ACCESS TUNNEL ENGINEERING TO OPTIMIZE THE CATALYTIC CYCLE OF CARBOHYDRATE HYDROLASES WITH BURIED ACTIVE SITE

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The active site of many enzymes is buried inside the protein core and is connected with the surrounding solvent by access tunnels. An emerging approach to optimize these enzymes properties is the engineering of structural features governing the exchange of ligands between the active sites and bulk solvent. However, it is still challenging to redesign the access tunnels of enzymes catalyzing biopolymers like carbohydrate hydrolases because of the extremely complicated substrate structure. In this study, structure-guided saturated mutagenesis was performed to reconstruct all three access tunnels of xylanase S7-xyl from *Bacillus halodurans* S7, which results in a mutant 254-RL1 with 3.4-fold increase in specific activity. Structural comparison and kinetic analysis revealed that products egress is the rate-limiting step in the catalytic cycle of S7-xyl. The products release tunnel in S7-xyl was experimentally validated, and not the tunnel radius but the length determining the products release efficiency. Application assessment showed that relieving the inhibition of reducing sugars on mutant 254-RL1 could accelerate the hydrolysis efficiency of cellulase on different pretreated lignocellulose materials, representing a good candidate in enzyme cocktails for lignocellulose biodegradation. In addition, the same strategy was successfully utilized to improve the specific activities of three other xylanases with buried active site, suggesting the general application of tunnel engineering to optimize carbohydrate hydrolases with buried active site.