BIOTRANS
LA ROCHELLE 2023

N°1121 / PC
TOPIC(s) : Enzyme discovery and engineering

Disulfide engineering of tryptophan halogenases

AUTHORS
Caroline BESSE / BIELEFELD UNIVERSITY, UNIVERSITÄTSSTRASSE 25, BIELEFELD
Norbert SEWALD / BIELEFELD UNIVERSITY, UNIVERSITÄTSSTRASSE 25, BIELEFELD

PURPOSE OF THE ABSTRACT

Flavin-dependent halogenases allow halogenation of electron-rich aromatic compounds under mild reaction conditions. Unlike chemical halogenation, flavin-dependent halogenases are highly regioselective even at electronically unfavoured positions. Flavin-dependent halogenases comprise several tryptophan halogenases that can halogenate positions 5, 6 or 7 of tryptophan. The resulting haloarenes are of interest to the pharmaceutical and agricultural industries and are also suitable for further functionalization, such as cross-coupling reactions. Although enzymatic halogenation was shown to provide gram amounts of halogenated tryptophan [1], improvement of the enzyme performance is still desirable.

Previous reports showed that several tryptophan halogenases, like PyrH, RebH and PrnA tend to form dimers in solution and in crystal structure. The tryptophan-6-halogenase Thal, while forming dimers in all published crystal structures, was found to exist as a monomer in solution. ESI-MS analysis of Thal and thermostable Thal variants have shown that the thermostable Thal variants have a higher affinity for homodimer formation than the wild type.[2]

Inspired by these results, the influence of dimerization on thermostability and activity was investigated using disulfide engineering. To generate a covalently bridged Thal dimer, two amino acids at the prospective dimer interface were mutated to cysteines to enable formation of disulfide bridges. Using size exclusion chromatography, SDS gel electrophoresis and mass spectrometric analysis under non-reducing conditions, it was demonstrated that the generated Thal variant exists as a disulfide-bridged dimer in solution. While the variant shows a significantly increased T50 value compared to the wildtype under non-reduced conditions, the T50 value under reduced conditions is decreased compared to the wild type. So, it could be shown that the dimerization is the driving force for the increased thermostability. The activity was not affected by the mutations and dimerization.

To investigate whether a similar effect can be achieved with other halogenases, homologous mutations were introduced into the tryptophan-5-halogenase PyrH. Since these resulted in significantly increased thermostability, further mutants were generated that form covalent dimers and exhibit increased thermostability compared to the PyrH wildtype. The generated variants are a good starting point for further enzyme engineering campaigns to increase activity or expand the substrate spectrum.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Disulfide Engineering | Halogenase

BIBLIOGRAPHY

[1] M. Frese; N. Sewald. Angew. Chem. Int. Ed. 2015, 54, 298-301.

[2] H. Minges; C. Schnepel; D. B□ttcher; M. S. Wei□; J. Spro□; U. T. Bornscheuer; N. Sewald, ChemCatChem 2020, 12, 818.