Complexes of LPMO9c from *Neurospora crassa* with small-molecule inhibitors

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Lytic polysaccharide monooxygenases (LPMOs) are oxidative copper metalloenzymes with a key role in the conversion of complex biomass to fermentable sugars. Despite the high interest in sustainable production of biofuels and biochemicals, the enzymatic conversion of recalcitrant polysaccharides was falsely thought to be promoted only by hydrolases and LPMOs were discovered relatively recently [1]. LPMOs use hydrogen peroxide or oxygen as co-substrate binding to copper(I) in the active site. Oxidized LPMO possessing copper(II) has shown practically no activity, thus initial activation by a reducing agent, *e.g.* ascorbic acid is necessary [2]. After activation it appears to react through a Fenton-like mechanism similar to hexaaquacopper but some observations remain inconsistent and the exact mechanism is still debated [3]. Also works focused on inhibitors of LPMOs are scarce despite their importance. To shed more light into the field, we propose several small inhibitors of LPMO9c from *Neurospora crassa*. For certain species, a 50% drop of the activity was observed with millimolar inhibitor concentration (IC50). Similar results were observed for two different substrates. Considering competitive inhibition, we have calculated binding energies of inhibition complexes using DFT/B3LYP approach including the GD3BJ dispersion correction, PCM solvation model and the basis set superposition error estimation. We have found that inhibitors bind strongly to the active site and the binding energies correlate reasonably with the IC50 values supporting the experimental measurements. By separating the binding process into subprocesses of dehydration, deformation and interaction, we have quantified corresponding energies and their effect on the total binding affinity. The largest variance was observed for the interaction energies. Contributions of dehydration and deformation seem to improve inhibition prediction. Using the NBO analysis, the strongest stabilizing interactions were identified. Several off-site hydrogen bonds were found in complexes with strong binders. This work serves as one of the first LPMO inhibition studies, identifies several candidates for reversible LPMO inhibition and proposes a methodology for the prediction of their affinity.

References

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