Motivation

FAD-dependent halogenases catalyze the regioselective halogenation of organic compounds under benign reaction conditions at room temperature and nearly pH 7 thereby merely requiring a halide salt and molecular oxygen. Their convenient handling is an enormous advantage to classical halogenation reagents that usually require hazardous reaction conditions. Biohalogenation is of particular interest for organic synthesis as it enables the preparative scale synthesis of reactive halogenated compounds with remarkable regioselectivity. Even though it was demonstrated that FAD-dependent tryptophan halogenases such as RebH are able to halogenate unnatural substrates and override directing electronic effects\textsuperscript{[33]}, the enzyme's limited substrate scope as well as its low activity and instability are factors that impede further applications in industrial processes. Therefore, the desired objective for this thesis is the modification and optimization of the tryptophan 6-halogenase Thal towards an improved thermostability, in order to obtain a catalyst with increased lifetime and activity at elevated temperatures. As previous results regarding an improved thermostability of RebH were achieved by several rounds of directed evolution\textsuperscript{[42]}, random mutagenesis of Thal in combination with a robust high-throughput fluorescence screening is considered as promising approach.

Apart from the well-established tryptophan halogenases a marine brominase, namely Bmp5 from \textit{Pseudoalteromonas luteoviolacea}, additionally turns into focus of this thesis. As Bmp5 is reported to catalyze the incorporation of bromide as well as iodide, it is of special interest for synthetic purposes, due to the fact that tryptophan halogenases are unable to accept iodide as substrate. After optimization of enzyme expression as well as examinations on purification strategies, Bmp5-catalyzed halogenations are planned to be performed on analytical as well as preparative scale. Here, the enzyme's applicability with immobilization methodologies such as combiCLEAs could be an interesting approach. Additionally, substrate screening revealing unnatural accepted compounds would be an important feature towards Bmp5's application for synthetic purposes. Finally, the establishment of a robust cofactor regeneration system, enabling continuous supply of necessary cofactors is another goal.
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