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Probing the catalytic mechanism of an antifibrotic copper metallodrug

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Angiotensin-1-converting enzyme (ACE) is a zinc metalloprotease consisting of two domains with distinct inhibitor binding affinities despite their 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. Selective N-domain inhibition thus shows potential for the treatment of fibrosis. Contrary to conventional competitive ACE inhibitors such as Lisinopril, catalytic metallodrugs irreversibly inactivate ACE through the oxidation of active site residues. Since the metallodrug is recycled from the irreversibly inactivated target, it is effective at sub-stoichiometric concentrations and less likely to cause side-effects/toxicity. The aim of this study was to elucidate the molecular mechanism responsible for N-selective ACE inactivation by Cu-Gly-Gly-His-Lisinopril (CuGGHLis).

Kinetic characterization of inhibitor binding with fluorogenic assays showed that CuGGHLis binding was potent and non-selective (Ndom Ki =44.94±1.84nM; Cdom Ki =15.57±1.30nM). Catalytic inactivation was subsequently studied by monitoring remaining enzyme activity over time in the presence of redox co-reactants and CuGGHLis at IC20. In the presence of H2O2 and ascorbate, minimal diffuse radical oxidation of the Ndom was observed. Upon addition of CuGGHLis, however, the Ndom was rapidly and completely inactivated. Interestingly, the Cdom displayed greater diffuse radical oxidation and none catalysed by CuGGHLis.

Insight into the mechanism was gained by characterizing residual activity through Michaelis Menten kinetics. This revealed that CuGGHLis catalyzed oxidation of Ndom residues involved in binding and hydrolysis of the non-domain selective substrate whereas Cdom residues were unaffected. No oxidative protein cleavage was detected by SDS-PAGE and silver staining, suggesting inactivation through side chain oxidation. Mass spectrometry will reveal the sites of metal-catalysed oxidation while X-ray crystallography will identify the CuGGHLis binding site.

Time-course kinetic assays described the Cdom's lower thermal stability, higher dependence on detergent stabilization and greater diffuse radical oxidation. This is thought to be due to the unique hinging behaviour and higher carbohydrate content of the Ndom. Both domains were fully glycosylated with complex glycans, explicitly solvated and subjected to molecular dynamics simulations. While the Cdom active site is exposed to diffuse radicals, the Ndom is shielded by a glycan interaction network surrounding the molecule.

This study provides further insight into the mechanism of N-selective irreversible inactivation by CuGGHLis. In future, these findings will aid the design of a new class of selective antifibrotic ACE inhibitors effective at sub-stoichiometric doses.

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 analysis was performed using VMD and the cpptraj module in Amber 14.

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