ESRF	**Experiment title:** Structural studies on MASP-1 and MASP-2: autoactivation and specific binding of peptide inhibitors	**Experiment number**: **MX-1249**
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Shifts: 2	**Local contact(s)**: Stéphanie Monaco	*Received at ESRF:*

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Report:

Our structural studies focuse on the first enzymes of the complement enzyme cascade system, the major element of innate immunity, exploring mechanisms of the first enzyme reactions of the lectin and classical pathways of activation, as well as specificity and regulation by inhibioion of the first enzymes. The aim of the present project was to explore the strucutral basis of two the functionally important characteristics of MASP-1 and MASP-2, first enzymes of the lectin pathway of complement ativation:

(1) We collected an 1.28 Å dataset from a crystal of MASP-2 in complex with its specific inhibitor, SGMI-2. (A dataset from a crystal of MASP-1/SGMI-1 complex was collected also at the ESRF, but under a different project number.)
(2) We collected a 2.5 Å dataset from a crystal of the proenzyme form of MASP-1.

(1) Reference

D. Héja, V. Harmat, K. Fodor, M. Wilmanns, J. Dobó, K. A. Kékesi, P. Závodszky, P. Gál, G. Pál:
Monospecific Inhibitors Show That Both Mannan-binding Lectin-associated Serine Protease-1 (MASP-1) and -2 Are Essential for Lectin Pathway Activation and Reveal Structural Plasticity of MASP-2.
J. Biol. Chem. 287: 20290–20300 (2012)

Abstract: The lectin pathway is an antibody-independent activation route of the complement system. It provides immediate defense against pathogens and altered self-cells, but it also causes severe tissue damage after stroke, heart attack, and other ischemia reperfusion injuries. The pathway is triggered by target binding of pattern recognition molecules leading to the activation of zymogen mannan binding lectin-associated serine proteases (MASPs). MASP-2 is considered as the autonomous pathway-activator, while MASP-1 is considered as an auxiliary component.We evolved a pair of monospecific MASP inhibitors. In accordance with the key role of MASP-2, the MASP-2 inhibitor completely blocks the lectin pathway activation. Importantly, the MASP-1 inhibitor does the same, demonstrating that MASP-1 is not an auxiliary but an essential pathway component. We report the first Michaelis-like complex structures of MASP-1 and MASP-2 formed with substrate-like inhibitors. The 1.28 Å resolution MASP-2 structure reveals significant plasticity of the protease, suggesting that either an induced fit or a conformational selection mechanism should contribute to the extreme specificity of the enzyme.

(2) Reference

M. Megyeri, V. Harmat, B. Major, A. Végh, J. Balczer, D. Héja, K. Szilágyi, D. Datz, G. Pál, P. Závodszky, P. Gál, J. Dobó:

Quantitative Characterization of the Activation Steps of Mannan-binding Lectin (MBL)-associated Serine Proteases (MASPs) Points to the Central Role of MASP-1 in the Initiation of the Complement Lectin Pathway.

J. Biol. Chem. 288: 8922–8934 (2013)

Abstract: Mannan-binding lectin (MBL)-associated serine proteases, MASP-1 and MASP-2, have been thought to autoactivate when MBL/ficolin_MASP complexes bind to pathogens triggering the complement lectin pathway. Autoactivation of MASPs occurs in two steps: 1) zymogen autoactivation, when one proenzyme cleaves another proenzyme molecule of the same protease, and 2) autocatalytic activation, when the activated protease cleaves its own zymogen. Using recombinant catalytic fragments, we demonstrated that a stable proenzymeMASP-1variant (R448Q) cleaved the inactive, catalytic site Ser-to-Ala variant (S646A). The autoactivation steps of MASP-1 were separately quantified using these mutants and the wild type enzyme. Analogous mutants were made for MASP-2, and rate constants of the autoactivation steps as well as the possible cross-activation steps between MASP-1 and MASP-2 were determined. Based on the rate constants, a kinetic model of lectin pathway activation was outlined. The zymogen autoactivation rate of MASP-1 is ~3000-fold higher, and the autocatalytic activation of MASP-1 is about 140-fold faster than those of MASP-2. Moreover, both activated and proenzyme MASP-1 can effectively cleave proenzyme MASP-2. MASP-3, which does not autoactivate, is also cleaved by MASP-1 quite efficiently. The structure of the catalytic region of proenzyme MASP-1 R448Q was solved at 2.5 Å . Proenzyme MASP-1 R448Q readily cleaves synthetic substrates,

During our experiment we had time to collect data from cystals of other proteins. These are still ongoing projects:

(3) Reference

I. Leveles, G. Róna, I. Zagyva, Á. Bendes, V. B. G. Vértessy:

Crystallization and preliminary crystallographic analysis of dUTPase from the helper phage Φ11 of Staphylococcus aureus.

Acta Crystallographica F 67: 1411-1413 (2011)

Abstract

Staphylococcus aureus superantigen-carrying pathogenicity islands (SaPIs) have a determinant role in spreading virulence genes among bacterial populations that constitute a major health hazard. Repressor (Stl) proteins are responsible for transcriptional regulation of pathogenicity island genes. Recently, a derepressing interaction between the repressor Stl SaPIbov1 with dUTPase from the Φ11 helper phage was suggested [Tormo-Mas et al. (2010). Nature 465, 779-782]. Towards elucidating the molecular mechanism of this interaction, this study reports expression, purification, and X-ray analysis of Φ11 dUTPase that contains a phage-specific polypeptide segment not present in other dUTPases. Crystals were obtained using the hanging-drop vapor-diffusion method at room temperature. Data were collected from one type of crystal to 2.98 A resolution. The crystal of Φ11 dUTPase belonged to the cubic space group I23, with unit-cell parameters a=98.16 Å, α=β=γ= 90.00°.

(4) Aeropyrum pernix acylaminoacyl peptidase in complex with a covalently bound inhibitor; 2.6Å resolution. Our aim was to study the effect on substrate binding on the conformation of the loop holding the catalytic histidine in different states of the enzyme (manuscript in preparation).

(5) ERK2 / docking peptide complex (MAP-kinase): 2.2Å resolution dataset, unfortunately crystal contacts interfered with complex interactions.