MS5-O4 An integrative approach targeting phosphatase-substrate complexes for new cancer drug design strategy

Andrew H. Wang[1], Kai-En Chen[1], Shu-Yu Lin[1], Meng-Ru Ho[1], Chia-Cheng Chou[1], Tzu-Ching Meng[1]

1. Institute of Biological Chemistry, Academia Sinica, Taipei 11581, Taiwan.

email: ahjwang@gate.sinica.edu.tw

Reversible phosphorylation is an essential post-translational modification in the regulation of numerous cellular activities. This fundamental mechanism is precisely controlled by protein kinases, protein phosphatases and thousands of their phosphoproteins in any given cells. Two examples will be presented on PTPN3 and two of its specific substrates, mitogen-activated protein kinase 12 (MAPK12/p38γ) and epidermal growth factor (EGFR) pathway substrate 15 (Eps15).

The first study involves the structural elucidation of the PTPN3-p38γ complex, which is known to be a novel target for Ras-dependent malignancies. We determined the molecular architecture of this phosphatase-kinase complex by employing a hybrid method combining X-ray crystallography, small-angle X-ray scattering and chemical cross-linking coupled with mass spectrometry. Our crystal structure of PTPN3 in complex with the p38γ phosphopeptide presents a unique feature of the E-loop that defines the substrate specificity of PTPN3 towards fully activated p38γ. The low resolution solution structure demonstrates the formation of an active-state complex between the phosphatase domain of PTPN3 and p38γ. We show a regulatory function of PTPN3's PDZ domain, which stabilizes the active-state complex through interaction with the PDZ-binding motif of p38γ. Binding of the PDZ domain to the PDZ-binding motif lifted the auto-inhibitory constraint of PTPN3, enabling efficient tyrosine dephosphorylation of p38γ to occur. Our findings emphasize the potential of structure-based drug design to antagonize Ras transformation via intervention in PTPN3-p38γ interaction.

In the second study, we have also determined the crystal structure of PTPN3 in complex with Eps15 phosphopeptide. Dephosphorylation of Eps15 by PTPN3 is important for the regulation of EGFR in non-small cell lung cancer. Binding of Eps15 phosphopeptide to the PTPN3 active site reveals a novel conformation, which is different to other PTP-phosphopeptide structures. Our phosphatase activity confirmed a high level of substrate specificity between PTPN3 and Eps15. Employing the biochemical approach, we have identified the key PTPN3 residue involved in the recognition of Eps15. (Chen KE et al., Science Signal. 2014 7(347):ra98, Structure, 2015, in press)

Keywords: phosphatase. synchrotron, cancer, drug discovery

MS5-O5 Towards a generalised approach for the time-resolved crystallographic study of enzymes

Diana C.F. Monteiro[1], Vijay Patel[1], Christopher P. Bartlett[1], Shingo Nozaki[2], Thomas D. Grant[3], James A. Gowdy[1], Gary S. Thompson[1], Arnout P. Kalverda[1], Edward H. Snell[3], Hironori Niki[2], Arwen R. Pearson[4], Michael E. Webb[5]

1. Astbury Centre for Structural Molecular Biology, University of Leeds, UK
2. Microbial Genetics Laboratory, Genetic Strains Research Center, National Institute of Genetics, Japan
3. Hauptmann-Woodward Medical Research Institute, Buffalo, NY, USA
4. Hamburg Centre for Ultrafast Imaging, Institute of Nanostructure and Solid State Physics, University of Hamburg, Germany
5. School of Chemistry, University of Leeds, UK

email: cm07dcfm@leeds.ac.uk

Protein motions and dynamics are essential for function. Static structural studies provide only very incomplete information about the active conformations adopted by proteins. In contrast, time-resolved crystallography enables the observation of both small-scale and large-scale protein conformational changes that occur during function.[1]

To "watch" enzymes function in real-time, the enzymatic reaction must be triggered quickly and cleanly across the crystal. We have developed a new set of general photoactive reagents to trigger enzyme reactions using light. These reagents are designed such that they decouple photoactivation chemistry from the enzyme reaction and can be easily attached to strategically placed cysteines on the protein surface.

We are testing these new reagents using the protein aspartate α-decarboxylase (ADC) as a model system. This enzyme catalyses the conversion of aspartate to β-alanine, a precursor of coenzyme A. ADC is expressed as an inactive zymogen which cleaves post-translationally, yielding the catalytic pyruvoyl group. However, the cleavage requires an additional activating partner, PanZ.[2] To understand how ADC activation is catalysed by PanZ, we solved the structure of the ADC-PanZ protein-protein complex at high-resolution. With the aid of complementary techniques (SAXS, ITC, MS, NMR, *in cellulo* studies) we showed that not only does this protein-protein interaction promote ADC activation "mechanically" but that it is also involved in regulating CoA production in bacteria.[3-4]

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