ENZYME ENGINEERING OF FUNGAL-DERIVED FAD-GDH BY CIRCULAR PERMUTATION

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The flavin adenine dinucleotide dependent glucose dehydrogenase (FAD-GDH; EC 1.1.5.9) comprises oxidoreductases that catalyze the initial oxidation of glucose and other sugar molecules, using FAD as the primary electron acceptor. FAD-GDH has received attention as biocatalyst for glucose monitoring, especially self-monitoring of blood glucose.

Narrowing the substrate specificity of FAD-GDH for glucose is desired for future application. In this study, we employed a technique called circular permutation (CP) to explore the effects on enzyme substrate specificity. CP is a method to create protein variants by connecting the native protein termini via a covalent soft linker and introducing new ends through the cleavage of an existing peptide bond (Fig 1) (1, 2).

Starting with FAD-GDH derived from *Aspergillus iizukae* (AiGDH), we explored various the amino acid linker to connect the original termini. Subsequently, 16 CP variants with new termini in selected parts of the protein structure were generated and tested for catalytic activity toward glucose. The activity of wild type AiGDH toward xylose is approximately 10% of that for glucose. Termini relocation in the leading CP variants resulted in a 1.5 to 2-fold reduction of relative activity for xylose over glucose. At the same time, wild type and CP variants exhibited only residual activity for maltose (<1%).

Thermostability of CP variants was measured by the T_{50} values, which is the temperature at which 50% residual activity is maintained following a heat treatment. The CP variants exhibit T_{50} values lower than wild-type. We tried the secondary engineering of CP variants to improve the thermostability. We found the thermostable variants through the introduction of a new disulfide bridge. The combination of CP with the introduction of a disulfide bridge showed the functional benefits, exhibiting T_{50} values higher than original CP variants.

Our results suggest that CP variants display reduced xylose interference and reduced cross-reactivity with a range of sugars. Secondary engineering of CP variants exhibit the functional benefits, which improve the thermostability. As such, they could be useful biosensors for self-monitoring of blood glucose.

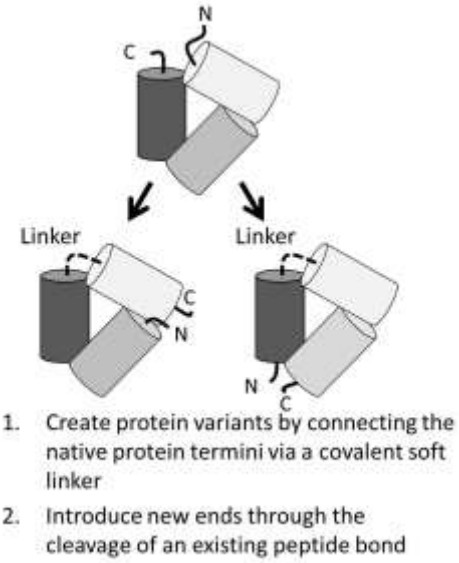

Fig 1. Schematic models of circular permutation.

References

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