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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 and increase their salt resistance.

Ghcol was expressed in Brevibacillus and purified from the supernatant. The crystal structures of ligand-free and Gly-Pro-hydroxyproline (Hyp)-complexed Ghcol were obtained. The structures revealed that the activator and peptidase domains exhibit a saddle-shaped structure with one zinc ion and four calcium ions. The activator domain comprises two homologous subdomains. In the ligand-complexed Ghcol, two Gly-Pro-Hyp molecules each bind at the active site and at two surfaces on the duplicate subdomains of the activator domain facing the active site. Analysis of variants with one of three active-site Tyr residues revealed that mutation of Tyr564 affected catalysis, while mutation of Tyr476 or Tyr555 affected substrate recognition. This was explained by the fact that Tyr564 is closer to the zinc ion than Tyr476 and Tyr555, and that Tyr476 and Tyr555 are closer to Gly-Pro-Hyp than Tyr564.

XynR was expressed in Escherichia coli and purified from the cells. The crystal structures of ligand-free and xylose or xylobiose-complexed XynR were obtained. The structures revealed a TIM-barrel structure consisting of eight α-helices and eight β sheets, which was the same as the ligand-free XynR. We previously selected T315N as an alkaliphilic variant of XynR from the site saturation mutagenesis library. In this study, we examined the effects of amino acid residue at 315 position of XynR on its alkaliphily and alkaline resistance. In the hydrolysis of beechwood xylan at pH 8.0, four variants (T315H, T315N, T315Q, and T315S) exhibited higher activity (90–110% of that of WT), while other 15 variants exhibited lower activity (less than 60% of that of WT). T315H, T315N, and T315Q exhibited a narrower bell-shaped pH dependence of stability at alkaline side than WT and T315S. These results suggested that at position 315, the amino acid residue whose side chain has an amido group (Asn, Gln) makes XynR alkaliphilic, while that whose side chain has a hydroxyl group (Thr, Ser) makes it alkaline-resistant.

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