

## Stability and activity modulation of *Bacillus circulans* xylanase based on the analysis of conserved flexible motions of surface residues

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The flexibility of residues has an important role in conformational changes for substrate binding and is also closely linked to protein stability. Role of flexible residues in enzyme activity could be investigated by analyzing flexible motions of enzymes. Here, we analyzed the flexibility of *Bacillus circulans* xylanase (BCX) by analyzing flexible motions of family 11 xylanases with molecular dynamic simulation. Family 11 xylanases have three conserved flexible loops and one surface loop (corresponding to 46~49 residues of BCX) connects active sites pocket with second substrate binding site far from active site. However, flexible motion of this loop was constrained by cooperative interactions with other residues (cation- $\pi$  interaction (W42-R49) and electrostatic interaction (D4-R49)). Saturation mutagenesis of R49 was conducted and stability and kinetic parameters were determined. Most mutants lost their stability due to disruption of cooperative interactions but gained higher catalytic power (up to 2 fold of  $K_{cat}/K_m$ ) due to increased flexibility. This implies that flexible loop (46-49), especially, R49, seems to have important role in modulating the stability and activity of xylanases.