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The glycan shell and its role in angiotensin converting enzyme structure and function.

Angiotensin converting enzyme (ACE) is a zinc metalloprotease consisting of two domains with distinct physiological functions despite their 60% sequence similarity and 90% active site identity. While the C-domain controls blood pressure, the N-domain is selective for cleaving the antifibrotic N-acetyl-Ser–Asp–Lys–Pro peptide. ACE is a highly glycosylated protein (30% carbohydrates by weight) with 17 potential *N*-linked glycosylation sites (PNGs). Mass spectrometric analyses revealed the presence of complex-type glycans at 9 N-domain and 6 C-domain PNGs. The location of these glycans are unique to each domain and could thus have domain-specific functions. It has been shown experimentally that removal of glycans at certain sites results in a decrease in protein melting temperature, possibly by reducing protein stability. ACE is inherently very dynamic and has been shown to undergo domain-specific hinging events upon inhibitor binding. Glycans are also required at specific sites for correct intracellular folding and secretion of the mature protein. It is also likely that the experimentally observed disparities in the domains' susceptibility to inactivation by diffuse reactive oxygen species (ROS) stems from these differences in glycosylation. The mechanism whereby these glycans function is, however, poorly understood. The aim of this study was therefore to elucidate the functional role of glycan molecules at each PNGs of ACE.

ACE catalytic inactivation was studied by monitoring remaining enzyme activity over time in the presence of redox co-reactants. Upon incubation with hydrogen peroxide and ascorbate, minimal diffuse radical oxidation of the N-domain was observed. Interestingly, the C-domain displayed greater diffuse radical oxidation. Time-course kinetic assays further described the C-domain's lower thermal stability and higher dependence on detergent stabilization. This is thought to be due to the unique hinging behavior and higher carbohydrate content of the N-domain. To test this hypothesis, both domains were fully glycosylated with complex glycans using the Glycam web server, explicitly solvated and subjected to molecular dynamics simulations in Amber. Analysis of the glycans' volume occupancy, radial distribution functions, correlated motions and hydrogen bonding over time revealed the presence of a glycan shield around the N-domain. This was shown to diminish access to the active site cleft by Caver Analyst and AQUA-DUCT analyses. Glycans with frequent hydrogen bonds to the N-domain were also identified and appear to be involved in protein folding and thermostability through stabilization of flexible active site loops. In contrast, the C-domain active site displayed a greater influx of solvent due to sub-optimal glycan shielding.

This study provides molecular insight into the role of ACE glycosylation and aids future investigations into the functional differences of glycoforms expressed in various human tissues.

HPC content

The two metalloprotein structures were built using the Schrodinger Suite, glycosylated using the Glycam web server and explicitly solvated using *tleap* in Amber 14. The ff14SB and GLYCAM06j-1 forcefields were used for protein and glycan residues, respectively, while a hybrid bonded/non-bonded model with custom forcefield parameters was used for the Zn-coordination site. Minimization, heating and equilibration was performed using Amber 14 on CPU while GPU acceleration of PMEMD was used for production dynamics. Trajectory analyses were performed using VMD, the Volutil package and the *cpptraj* module in AmberTools 15. Differences between the domains' active site solvent access was visualized by computing solvent tunnels using Caver Analyst v1.0. Resulting tunnels were visualized in PyMOL v2.0.6 and VMD v.1.9.3. The newly developed AQUA-DUCT tool (custom-installed at CHPC) was further used to quantify tunnel usage during the simulations of explicitly solvated N- and C-domain. The number of TIP3P water molecules which entered or exited the active site via tunnels were quantified in each frame and their pathways mapped.

Presenter Biography

Primary author: Ms LUBBE, Lizelle (University of Cape Town)

Co-author: Prof. STURROCK, Ed (University of Cape Town)

Presenter: Ms LUBBE, Lizelle (University of Cape Town)

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