging-drop vapour-diffusion technique. The crystal diffracted to beyond 2.0 Å resolution and belonged to space group $P3_221$, with unit-cell parameters a=b=66.85 Å c=187.89 Å and one molecule per asymmetric unit.

Structural and Functional Study of XIAP-Associated Factor 1 (XAF1) - Identification and Characterization of a 13 kDa Structural Domain

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Abstract

Apoptosis, referring to the biological process of programmed cell death, plays an essential role in developmental and cellular homeostasis of mammalian cells. The process culminates in the activation of caspases cascade to degrade the cellular machinery. The X-linked Inhibitor of Apoptosis (XIAP), belonging to the family of intrinsic inhibitor apoptosis protein (IAP), is the most potent and versatile inhibitor of caspases and apoptosis.

XIAP-associated factor 1 (XAF1) is a 37kDa nuclear protein, which was reported to antagonize and interact with recombinant XIAP *in vitro*. Overexpression of XAF1 was shown to trigger the redistribution of cytoplamic XIAP to the nucleus, leading to XIAP-suppression, caspases activation and apoptosis. On the other hands, the loss of endogenous XAF1, by utilizing the adenovirus infection of antisense XAF1 mRNA, was reported to enhance cellular resistance to apoptosis.

Protein sequencing analysis of the nuclear XAF1 protein revealed the existence of TRAF zinc finger motif within H23-E99. However the domain architecture and structure of XAF1 is still not fully understood. In the present work, a 13kDa domain was identified by using limited proteolysis and mass spectroscopy. Circular Dichroism (CD), nuclear magnetic resonance (NMR) and metal titration study demonstrated that the domain is α-helical rich and have Zinc-binding property. The zinc binding properties of XAF1 may involve in biochemical function such as XIAP interaction.

This work was supported by grants from the Hong Kong University Research Grant (KH Sze), the Research Grants Council of Hong Kong for KH Sze (HKU 7350/04M, HKU 7533/06M).

Structural Modification of OFP - from Tetramer to Monomer

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Abstract

Fluorescent protein is one of the most popular biotechnological tools used in cell biology research today. They are frequently used as fluorescent tag to label the protein of interest so that one can monitor the trafficking of labeled proteins in living cell. Advanced in molecular life cell imaging techniques has increased the demand of new fluorescent proteins with distinct spectral and structural properties. Previously a new orange color GFP-like protein named OFP was cloned from tube anemone *Cerianthus* sp. Crystallographic analysis indicated that it is tetrameric. By using site-directed mutagenesis technique, we have successfully created two structural mutants of OFP. A dimeric variant OFP2 was created with W118K and V120E double mutations. Further engineering of OFP2 with K79R/T141A/T144H/F155V/Y169A/Y188P/N199A/K192S/R194S mutations yield a monomeric OFPm. The fluorescence quantum yields of OFP2 and OFPm to the wild-type are 0.64 and 0.32 respectively.

Crystal Structure of Human ERp44

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Abstract

The endoplasmic reticulum (ER) is a multifunctional organelle containing many molecular chaperones and enzymes, and provides a unique environment for oxidative folding, post-translational modifications and quality control of membrane and secretory proteins. ERp44, an ER-resident protein, is a member of the protein disulfide isomerase (PDI) family. ERp44 mediates thiol-dependent retention in the early secretory pathway, forming mixed disulfides with unassembled proteins through its conserved -CRFS- motif. It also regulates Ca^{2+} outflow binding inositol 1, 4, 5-trisphosphate receptor type 1. Here we present the crystal structure of human ERp44 (hERp44) at 2.6 Å, the first full length structure of a member of the mammalian PDI family. hERp44 contains three Trx-fold domains arranged in the order **a-b-b'** with a C-terminal tail. Three thioredoxin (Trx) domains are arranged in a clover-like structure. The C-terminal tail bridges domains **b'** and **a**, and fills the space between them. Domain **a** of hERp44 is very similar to that of yeast PDI (yPDI) but with -CRFS- instead of the canonical -CXXC- motif. Around the -CRFS- motif there is a hydrophobic patch. Structure superposition of the **b'** domains of hERp44 and yPDI clearly reveals a hydrophobic pocket in hERp44. This hydrophobic pocket in the **b'** domain is close to the hydrophobic patch around the -CRFS- motif in the **a** domain, which binds covalently client proteins. These hydrophobic areas could act as a docking site for substrate binding. However, they are both largely covered by the C-terminal tail.

A Brain-Specific PACAP in Fish Model: Solution Structure by Nuclear Magnetic Resonance Spectroscopy and Effects on Growth Hormone Release and Gene Expression

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Abstract

Pituitary adenylate cyclase-activating polypeptide (PACAP) has been proposed to be the ancestral growth hormone (GH)-releasing hormone. Recently, using grass carp as a model for modern-day bony fish, we have demonstrated that PACAP nerve fibers are present in close proximity to carp somatotrophs and mammalian PACAPs can induce GH secretion in carp pituitary cells. To further examine the role of PACAP as a GH-releasing factor in fish, the structural identity of grass carp PACAP has been established by 5'/3' RACE. The newly cloned cDNA is 984 bp in size with a 173 bp 5'UTR, 528 bp ORF, and 283 bp 3'UTR encoding a 175 a.a. preprohormone. Grass carp PACAP is a single-copy gene and expressed exclusively in the brain but not in other tissues. The mature peptides of PACAP preprohormone, namely $PACAP_{27}$ and $PACAP_{38}$, were then synthesized according to the sequence of the carp PACAP cDNA. Structural analysis by circular dichroism has shown that the solution structures of $PACAP_{27}$ and $PACAP_{38}$ are dominated by α helixes but not β sheets. As revealed by nuclear magnetic resonance (NMR) spectroscopies, grass carp $PACAP_{38}$ is composed of a flexible N-terminal from His^{1} to Ile^{5}, an extended central helix from Phe^{6} to Val^{26}, and a short helical tail in the C-terminal from Arg^{29} to Arg^{34}. The C-terminal helix is highly basic and located after a hinge region at Leu^{27} to Gly^{28}. The solution structure of $PACAP_{27}$ is comparable to that of $PACAP_{38}$, except that the C-terminal helix is missing. The central helix for $PACAP_{27}$ and $PACAP_{38}$ both exhibits an amphipathic characteristic with basic residues clustered mainly on one side of the helical structure whereas the hydrophobic residues forming a loose core on the other surface. In grass carp pituitary cells, the two forms of PACAPs were both effective in stimulating GH release, GH mRNA expression, and GH promoter activity. These stimulatory effects occurred with parallel rises in cAMP production and Ca^{2+} entry through voltage-sensitive Ca^{2+} channels. In these functional studies, $PACAP_{38}$ was found to have a higher potency and efficacy of stimulation compared to $PACAP_{27}$, and these results are consistent with the pharmacological properties of type IB PACAP receptor reported in mammals. This study represents the first report for the solutions structures of non-mammalian PACAPs and provides evidence that a brain-specific isoform of PACAP in fish can stimulate GH synthesis and secretion at the pituitary level, presumably by activating the cAMP- and Ca^{2+}-dependent signaling mechanisms.

The project was sponsored by RGC grants, Hong Kong.

Proteins with Multiple Biological Activities from the Bacteria *Bacillus amyloliquefaciens* and *Bacillus subtilis*

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Abstract

An antifungal protein designated as baciamin, with a molecular mass around 50 kDa, was purified from the bacterium *Bacillus amyloliquefaciens*. This antifungal protein was isolated by ion exchange chromatography on Mono Q and gel filtration on Superdex 200. Baciamin manifested a broad spectrum of antifungal activity with IC_{50} in the nanomolar range. Baciamin could induce membrane permeabilization of tested fungi, but it failed to induce membrane permeabilization on rabbit red blood cells. Its antifungal activity was retained after incubation with trypsin and EDTA. Various ions tested did not affect its antifungal activity. Baciamin reduced the activity of HIV-1 reverse transcriptase with an IC_{50} of 480 nM. It also inhibited proliferation of hepatoma, breast cancer and colon cancer cell lines with an IC_{50} of 100, 80, 110 nM, respectively. Baciamin did not elicit mitogenic response from murine splenocytes, but it augmented nitric oxide production by mouse macrophages. A comparison of baciamin from *B. amyloliquefaciens* with bacisubin from *B. subtilis* revealed that the former was devoid of ribonuclease and hemagglutinating activities which were present in the latter although both possessed antifungal activity. The two *Bacillus* proteins were different in molecular mass and N-terminal sequence.

Structural Study of *Helicobacter Pylori* HypA, a Nickel and Zinc Binding Accessory Protein

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Abstract

Several accessory proteins are required for the maturation of urease and hydrogenase in gastric pathogen *Helicobacter pylori*. Among all these accessory proteins, HypA, a putative nickel and zinc binding protein is required for the full activity of both the hydrogenase and urease enzymes [1]. HypA is a small metalloprotein with 117 residues. Previous studies indicate that the protein has two distinct metal-binding sites, a high-affinity Zn^{2+} site and a lower-affinity Ni^{2+} site [2] and the Zn site may undergoes a structural change in response to Ni binding [3]. Recent study shows that HypA could interact with another accessory protein UreE, which facilitates the nickel transfer eventually to the apo-urease [4]. However, until now no protein structure of HypA or its homologues has been reported. We has been over-expressed HypA in *E. coli* BL21(DE3). Isotopically enriched (^{15}N, and $^{13}C/^{15}N$) protein was prepared by growing cells in modified M9 medium containing $^{15}NH_4Cl$ and/or ^{13}C-glucose followed by protein purification. A single peak for each residue in the well-dispersed 2D [^{1}H,^{15}N] HSQC spectrum indicates a uniquely folded structure of the protein. A series of double and triple resonance experiments, e.g., HNCO, HN(CA)CO, HNCACB, CBCA(CO)NH, were performed for the assignment of the protein backbone. For the side-chain assignments, HBHA(CO)NH, CC(CO)NH, (H)CC(CO)NH, HCCH-TOCSY, HCCH-COSY experiments have been carried out. NOE data for structure determination were extracted from three-dimensional ^{15}N- and ^{13}C-edited NOESY spectra. The structure of the protein will be described in detail.

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Chemiluminescence Detection of Proteins on Membranes Employing DNA Probe and TMPG Reagent

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Abstract

Biogenic proteins or enzymes in cells generally play important roles in biochemical pathways. The detection and identification of these proteins provide a better insight into their bio-functional roles, such as enzyme-related diseases and biological mechanism. Since those proteins are present at very low concentration, a highly sensitive and selective method has been eagerly desired. In order to establish simultaneous determination of various proteins on a microchip, we have studied a comprehensive method for chemiluminescence detection of biotinylated IgG antibody (b-Ab) on a membrane employing biotinylated DNA (b-DNA) as a detection probe and a chemiluminogenic reagent, 3',4',5'-trimethoxyphenylglyoxal (TMPG). The chemiluminescence from the b-DNA was generated by the chemical reaction with TMPG under the room temperature for a few minute in the presence of tetra-propyl ammonium phosphate and dimethylformaldehyde. This chemiluminescence reaction was specific for the guanine moiety of DNA. b-Ab spotted on a nylon membrane was incubated with avidin and b-DNA that was synthesized using biotin-AC5 hydrazide. Then this macromolecular complex of b-Ab, avidin and b-DNA was detected by the TMPG reagent. By means of our developed technique, 670 fmol b-Ab on the nylon membrane was detectable. We are currently developing an immnoblotting method for the chemiluminescence detection of various proteins of cytochrome P450 family.

Taking the Edge Off: The Softer Side of In-house SAD Phasing

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Abstract

The phase problem in macromolecular crystallography has been mitigated dramatically in recent years by advances in methodology and instrumentation. SAD phasing has now become the primary *de novo* phasing method. A search of the PDB of structures released in 2006 reveals the number of structures solved by SAD phasing exceeds those solved by MAD for the first time. A number of these examples of successful S-SAD and Se-SAD phasing used Cr radiation (λ = 2.29 Å), which can double the anomalous signal of sulfur and selenium compared to Cu radiation.

This report reviews recent results from phasing with the enhanced anomalous signal provided by Cr radiation to demonstrate this longer wavelength can be used to solve *de novo* structures. Selenium, as the heavy atom, with Cr radiation can provide sufficient anomalous scattering for routine phasing. Cr radiation opens a new path to extracting the weak anomalous signal from sulfur to phase native protein data. With the addition of Cr radiation to the crystallographer's toolkit, in-house X-ray sources can routinely provide at least two wavelength options. The combination of diffraction data collected using both Cu (λ = 1.54 Å) and Cr radiation can improve the electron density tremendously. Anomalous scattering from sulfur can also assist in molecular replacement solutions. Finally, the data collected with Cr radiation can be used to refine a structure. Ultimately, this makes it possible to solve a protein structure with a single data set.

This in-house phasing approach we describe has been given the label "**know before you go**" by John Rose and B.C. Wang at the University of Georgia. This method improves the efficiency of the solution of macromolecular crystal structures and usage of the synchrotron beam time.

Resonance Assignments and Solution Structure of a 28 kDa Active Mutant of Maize Ribosome-Inactivating Protein (MOD) Studied by NMR Spectroscopy

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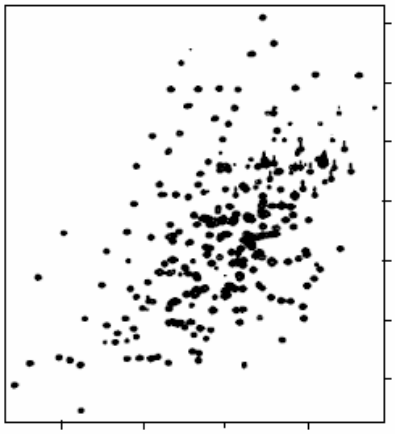
Abstract

N-glycosidase ribosome-inactivating proteins (RIP) cleave the N-glycosidic bond of A4324 in the 28S ribosomal RNA. Removal of this base abolishes the binding of eukaryotic elongation factor EF1 and EF2 to the ribosome and inhibits protein synthesis. RIPs are therefore highly cytotoxic, and they can be used to design immunotoxins, anti-viral and anti-tumor agents. Maize ribosome-inactivating protein belongs to a unique class of RIP with an internal 25-amino acid inactivation fragment. It is synthesized and stored in the kernel as a 34 kDa inactive precursor (Pro-RIP). During germination, the 25-amino acid fragment is removed to generate a two-chain biologically active RIP (MOD). We have cloned MOD with an engineered short loop linking the two chains and expressed the protein in *E. coli*. Overall, 92.6% of ^{13}CO, NH, ^{15}N, C_α and $C_\alpha H$ backbone resonances were assigned. For side chain assignment, 91.4% of ^{1}H, ^{13}C and ^{15}N chemical shifts were assigned. The 3D solution structure of MOD and its comparison with the structures of other RIPs will be discussed in the present study. Figure 1 displays the full ^{1}H, ^{15}N-HSQC spectrum of MOD recorded at 310 K showing very good ^{1}H-^{15}N cross peaks dispersion.

This work was supported by grants from the Hong Kong University Research Grant (KH Sze), the Research Grants Council of Hong Kong for KH Sze (HKU 7350/04M, HKU 7533/06M) and PC Shaw (CUHK4606/06).

Figure 1. ^{1}H-^{15}N HSQC spectrum of MOD recorded at 310 K



Structural Determination of Human Common-type Acylphosphatase

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Abstract

Acylphosphatase is a small enzyme that catalyzes the hydrolysis of carboxyl-phosphate bond in acyl phosphates. It is widely distributed in all three domains of living organisms: eukarya, prokarya and archaea. Mammalian acylphosphatases are classified into two isoforms, muscle-type and common-type. Acylphosphatases have been studied extensively as a paradigm for the formation of insoluble protein aggregates known as amyloid fibrils; and its correlation with simple biophysical parameters such as hydrophobicity, secondary structure propensity and net charges. Acylphosphatases have also been used to demonstrate the activity, stability and flexibility relationships of enzyme. Despite being studied extensively, the structure of any human acylphosphatase is not known. In this study, crystal structure of the human common-type acylphosphatase is determined. Crystals of human common-type acylphosphatase were grown by sitting-drop-vapor-diffusion method at 289K. The best crystals were obtained by mixing 1 μl of 10% isopropanol, 20% PEG 4000, 0.1M Na HEPES buffer at pH 7.5 with 2 μl of 10 mg ml^{-1} protein sample. Diffraction data was collected to 1.45Å resolution at 100K. The crystals belong to space group $P2_12_12_1$, with unit-cell parameters a=42.58, b=47.23, c=57.26 Å. Human common-type acylphosphatase was in monomer form in the crystal structure, and it adopts an α/β sandwich fold that is common to other acylphosphatase.

This work was supported by a grant from the Research Grants Council of the Hong Kong Special Administrative Region, China (project No. CUHK4590/05M) and a Direct Grant (project No. 2030324) from the Research Committee of the Chinese University of Hong Kong.

Expression, Purification and Crystallization of the Human Phox Homology (PX) Domain Protein SNX9

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Abstract

Sorting nexin 9 (SNX9) has a Src homology 3 domain in its amino terminus and a region with predicted low complexity followed by a carboxyl-terminal part containing the phox domain. Previous studies have indicated that sorting nexin 9 participates in regulation of EGFR degradation. SNX9 interacts with clathrin-coated vesicle adaptor protein AP-2, clathrin and Dynamin-2 and has been identified as an interactive protein of the metalloprotease disintegrins MDC9 and MDC15. But the exact function of sorting nexin 9 in vesicle trafficking is still not clear. The PX domain has been recently identified as an inositol phospholipid-binding module. This feature may associate the function to the interaction with membrane vesicles and regulation of intracellular vesicle trafficking. To understand the structure-function relationship, SNX9 was over-expressed as a soluble protein in *E.coli* and purified by two chromatographic steps including affinity and gel filtration chromatography. The purity of protein was checked by SDS-PAGE, Native-PAGE. The his-tag at the N-terminus of SNX9 was not removed. Crystallization experiments were performed at 277K or 298K by the sitting-drop vapor diffusion method using 96-well microplates and optimized by hanging-drop vapor diffusion method using 24-well plates.

Highly Selective Fluorometric Assay for HIV-1 Protease Activity

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Abstract

Human immunodeficiency virus type 1 (HIV-1) protease plays an essential role in the viral life cycle by cleaving Gag and Gag-Pol proteins into structural and functional proteins necessary for viral assembly and maturation. Therefore, HIV-1 protease is a prime target of drugs developed to control HIV/AIDS, and HIV-1 protease inhibitors are used as medicines against HIV/AIDS today. The highly sensitive assay for HIV-1 protease is important for screening of inhibitors and/or understanding the mechanism of AIDS. Recently, we found that a novel selective fluorogenic reaction for peptides. In this reaction, the peptides reacted with catechol in the presence of sodium periodate, and formed a fluorescent product in a neutral borate aqueous solution. In this study, we tried to apply this fluorescence reaction to HIV-1 protease assay. The strategy of our assay is followed; N-terminal acetylated substrate is digested by HIV-1 protease, and then the generated product, that has unmodified N-terminus, is converted to a fluorescent compound by our fluorogenic reaction. In this assay, we used crude extracts from *E. coli,* which express recombinant HIV-1 protease. Firstly, we optimized the reaction conditions, therefore, the maximum enzyme activity is obtained in the presence of 1.0 M NaCl at pH 5.5, 37˚C. The sensitivity of our assay is comparable to that of a commercial HIV-1 protease assay kit. Our HIV-1 protease assay method is sensitive, simple, convenient, and economic， and this assay system is also readily adaptable to other proteolysis enzymes and their substrates.

Evaluation of Dextran-Based Polymeric Chemiluminescent Compounds for the Direct Detection of Proteins on a Membrane

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Abstract

Chemiluminescence (CL) imaging has played significant roles in scientific fields due to its high sensitivity, high selectivity, and simple instrumentation. The CL signal is measured by a charge-coupled device (CCD) camera and then quantified by imaging software. At the present, the demand for a rapid and a sensitive detection of biological samples have been increased. Here we report a simple method to synthesize a sensitive CL compound based on dextran. The dextran probe was tethered with a small amount of biotin as a linker and a large amount of luminol or isoluminol as a CL emitter. Although the CL detection was initially found to be minimal, this was subsequently improved with our developed acetonitrile-tetrapropylammonium hydroxide (TPA)-H_2O_2 system. Its CL intensity was further increased by employing 0.45 mM Fe (III) as a catalyst. We thought that the addition of avidin can mediate the formation of polymeric CL dextran complex, which enhanced the CL intensity due to an increasing number of isoluminol (or luminol) units. We successfully employed this dextran-based CL macromolecule for the direct detection of proteins on a PVDF membrane. This newly developed CL compound provides a rapid, simple and sensitive technique for proteins CL imaging.

A50

NopL, an Effector Protein of *Rhizobium* sp. NGR234, Interferes with MAP Kinase Signaling

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Abstract

Many pathogenic bacteria use type 3 secretion systems to deliver type 3 effectors into eukaryotic host cells. Type 3 secretion systems have been also found in nitrogen-fixing *Rhizobium* species that establish nodule symbiosis with legumes. Nodulation outer protein L (NopL) has been identified as a first type 3 effector secreted by *Rhizobium* sp. NGR234, but its molecular function in plants remains elusive. Up to now, no homologous proteins to NopL have been described for bacterial pathogens, suggesting that NopL is a *Rhizobium*-specific type 3 effector. Previous studies with tobacco plants expressing *nopL* showed that NopL suppressed activation of pathogen-related defense genes (Bartsev et al. 2004, Plant Physiol. 134, 871). Here, we used tobacco leaves to perform *Agrobacterium*-mediated expression studies with *nopL* and the tobacco MAP kinase gene *SIPK*. Co-expression of *nopL* and *SIPK* partially suppressed the SIPK-induced hypersensitive response, suggesting that NopL interferes with MAP kinase signaling. In transgenic *Arabidopsis* plants, expression of *nopL* induced disease-like symptoms. Studies with alkaline phosphatase followed by Western-blot analysis with antibodies raised against NopL showed that NopL was phosphorylated in transgenic plants that expressed *nopL*. *In silico* analysis predicts that NopL has nine putative Ser/Thr phosphorylation sites for MAP kinases. It is hypothesized that MAP kinases phosphorylate NopL instead of their natural target proteins. To better understand the structure-function relationship of NopL, we currently make efforts to crystallize NopL in order to determine its structure by X-ray crystallography.

Understanding the Transmembrane Signal Transduction of Histidine Kinases by a Structural Genomics Approach

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Abstract

Histidine kinases (HK) are part of the two-component signal transduction system (TCS), which is the major pathway for bacteria to respond to environmental stimuli. No TCS system was found in eukaryote cells, making it a potential target for novel antibiotics. Histidine kinases are usually composed of one extracellular sensor domain, two transmembrane helices, one intracellular dimerization domain and one intracellular kinase domain. The C-terminal kinase domains are highly conserved throughout the system, while the extracellular sensor domains are very versatile, which corresponds with their function as sensors of different substrates. The molecular mechanism of the transmembrane signaling remains elusive even though several structures of the kinase domains and the sensor domains have been solved. A bioinformatics analysis was done previously on the sensor domains of all known and predicted HKs. Over 400 distinct families have been identified based on cutoff at E-value EXP-3 and the 5 most populated families contain at least 30 members each. These proteins have distinctive fold including all-alpha, alpha/beta or all-beta, which is the unique feature of Family 3. In order to complete the structural landscape of the sensor domains of HKs, I solved 3 structures from family 1 and am working on family 3 and 5. The broader view should bring more information to our understanding of this important signaling pathway. In addition, the comparison of 3D structures from different proteins in a same family will shed light on our understanding of the sequence-structure relationship of proteins.

Signal Perception and Transduction Mechanism Revealed by Crystal Structure of a Sensory Histidine Kinase, DctB

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Abstract

How environmental signals are perceived and then transmitted across a lipid membrane from the bacterial periplasm into the cytoplasm is not currently understood at the mechanistic level. In this report, crystal structures of the periplasmic domain of *Sinorhizobium meliloti* DctB, a typical periplasmic sensing histidine kinase, were solved with and without a signal-ligand, succinate. The dimeric structures revealed that the ectodomain of DctB consists of an N-terminal helical domain, mainly participating in dimerization, and two PAS domains (distal and proximal relative to the membrane). Upon ligand binding, significant conformational changes take place within the binding pocket, causing rearrangements of the dimer. Combined with mutagenesis results, we propose that these series of conformational changes upon signal sensing can be transmitted across the membrane by helical bundles to trigger the rotation of the cytoplasmic HAMP (histidine kinases, adenylyl cyclases, methyl-accepting chemotaxis proteins, and phosphatases) linker, thus to further activate the histidine kinase domains.

Histidine-tagged Human Augmentor of Liver Regeneration (hALR) protein: Preparation and Characterization

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Abstract

Human augmenter of liver regeneration (hALR; Hepatopoietin) is a novel hepatotrophic growth factor that stimulates hepatocyte proliferation. It is a protein that consists of 125 amino acids. hALR belongs to Erv1p/ALR protein family, whose members have essential functions in the biogenesis of mitochondria. hALR has also been identified as a FAD-linked sulfhydryl oxidase and protects the liver in a liver specific manner. To develop hALR as a protein drug for liver diseases, we produced a histidine-tagged hALR protein in an Escherichia coli expression system. This system produced high yield of histidine-tagged hALR of about 150 mg/l and up to 85% purity which is 100 mg/l higher yield and 15% more pure than the native hALR. It also allows single-step affinity purification. Sulfhydryl oxidase activity assay indicated that native hALR has the highest sulfhydryl oxidase activity whereas histidinetagged hALR shows only half of the sulfhydryl oxidase activity. In liver cell proliferation assay, the two proteins showed similar activity. This activity is also similar to that of hepatocyte growth-promoting factor (pHGF), a commercially available drug in China to treat liver failure. Our data suggest that the 6xHis-tag at the C-terminus of hALR might affect the redox active disulfide pair formation but not the binding of protein to the hALR specific receptor on the cell surface. These data will certainly facilitate the development of the recombinant hALR as a pharmacological agent in the treatment of liver failure.

Class A beta-lactamase-based biosensors for the detection of beta-lactam antibiotics: Preparation and characterization

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Abstract

The extensive use of antibiotics in veterinary and clinical applications has lead to food contamination and reduced efficacy of antibiotics as bacteria becomes antibiotic-resistant due to its evolution. Beta-lactamase of *Bacillus licheniformis* 749/C (PenP) with site-directed mutagenesis on E166C, in which the Glu-166 residue in the Ω loop is replaced with a cysteine residue, was expressed in *E. coli* and purified by two chromatographic steps including affinity and ion-exchange chromatography. The six-His tag of PenPE166C was cleaved by TEV Protease and the digested PenPE166C was purified by nickel affinity chromatography. The mutant E166C was labelled with two thiol-reactive fluorophores, fluorescein-5-maleimide and badan, to form PenPE166Cf and PenPE166Cb. The two mutants can be used as a biosensor that can detect trace amount of beta-lactam type of antibiotics. The fluorescence profiles of the two mutants with addition of penicillin G and other beta-lactams were illustrated. It was found that both PenPE166Cf and PenPE166Cb give significant changes in fluorescence intensity upon binding with penicillin G, even when the penicillin G concentration is as low as 0.01 μM. The crystallization conditions for both PenPE166Cf and PenPE166Cb were examined. Both mutants were crystallized by hanging-drop vapour diffusion method. Enzyme without removal of His-tag was unable to crystallize. Crystals of PenPE166Cb, both with and without addition of penicillin G were taken for X-ray data collection, which gave a resolution of up to 1.8 Å. Crystals of PenPE166Cf, both with and without addition of cefotaxime gave resolutions of 1.9 Å and 3.0 Å, respectively.

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Crystal Structure of the YOR391p from *S. cerevisiae*, a possible chaperone and cysteine protease

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Saccharomyces cerevisiae gene YOR391p produces a 25.9 kDa protein whose expression level rises several-fold in response to various stress condition. It belongs to DJ-1/ThiJ/PfpI family whose member called human DJ-1 is mutated in autosomal recessive early-onset Parkinson's disease. Because YOR391p's biochemical function is still unknown, we present here the crystal structure of Hsp33 at 2.7 angstrom resolution. Overall structure shows YOR391p has one cap domain and one core domain, which shares nearly the same topological structure with Hsp31 from *E.coli*. Former study have approved that Hsp31 from *E.coli* has chaperone and peptidase activity, whose peptidase catalytic triad Cys-His-Glu locates in one narrow concave on the surface. But the entrance diameter of YOR391p's concave is slightly bigger than that of *E.coli* Hsp31, which imply YOR391p may have a more abroad substrate specificity. Future work will focus on the peptidase activity detection of YOR391p and its diverse expression level under different oxidative stress.