s1.m8.p14 **Structures of the zymogen and active forms of the catalytic fragment of MASP-2.** Veronika Harmat,[a] Péter Gál,[b] Tünde Bián,[b] Géza Ambrus,[b] Gábor Náray-Szabó[a] and Péter Závodszky[b], [a]*Protein Modelling Group, Hungarian Academy of Sciences - Loránd Eötvös University, Hungary, and* [b]*Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Hungary. E-mail: harmatv@ludens.elte.hu*

Keywords: Complement activation; Serine protease; Hinge bending

A family of serine proteases mediate the proteolytic cascades of several defense mechanisms in vertebrates, such as the complement system, blood coagulation and fibrinolysis. These proteases usually form large complexes with other glycoproteins. Their common features are their modular structures and restricted substrate specificities. Mannan-binding lectin-associated serine protease 2 (MASP-2) is the key enzyme of the lectin activation pathway of complement. In its complex with MBL, a dimer of MASP-2 is able to perform all the functions of the $C1r_2C1s_2$ tetramer in the C1 complex of the classical pathway.

We present the structures of catalytic fragment of MASP-2 both in its zymogen form (encompassing two complement control protein modules and the chymotrypsin-like serine protease domain: CCP1-CCP2-SP) and activated form (CCP2-SP fragment).

The structures show three different domain orientations reflecting increased modular flexibility at the CCP2/SP joint. The detected interdomain flexibility is likely to be important in keeping the SP domains of the dimer close to each other in correct position for autoactivation and subsequently to make them accessible for its bulky substrates. This flexibility may partly explain the ability of the MASP-2 dimer to perform all of its functions alone, which are mediated the by $C1r_2$-$C1s_2$ tetramer in the C1 complex. Structural details of the surfaces of the CCP1 and SP domains may explain why MASP-2 is not able to form dimers via the CCP1 and SP regions of the monomers, like C1r.

Surprisingly, some surface loops of the SP domain forming substrate binding subsites are similar to those of trypsin or thrombin, and show significant differences if compared with those of C1s. That indicates the nearly identical substrate specificities of C1s and MASP-2 are realized through different sets of enzyme-substrate interactions.

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s1.m8.p15 **Carbohydrate Recognition by α-Amylase Isozymes: Their Crystal Structures Reveal New Binding Sites and Remarkable Functional Differences.** Richard Haser[a], Samuel Tranier[a], Xavier Robert[a], Birte Svensson[b] & Nushin Aghajari[a], [a]*Institut de Biologie Et Chimie des Protéines Umr 5086, Laboratoire de Biocristallographie, Ifr 128 " Biosciences ",Cnrs And Université Claude Bernard Lyon I, 7 Passage du Vercors, 69367 Lyon Cedex 07, France,* [b] *Danish Technical University, Biochemistry and Nutrition Group, Biocentrum Soltofts Plads, Dk-2800 Kgs Lyngby, Denmark. E-mail: r.haser@ibcp.fr*

Keywords: Glycoside-Hydrolase; Protein/Sugar Interactions; Crystal Structure

The Barley α-amylase isozymes, **AMY1** and **AMY2**, share nearly 80% sequence identity but display quite different physico-chemical properties. The crystal structures of both **AMY2** and **AMY1** were established in the native state, but also in complex with various inhibitors (like the diabetic drug acarbose) and substrate analogues, as for **AMY2** with the endogenous bifunctional barley α-amylase/subtilisin protein inhibitor **BASI** which only inhibits amy2. Remarkably, these isozymes have very similar **3D** structures but behave differently when interacting with substrates and inhibitors. **A** subtle, but very significant conformational change occurs when sugars bind, leading to the identification of an additional sugar recognition site in the **c**-terminal domain of amy1 named "the pair of sugar tongs", not present in **AMY2** (Robert *et al*.(2003) structure). A crucial role is observed for a tyrosine contributing to capture the polysaccharide. Changes in the immediate vicinity of this key tyrosine together with differences in the electrostatic surface potential confer to the **c**-terminal domain a new function as a carbohydrate-binding module. Interestingly, this extra carbohydrate site in **AMY1** is consistent with an increased affinity (compared to **AMY2**) when acting on starch granules. This site is also confirmed by the crystal structure of the complex between the inactive mutated enzyme and a true substrate, maltoheptaose. In contrast to the active site, the "sugar tongs" region reveals the circularization of the sugar substrate, probably controlled by the key tyrosine side-chain. Moreover, we very recently have established the first **3D**-structures of an α-amylase (**AMY1**) in complex with a cyclodextrin, this ligand binding only at the new "sugar tongs" site. Therefore we propose that the **c**-terminal domain, until now function-less appears, with the new carbohydrate site, to contribute to the fine-tuning of the enzyme/polysaccharide interactions and to be a major determinant for the barley α-amylase isozyme specificity, and eventually for other enzymes that work on α-linked glucopyranose-derived substrates.