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Highly active and enantioselective HheG variants obtained by flexible loop engineering

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PURPOSE OF THE ABSTRACT

G-type halohydrin dehalogenases, such as HheG from Ilumatobacter coccineus, are privileged enzymes among halohydrin dehalogenases (HHDHs) due to their ability to convert also sterically more demanding internal epoxide substrates (cyclic as well as acyclic ones) with a variety of anionic C-, N-, O-, S- and halide nucleophiles [1-3]. Their rather low stability and often only moderate enantioselectivity, however, constitute major limitations for practical application. While we recently solved the stability issue by generation of highly stable and active cross-linked enzyme crystals of HheG [4,5], we now attempted to create also highly enantioselective variants by protein engineering. Thus, a highly flexible active-site loop was found to influence HheG's enantioselectivity tremendously. A site-saturation library containing all possible amino acid exchanges at three selected residues was generated and screened in ring opening reactions of different epoxide substrates in combination with two different nucleophiles. This way, several variants with largely increased or even inverted enantioselectivity towards the tested substrates could be obtained. The most impressive one did not only yield (1S,2S)-2-azidocyclohexan-1-ol with ≥96% enantiomeric excess, but displayed also a 10-fold higher specific activity compared to wild-type HheG (Figure 1). Kinetic data indicate that this improvement in enzymatic activity is caused by a largely increased kcat as well as enhanced binding of the nucleophile. Overall, we are convinced that this loop represents a major target for enantioselectivity engineering not only in HheG but also other related G-type halohydrin dehalogenases.
Currently, we are trying to rationalize on a structural level the impact of that loop on enzyme activity and enantioselectivity by performing molecular dynamics (MD) simulations in collaboration with the group of Silvia Osuna.

FIGURES

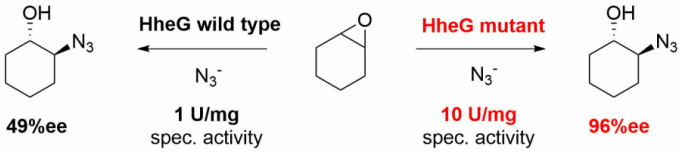

FIGURE 1

Figure 1.

Azidolysis of cyclohexene oxide catalyzed by HheG wild type and a mutant with enhanced enantioselectivity.

FIGURE 2

KEYWORDS

protein engineering | enantioselectivity | halohydrin dehalogenase | epoxide ring opening

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