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Crystal structure of human alpha-L-iduronidase

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Alpha-L-iduronidase (IDUA, EC: 3.2.1.76) is a lysosomal enzyme that cleaves terminal α-iduronic acid residues from glycosaminoglycan, heparin sulphate, and dermatan sulphate. The deficiency of this enzyme causes Mucopolysaccharidosis Type I (MPS I) disease, also known as Hurler/Scheie syndrome. MPS I disease is appeared in child age and characterized by progressive mental retardation, gross facial features, enlarged and deformed skull, small stature, corneal opacities, hepatosplenomegaly, valvular heart defects, thick skin, joint contractures, and hernias. The treatment of MPS I is now mainly by an enzyme replacement therapies which introduce the recombinant IDUA to the blood by intravenous infusion. To date, many mutations of IDUA found in MPS I patients have been reported, however, details of relationship between the mutations and MPS I disease is poor. To elucidate the structural basis of MPS I and structure-based drug development, we have crystallized and determined the crystal structure of human IDUA at 2.5 Å resolution by SIRAS phasing. The crystal of human IDUA belongs to R3 spacegroup with its unit cell parameters as a=259.22 Å, b=259.22 Å, c=71.8 Å, and we have refined the model to Rwork=17.3%, Rfree=22.6%. The structural model includes almost the whole length of the polypeptide chain (27-642) and four oligosaccharide chains. The overall structure of IDUA is almost the same topology as XynB, which belongs to the same family in CAZy database (GH39). We could clearly observe a high-mannose chain, covalently attached at Asn372, is prolonged toward to the catalytic site. So, this oligosaccharide chain is suggested to be important for the enzymatic activities. The linkages of the mutation and phenotype of disease are discussed.

Keywords: glycoprotein, enzyme_therapy, crystal_structure

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Structural identification of nucleoprotein-nucleozin binding sites

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Influenza A virus is one of the most common pathogens that threaten the health of humans and animals. It is a typical orthomyxoviruses, and its genome comprises eight separated pieces of segmented negative-sense RNA, encoding for 11 proteins [1], [2]. Among them, nucleoprotein (NP), is a structural protein which contains about 500 aa. It has multiple functions during the virus life cycle, such as acting as an essential adaptor molecule for interaction between virus and host cells, or interacting with a great variety of viral and cellular proteins, while its most important role is to form ribonucleoproteins together with PA, PB1, PB2 and genomic RNA for transcription, replication and packaging [3].

Recently, Kao *et al*. identified NP as a druggable target and found that nucleozin could lead to the NP aggregates formation as well as antagonize its nuclear accumulation, which in turn cause the cessation of viral replication [4]. Since the interactions between nucleozin and NP are still not clearly known, it's our aim to identify the binding sites using X-ray crystallography.

The full length influenza A/WSN/33 (H1N1) NP gene was cloned into pET28a vector, with His-tag in its C-terminus [4] and overexpressed in *E.coli* BL21 Rosetta. Cell culture was purified by HisTrap HP and Superdex 200 gel filtration columns. Crystals were grown using the vapour diffusion method and the NP-nucleozin complex was prepared by soaking native crystal in solution containing 0.25mM nucleozin for 2h. Crystals of the complex can diffract to 3.8 Å at the Shanghai Synchrotron Radiation Facility. The structure of NP was determined by molecular replacement and it belongs to space group C2221 with a trimer per asymmetric unit. Possible nucleozin binding sites have been found and will be determined after further refinement.

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Crystal structure of NK2-heparin complex

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Hepatocyte growth factor (HGF/SF) is an activating ligand of the Met receptor tyrosine kinase, whose activity is essential for normal tissue development and organ regeneration but normal activation of Met has been implicated in growth, invasion, and metastasis of many types of solid tumours.

NK2 is a natural splice variant of HGF/SF, which consists of the N-terminal domain (N) and the two first kringle (K1 and K2) domains and requires heparan sulfate or soluble heparin for its activity, in the absence of the polysaccharides acts as a Met antagonist [1]. We describe the X-ray crystal structures of NK2 complex with heparin oligosaccharides containing six (dp6) and ten (dp10) subunits. We have found that dp6 and dp10 bind to NK2 inducing the dimmeritzation of NK2 N-terminal domain.

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Keywords: HGF/SF, NK2, heparin

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Preliminary neutron crystallographic study of mutant Transthyretin

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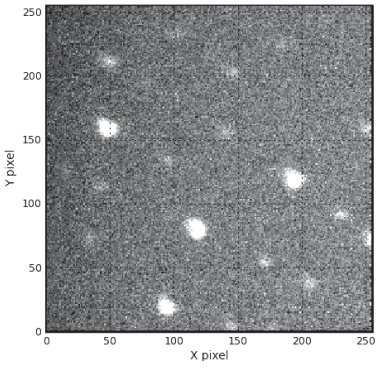
Transthyretin (TTR) is one of more than 30 nonhomologous proteins linked to human amyloid disease. TTR is found in the blood and cerebrospinal fluid, and is composed of four identical 127-aminoacid β-sheet-rich subunits. Dissociation of TTR appears to be the rate-determining step that allows subsequent partial misfolding and misassembly, leading to the formation of cross β-sheet amyloid fibrils, as well as several other aggregate morphologies. Deposition of TTR amyloid fibrils is linked to four different amyloid diseases: senile systemic amyloidogenesis, familial amyloid cardiomyopathy, familial amyloid polyneuropathy, and central nervous system selective amyloidosis. In spite of the many high-resolution crystal structures of wild-type and amyloidogenic mutant TTR, comparisons of mutant amyloidogenic TTR structures to native TTR structures do not reveal any significant structural diversity that could account for amyloidogenesis. In order to investigate molecular property of TTR, the neutron diffraction experiments of N-terminal truncated mutant TTR crystals were performed at J-PARC BL-03 iBIX, Japan. The mutant TTR crystals were grown using sitting drop vapor diffusion method up to 2.5mm^3 in volume after 3 months. Measurement conditions are as follow: the accelerator beam power: 120-220kW, the pulse repetition: 25Hz, the range of wavelengths: 2.5~6.5 Å (the 1st frame), 5.0~9.0 Å (the 2nd frame), the number of measurement settings: 82 settings (1st frame: 41 settings, 2nd frame: 41 settings). The neutron diffractions were observed up to 2.5-3.0Å resolution (Figure). We present the detailed data statistics of the preliminary neutron crystallographic experiments including the data processing and the structural refinement.



(Figure) TOF diffraction image of mutant TTR (2θ=51.13˚).

Keywords: transthyretin, neutron protein crystallography, amyloidosis

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Structural study of protein-bound CNG-repeats

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CNG repeat expansions in DNA cause many human diseases. The CNG-repeating RNAs produced during transcription are hypothesized to trigger these diseases via the ability to form double-helical hairpins and subsequently intervene with the cell silencing machinery [1], [2].

We performed the crystallographic study of double-helical RNAs that contained 5 to 6 consecutive CNG repeats (N = U, A, G or C) bound to silencing suppressor p19.

All CNG-repeats were found to form A-RNA double helix with N•N mismatches flanked by Watson-Crick G•C base pairs at both ends. The dominant stabilizing feature of these RNA structures is an intensive intrastrand stacking-interaction between two consecutive base pairs, observed at the GC/GC steps, which are periodically located at each third position of all CNG-repeating sequences. Stabilized by these interactions, the double helix easily accommodates N•N mismatches, with Pyr-Pyr 'pairs' showing no regular H-bonding between bases, but being supported by short repulsive contacts. In contrast, Pur-Pur arrangements are stabilized by hydrogen bonds, with G•G and A•A displaying *anti-syn* and *anti-anti* alignments, respectively. Interestingly, our CAG- and CUG-repeating RNA structures differ from protein-unbound short counterparts described in [3], [4], although overall RNA structures are similar. The observed structural differences between protein-bound and protein-free N•N 'pairing' in CNG-repeating RNAs point on adaptive role of these mismatches in facilitating the process of protein-RNA interaction in case of these sequences. In particular, the helical bending observed in [5], [6] for siRNA bound to p19 is most likely to be provided by the structural 'mobility' of these mismatches in case of CNG-repeating RNAs.

This structural study of CNG-repeating RNAs bound to p19 further supports the hypothesis of possible involvement of RNA silencing machine in development of human diseases caused by the trinucleotide expansions in genes. Our high-resolution structures of CNG-repeating RNAs complexed with p19 shed the light on what is happening with these repeats when they are getting bound to a protein.

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Structural characterization of atypical PKC iota complexes with substrates

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PKC iota is a human oncogene, a useful prognostic marker and validated target for chemical intervention in non-small cell lung cancer (NSCLC) and ovarian cancer [1]. This atypical PKC isoform has a C-terminal serine/ threonine protein kinase domain (AGC class) that is auto-inhibited by a pseudosubstrate (PS) region until a poorly characterized allosteric input releases it from the kinase active site. PKC iota associates with the polarity proteins PAR-3 and PAR-6 to form a conserved apical polarity complex found in both vertebrates and invertebrates [2].

The PKC iota kinase domain interaction with substrate and pseudosubstrate (PS) peptides has been characterized using fluorescence polarisation assay and kinetic assays. Optimized peptide/kinase domain complexes were then screened in crystallization trials and crystals were obtained diffracting to 4.0 Å. The peptide optimization was essential to obtaining crystals of the peptide/kinase complex.

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