Improving Nitration Activity of Fused TxtE-CYP102AI Reductase Domain by Optimizing the Linker Length

Reddy, Padmavathi

Cytochrome P450 monooxygenases are highly selective in substrate oxidation and have the promising potential as biocatalysts for industrial processes. A unique P450 enzyme known as TxtE catalyzes the nitration of l-tryptophan to 4-nitro-l-tryptophan, a reaction that is essential in producing pharmaceuticals, herbicides, dyes, food additives and pesticides. To develop this enzyme as nitration biocatalyst, the university laboratory has created a self-sufficient enzyme by fusing TxtE with the reductase domain of a naturally self-sufficient CYP102A1, or P450BM3. However, the low coupling efficiency of the fusion enzyme is a limiting factor in practical biocatalytic applications. The purpose of this research was to address this limitation by optimizing the length of a linker that connects TxtE and the P450BM3 reductase domain (BM3R). Six fusions with varying linker lengths were constructed and cloned into an expression plasmid pET28b using molecular biology tools including PCR amplification, restriction enzyme digestion and ligation. The constructs were transformed into E. coli cells through electroporation and the recombinant proteins were expressed and purified for in-vitro activity assessment with l-tryptophan as the substrate. The results suggested that while short linker lengths (4 -10 amino acids, AAs) led to misfolded proteins, moderate linker lengths (12-15 AAs) resulted in the active TxtE-BM3R enzymes, some of which was more active than the wild type. This research laid out a framework towards the development of industrially applicable nitration biocatalysts.

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