ASSESSMENT OF C-TYPE HALOHYDRIN DEHALOGENASE STABILITY

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Halohydrin dehalogenases (HHDHs) are important enzymes that display excellent selectivity and activity in reversible dehalogenation of vicinal halohydrins. Furthermore, HHDHs may employ wide range of unnatural nucleophiles in ring-opening reactions, extending the possibilities for the synthesis of enantiopure β-alcohols[1]. On account of these desirable properties, the variant of the HheC enzyme is employed in the synthesis of Lipitor, one of the best-selling pharmaceuticals in the world[2]. However, insufficient is known about the enzyme kinetic stability that is a prerequisite for further industrial implementations[2,3]. In this work, we assessed the stability wild-type HheC and two mutants with enhanced kinetic properties. First, freeze-thaw cycle testing was performed via activity measurements, whereby HheC proved to be very stable, facilitating protein handling procedures. The kinetic stability of different biocatalyst forms (whole cells, cell-free extract, and purified enzyme) in buffer was determined through activity tests, which led us to the conclusion that the cell wall and other proteins in the extract protect the enzyme and keep it active for longer period. Size distribution of purified protein was also investigated by dynamic light scattering methods to establish existence of larger protein particles formed due structural changes and aggregation. The stability of mutants during incubation with substrates and unnatural nucleophiles (azides, cyanides) was monitored to determine whether the components of the reaction mixture have an effect on enzyme activity. It was found that at higher concentrations of fluorinated styrene oxide derivatives, the enzyme loses activity faster, which was later reflected in the results of enzyme operational stability decay in the batch reactor. The co-dependency of operational stability decay rate constant on substrate initial concentration (Figure 1) was mathematically described by Eq 1. Understanding and quantifying the influence of compounds concentrations on enzyme behavior is of a great importance for larger scale processes[3]. Presented results give a better insight into the limitations of HheC application and help with biocatalysts handling for prolonging its longevity in reactors.

$$k_d = \frac{c_{0,\text{epoxide}} \cdot a}{c_{0,\text{epoxide}} + b} \qquad \text{(Eq 1)}$$

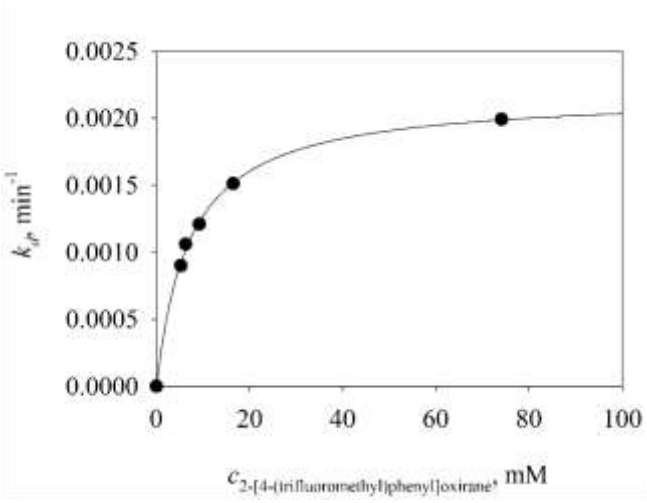

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Figure 1 – Dependence of HheC-W249P deactivation constant on rac-2-[4-(trifluoromethyl)phenyl]oxirane initial concentration.