

MECHANISM OF THE GLUCURONOYL ESTERASE *OtCE15A*: AN α/β HYDROLASE INVOLVED IN BIOMASS CONVERSION

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BACKGROUND

Lignocellulose is a complex and heterogeneous resource which could act as feedstock for production of clean and renewable fuels, materials, and fine chemicals (Figure 1A). Glucuronoyl esterases (GEs) from the Carbohydrate Esterase 15 (CE15) family are α/β hydrolases which cleave an important ester linkage connecting lignin to glucuronoxylan^{1,2}, and these enzymes could be exploited for green chemistry techniques to help reduce recalcitrance in biomass conversion strategies (Figure 1B).

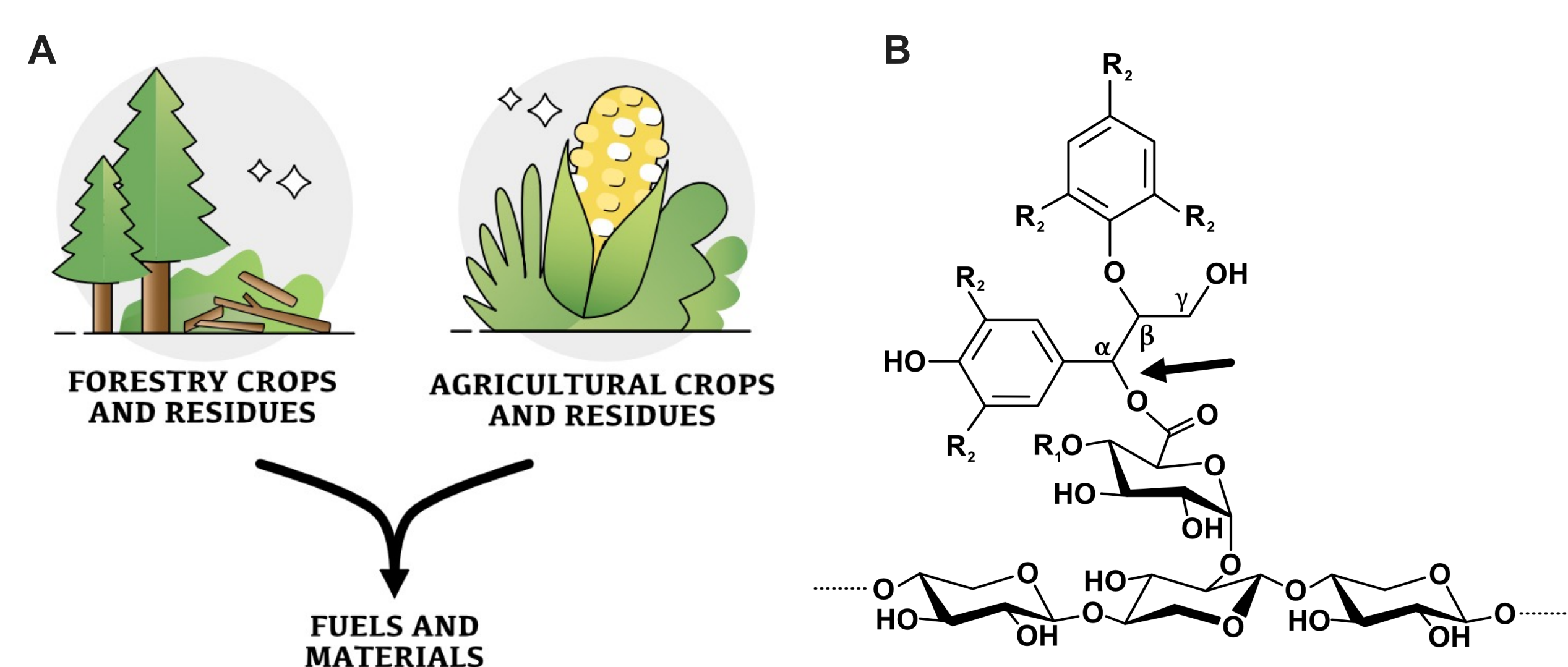


Figure 1. Conversion of plant biomass to address carbon needs. A) Better strategies for the utilization of recalcitrant forestry and agricultural wastes are needed to reach our sustainability goals. B) GE catalyze the cleavage of lignin-carbohydrate complexes (LCCs) thereby reducing biomass recalcitrance.

We've determined structures of a GE from the soil microbe *Opitutus terrae* in complex with biomass compounds^{2,3} (*OtCE15A*; Figure 2) which revealed two unique features of this α/β hydrolase: 1) it comprises two putative catalytic acids as part of its catalytic triad (Ser-His-acid), and 2) it contains an arginine residue, conserved amongst other GEs, which could support the oxyanion formed in the transition state analogous to other α/β hydrolases. Presented here is our investigation into the mechanism of action of *OtCE15A* by MM/QM simulations and kinetic characterizations which advances our understanding of this enzyme and its role in biomass turnover⁴.

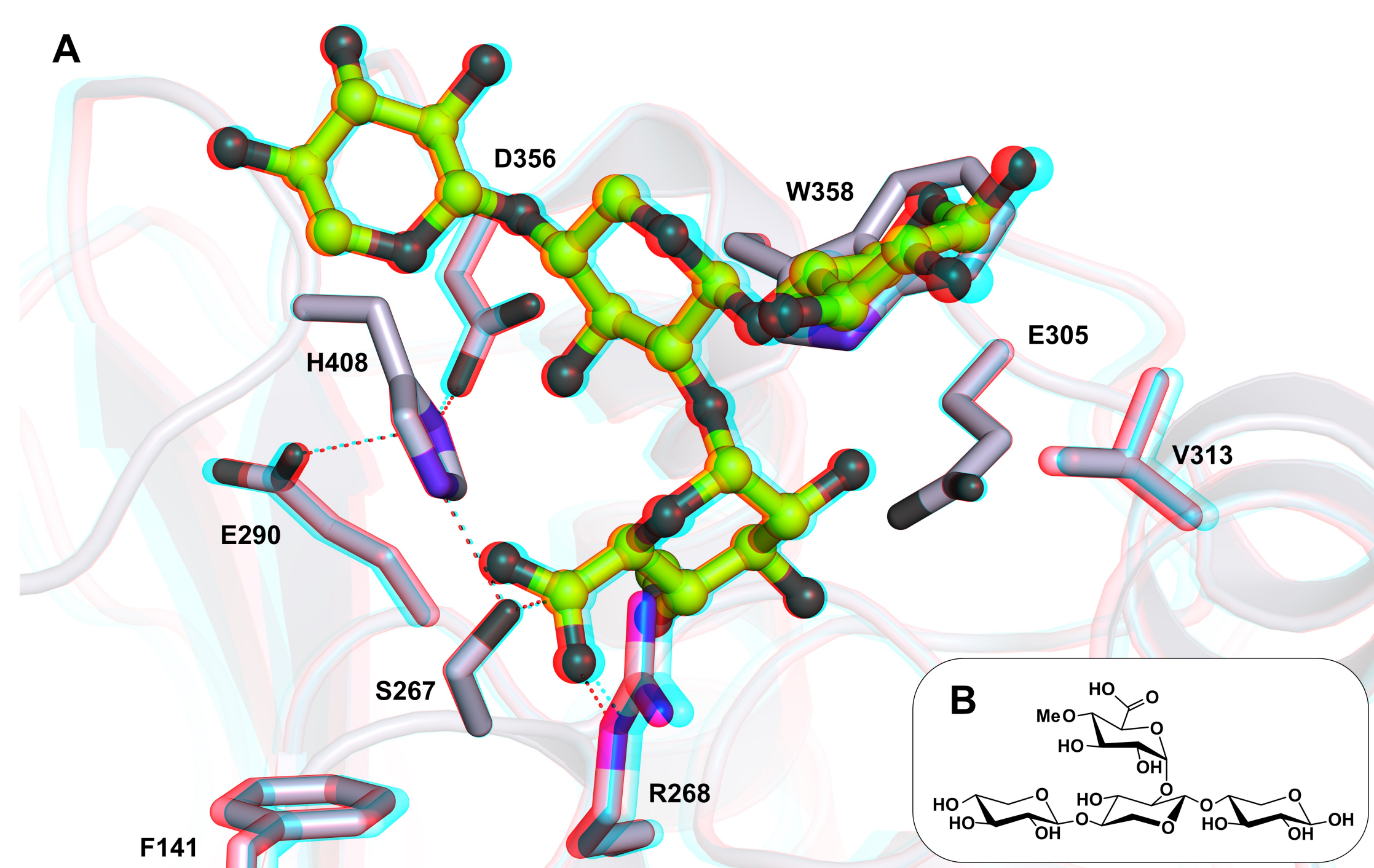


Figure 2. *OtCE15A* in complex with biomass fragment. A) Complex with aldotetrauronic acid (XUX; PDB: 6T0I) revealing Glu290 and Asp356 as potential acids and Arg268 as a potential stabilizer of the tetrahedral oxyanion transition state. B) Chemical structure of the bound XUX ligand.

RESULTS

The reaction catalyzed by *OtCE15A* was assumed to proceed through a covalent intermediate, going through tetrahedral transition states as in classical α/β hydrolases. We explored this mechanism by quantum and molecular mechanic simulations (QM/MM) (Figure 2) and quantified the contributions of each putative acids and active site arginine in the reaction mechanism (Tables 1 and 2).

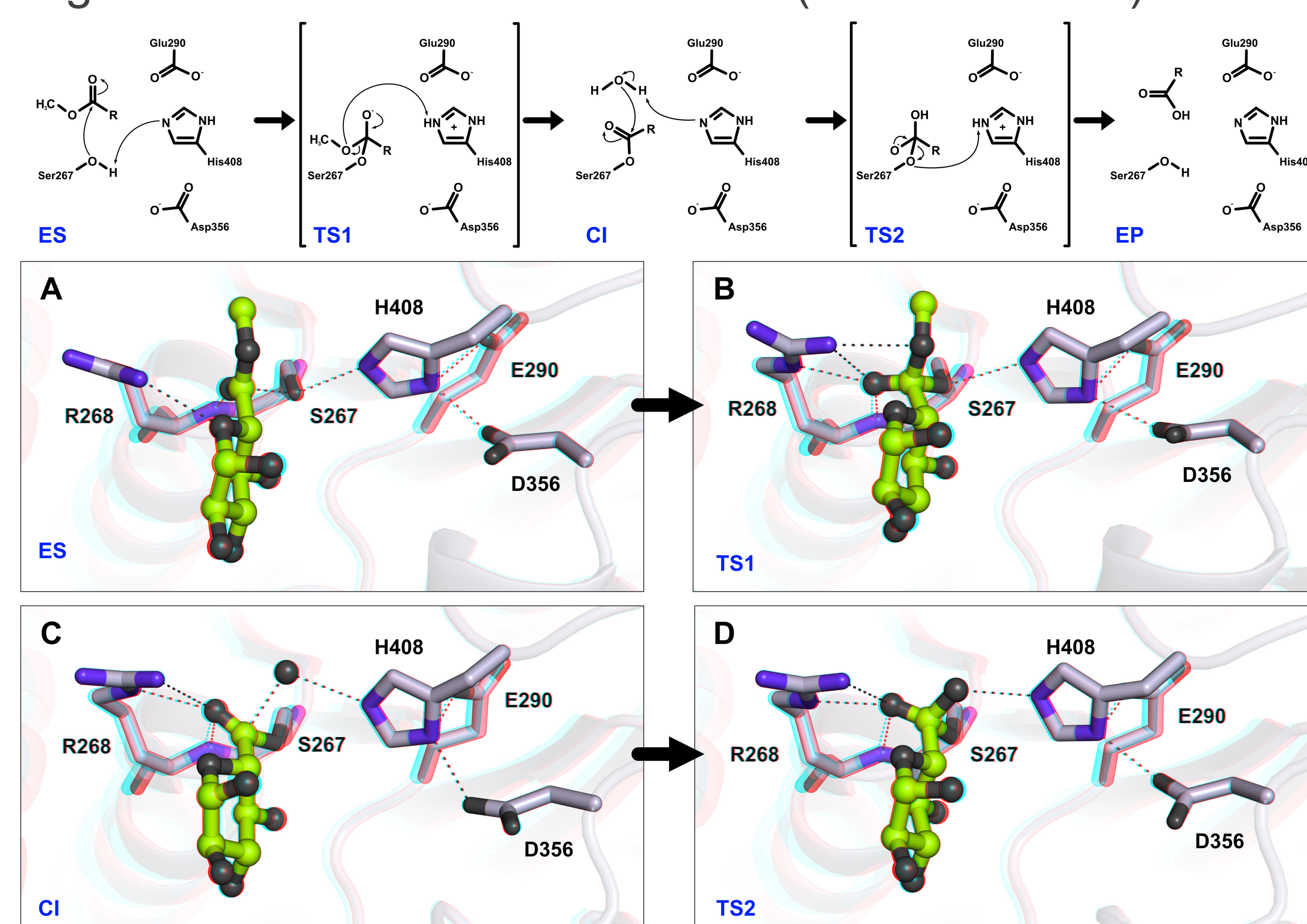


Figure 2. Reaction of *OtCE15A* proceeds through acylation and deacylation steps. Representative binding poses observed in QM simulations. ES (A) and EP denote enzyme-substrate and enzyme-product complexes, respectively. TS1 (B) and TS2 (D) denote transition states in acylation and deacylation, respectively, and CI (C) represents the covalent intermediate.

Reaction	WT-SHED	D356A-SHE	E290A-SHD	R268A-SHED
Acylation	6.8 ± 0.6	9.3 ± 0.3	19.2 ± 1.7	9.6 ± 0.8
Deacylation	7.5 ± 0.7	18.4 ± 0.4	32.0 ± 0.7	>110

Table 1. Quantification of residue contribution by QM simulations. ΔG (kcal/mol) against initial proton transfers in acylation and deacylation of WT *OtCE15A* and *OtCE15A*-D356A, -E290A, and -R268A variants with the MeGlcA substrate using units of SHE, SHD, and SHED, respectively.

Enzyme	K_m (mM)	k_{cat} (s ⁻¹)	k_{cat}/K_m (s ⁻¹ M ⁻¹)
Wild Type	3.57 ± 0.091	16.6 ± 0.11	4.65E+03 ± 1.2E+02
S267A	2.83 ± 0.66	0.000983 ± 0.000059	3.47E-01 ± 8.3E-02
R268A	0.408 ± 0.051	0.204 ± 0.0070	5.01E+02 ± 6.5E+01
E290A	2.03 ± 0.11	10.3 ± 0.11	5.07E+03 ± 2.8E+02
D356A	1.86 ± 0.11	5.21 ± 0.073	2.08E+03 ± 1.7E+02
E290A/D356A	0.502 ± 0.034	0.196 ± 0.0030	3.90E+02 ± 2.7E+01

Table 2. Kinetic characterization of wild type *OtCE15A* and substitution variants with the model substrate benzyl glucuronate. Assays completed in 100 mM sodium phosphate pH 7.5 at 25°C with the rate of glucuronate produced quantified by coupling to uronate dehydrogenase.

CONCLUSIONS

Both acidic residues contribute to positioning and deprotonating the catalytic histidine, while the active site arginine is stabilizing the oxyanion. The energetic barrier between acylation and deacylation is small and residue substitutions most greatly effects deacylation.

References

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