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Time-resolved serial crystallograhy to capture reaction intermediates of a glucuronyl esterase

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Glucuronyl esterases (GEs) from the carbohydrate esterase family 15 (CE15) are involved in degrading lignocellulosic biomass, by catalyzing the hydrolysis of an esterbond connecting lignin and hemicellulose in the plant cell wall (1). In order to utilize biomass in biorefineries, efficient methods are needed to separate cellulose, hemicellulose and lignin. Studying GEs to better understand their reaction mechanism can aid in improving existing biological preteatment methods used in biorefineries to be able to make better use of this renewable energy source. The bacterial GE from Opitutus terrae (OtCE15A) has previously been structurally determined at cryo-temperature and a reaction mechanism for the acylation and deacylation reactions has been proposed (2,3). Various glucuronate- and galacturonate esters have been used as model substrates for the lignin-hemicellulose linkage, and the substrates have been soaked into the crystals. However, attempts to capture the binding of the substrates prior to hydrolysis of the ester bond have so far been unsuccessful, but a covalent reaction intermediate has been obtained using enzymes with mutations at the catalytic site. In attempts to capture the binding of substrates prior to hydrolysis and to determine reaction intermediates, we have collected serial synchrotron X-ray crystallography (SSX) data at BioMAX (MAX IV, Lund), and conducted initial time-resolved SSX experiment at P14.EH2 (T-REXX of PETRA III, Hamburg). We have obtained high resolution (1.7Å) SSX data of OtCE15A at BioMAX and observed binding of the cleaved substrate of benzyl glucuronoate after a soaking time of 5 minutes. For time-resolved SSX experiments at T-REXX, we have tested and are planning to use the hit-and-return (HARE) method (4).

References
1. Larsbrink, Johan, and Leila Lo Leggio. “Glucuronoyl esterases–enzymes to decouple lignin and carbohydrates and enable better utilization of renewable plant biomass.” Essays in Biochemistry 67.3 (2023): 493-503.
2. Mazurkewich, Scott, et al. “Structural and biochemical studies of the glucuronoyl esterase OtCE15A illuminate its interaction with lignocellulosic components.” Journal of Biological Chemistry 294.52 (2019): 19978-19987.
3. Zong, Zhiyou, et al. “Mechanism and biomass association of glucuronoyl esterase: an α/β hydrolase with potential in biomass conversion.” Nature Communications 13.1 (2022): 1449.
4. Schulz, Eike C., et al. “The hit-and-return system enables efficient time-resolved serial synchrotron crystallography.” Nature methods 15.11 (2018): 901-904.

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