exogenous recombinant 'truncated' Galectin-3 (analogous to the MMP-processed form), but not full length Galectin-3, dramatically increases migration of the human breast cancer cell line BT-549. These results suggest that the MMP-cleaved Galectin-3 and the resulting structural changes are responsible for pro-metastatic properties of Galectin-3. We have obtained crystallographic data for CRD of Galectin-3 in complex with a pentasaccharide to which Galectin-3 has higher binding affinity compared to other galectin family members. This structural information may be utilized in the design of Galectin-3 specific inhibitors targeting the carbohydrate-binding site. We have also explored the structural differences resulting from MMP cleavage of Galectin-3 using SAXS.

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Keywords: carbohydrate binding, crystal structure, SAXS

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Hinge-loop mutation can be used to control domain swapping of human cystatin C

Marta Orlikowska,[a] Elżbieta Jankowska,[a] Izabela Behrendt,[a] Dominika Borek,[b] Zbyszek Otwinowski,[b] Piotr Skowron[c] Aneta Szymańska,[a] [a]*Department of Medicinal Chemistry, Faculty of Chemistry, University of Gdańsk, Gdańsk, (Poland).* [b]*Department of Biochemistry, UT Southwestern Medical Center, Dallas, (USA).* [c]*Division of Environmental Molecular Biotechnology, Faculty of Chemistry, University of Gdańsk, Gdańsk, (Poland).* E-mail: martaorlikowska07@gmail.com

Human cystatin C (hCC) is a low molecular mass protein (120 amino acid residues, 13,343 Da) that belongs to a family of single chain, reversible inhibitors of papain-like (C1 family) and legumain-related (C13 family) cysteine proteases [1]. In pathophysiological processes, which nature of is not understood, hCC is codeposited in the amyloid plaques of Alzheimer's disease or Down's syndrome. The amyloidogenic properties of HCC are greatly increased in a naturally occurring L68Q variant, resulting in fatal cerebral amyloid angiopathy in early adult life [2]. At physiological conditions wild-type hCC is a monomeric protein, but under crystallization conditions (pH 4.8) forms a domain-swapped dimer [3]. The dimerization process is facilitated by the presence in the hCC structure of a flexible region created by the loop L1 (55-59, QIVAG) connecting protein subdomains undergoing the exchange process. This loop is the only part of hCC which undergoes significant structural changes during the dimerization process and, according to experimental [4], [5] and theoretical [6], [7] studies, these changes are driven by the conformational constraints attributed to the located near the top of the loop Val residue (Val57 for hCC).

With the aim to check implications of grater or decreased stability of this loop on dimerization and aggregation propensity of human cystatin C, we designed and constructed hCC L1 mutants with Val57 residue replaced by Asp, Asn [8], Gly (residues favored in this position of β-turns) or Pro, respectively. By applying this rational mutagenesis approach we were able to obtain hCC variants stable in the monomeric form both in solution and in the crystal (V57G, Figure 1), monomeric in solution but dimeric in the crystal (V57D) and dimeric in solution and oligomeric in the crystal (V57P). The results of structural studies of hCC L1 mutants will be presented.

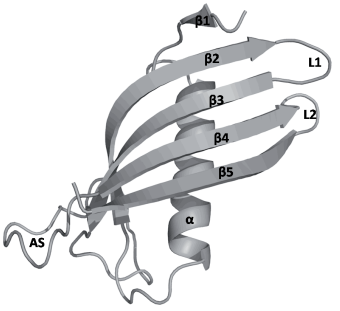

Figure 1. Crystal structures of monomeric hCC mutant V57G

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How group A streptococcus alpha-enolase interacts with human plasmin(ogen)

Daniel Ericsson,[a] Amanda J. Cork,[a] Eugene Valkov,[a] Anna Stamp,[a] Andrew Aquilina,[b] Mark J. Walker,[a] and Bostjan Kobe,[a] [a]*School of Chemistry and Molecular Biosciences, University of Queensland, (Australia).* [b]*School of Biological Sciences, University of Wollongong, New South Wales, (Australia).* E-mail: d.ericsson@uq.edu.au

The bacterium *Streptococcus pyogenes* causes a wide range of diseases in humans. More commonly known as Group A Streptococcus (GAS), this pathogen spans a huge spectrum of clinical conditions; from trivial to life threatening. Very common, non-invasive diseases like pyoderma and pharyngitis aside, this is also a causative agent of toxic-shock like syndrome, rheumatic heart disease, and necrotizing fasciitis. A prerequisite for these invasive conditions is that GAS has to migrate to and proliferate in areas of the body that are normally sterile.

It has been shown that the ability of GAS, and other bacteria, to interact with human plasminogen, a zymogen that circulates in our plasma at high concentrations, plays a major role in the invasive process. Plasmin, the active form of plasminogen, is a broad spectrum serine protease implicated in breaking down extracellular matrix and blood clots. The conversion of plasminogen to plasmin is normally under tight control; however this is subverted by GAS which activates this conversion by secreting the virulence determinant streptokinase. By forming a stable complex with either plasminogen or plasmin, streptokinase makes either complex display plasmin activity. GAS can bind the plasmin(ogen)-streptokinase complex to its surface via three known receptors; one of which is the streptococcal surface enolase (SEN).

SEN contains 435 residues per polymer and takes the form of an octameric ring, with a central 4-fold symmetry axis, and is found on the surface of GAS. Recent work [1] has focused on exploring the proposed plasmin(ogen) binding motifs in both wild type SEN, and in a range of point mutated residues. Choice of mutants was aided by creating an