

Engineering novel disulfide bond in bacterial α -type carbonic anhydrase for thermostabilization

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Exploiting carbonic anhydrase (CA), an enzyme that rapidly catalyzes carbon dioxide (CO₂) hydration, is an attractive route for carbon sequestration. However, the industrial applications of CA are hampered by the unstable nature of enzymes. We introduced *in silico* designed, *de novo* disulfide bond in α -type CA from *Neisseria gonorrhoeae* (*ngCA*) to enhance thermostability. Three *ngCA* variants (T133C-D197C, P56C-P156C, and N63C-P145C) were selected and expressed in *Escherichia coli*. The thermostability of N63C-P145C was greatly increased, showing 8-fold higher half-life at 70 °C. The melting temperature of N63C-P145C was 7.8 °C higher than that of the wild-type. This difference could be attributed to the loss of conformational entropy of the unfolded state. N63C-P145C showed an upward-shifted optimal temperature and was thermoactivated, which compensated for the lowered activity at 25 °C. Collectively, *de novo* disulfide engineering was successfully applied to improve the thermostability of CA and the constructed *ngCA* variant N63C-P145C can be used as an efficient biocatalyst for CO₂ sequestration under high temperature conditions.