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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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Preliminary neutron crystallographic study of mutant Transthyretin

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