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Increase in Salt Resistance of Collagenase and Xylanase Based on Their Structural Analysis

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Summary

It is thought that in order to expand the industrial use of collagenase and xylanase, not only high activity and stability but also high salt resistance is necessary. In this study, we aim to determine the X-ray crystal structures of *Grimontia hollisae* collagenase (Ghcol) and *Bacillus* GH10 xylanase XynR to increase their salt resistance.

Ghcol was expressed in *Brevibacillus* and purified from the supernatant. To explore its catalytic mechanism, its substrate (Gly-Pro-Hyp-Gly-Pro-Hyp, GPOGPO)-complexed crystal structure was determined at 2.0 Å resolution. A water molecule was observed near the active-site zinc ion. Since this water was not observed in the product (GPO)-complexed Ghcol, it was hypothesized that the GPOGPO-complexed Ghcol structure reflects a Michaelis complex, providing a structural basis for understanding the catalytic mechanism. Analyses of the active-site geometry and site-directed mutagenesis of the active-site tyrosine residues revealed that Glu493 and Tyr564 were essential for catalysis, suggesting that Glu493 functions as an acid and base catalyst while Tyr564 stabilizes the tetrahedral complex in the transition state. These results shed light on the catalytic mechanism of bacterial collagenase.

XynR was expressed in *E. coli* and purified from the cells. In the hydrolysis of beechwood xylan, all 19 variants at position 315 exhibited bell-shaped pH-activity profiles. T315H, T315N, T315Q, and T315S exhibited a broader bell-shaped pH-dependence of activity than WT. Crystallographic analysis revealed that the Ca^{2+} ion near position 315 in WT was absent in the T315Q variant. We accordingly hypothesized that the enhancement of alkaliphily in T315Q, and probably also in the T315H, T315N, and T315S variants, could be ascribed to an activity-stability trade-off associated with a reduction in stability due to the lack of this Ca^{2+} ion. Consistent with expectations, the alkaline resistance of T315H, T315N, T315Q, and T315S was found to be lower than that of WT. In addition, the thermostabilities of these four variants, as assessed using the denaturing temperatures (T_m) at 0 mM $CaCl_2$ based on ellipticity at 222 nm in circular dichroism measurements, were lower than that of WT.

Screening of Ghcol and XynR with higher activity, thermostability, and/or salt resistance is currently underway.