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X-ray photoreduction of the active site copper in the fungal lytic polysaccharide monooxygenase LsAA9A

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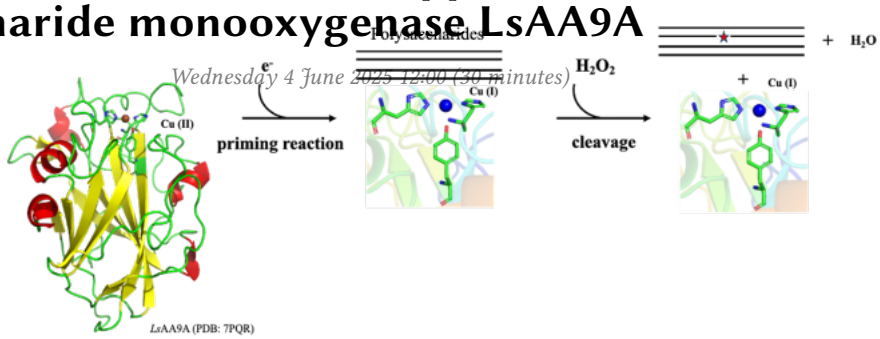

Figure 1: enter image description here

Lytic polysaccharide monooxygenases are enzymes [1] binding their active-site copper through the characteristic His-brace motif shown above including two His –one N-terminal –and often also a Tyr. The reaction cycle starts with reduction of the resting state Cu(II) to Cu(I) –in the laboratory usually using ascorbate as small molecule reductant. Despite the name, most LPMOs prefer hydrogen peroxide as co-substrate, to subsequently oxidatively cleave the glycosidic bonds in saccharides.
We have previously –through crystal cryo-structures of the model enzyme LsAA9A determined at high and low X-ray doses, and based on the hypothesis that X-ray induced photoreduction mimics natural priming reaction - reconstructed possible changes in geometry during the catalytic cycle and identified a small shortening of the Cu(II)-Tyr distance [2].
Aside from uncertainty on the biological significance of such shortening [3], we wanted to address concerns regarding the ability of macromolecular crystallography to reliably detect differences in the order of 0.1-0.2 Å. We thus carried out additional triplicate independent structure determination representing Cu(I)/Cu(II) states with/without the model substrates cellotriose, showing statistically significant differences only for the Cu(II)-Tyr distance with/without saccharide, but no other Cu-protein distance.
In order to assess whether additional general X-ray damage obscures similar shortening in the Cu(I) state induced by photoreduction, we are now comparing with cryo data collected after priming by chemical reduction with ascorbate at low X-ray doses.
Finally since X-ray-induced photoreduction of the active-site copper may closely approximate the chemical priming reaction, it holds potential as a trigger for time-resolved studies. To explore this possibility further, we are currently investigating the photoreduction process at room temperature.

References

[1] Tandrup, T., Frandsen, K.E.H., Johansen, K.S., Berrin, J.-G., Lo Leggio, L. (2018) Biochem. Soc. Trans. 46, 1431–1447
[2] Tandrup, T., Muderspach, S.J., Banerjee, S., Santoni, G., Ipsen, J.Ø., Hernández-Rollán, C., Nørholm, M.H.H., Johansen, K.S., Meilleur, F., Lo Leggio, L. (2022) IUCR Journal 9, 666-681.
[3] Wieduwilt, Lo Leggio and Hedegård (2024), Dalton Trans, 53, 5796-5807.

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