ACTIVITY AND STABILITY IMPROVEMENT OF CYCLOHEXANONE MONOOXYGENASE BY PROTEIN ENGINEERING

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INTRODUCTION

Among the great number of flavin-dependent monooxygenases, Baeyer–Villiger monooxygenases (BVMOs) have been studied most for their application as a biocatalyst. These interesting biocatalysts are capable of oxidizing carbonyl atoms into the corresponding esters or lactones[1] and performing heteroatom oxidation reactions, like sulfoxidations. BVMOs usually exhibit high chemo-, regio- and/or enantioselectivity while converting a wide variety of substrates. The prototype BVMO, cyclohexanone monooxygenase (CHMO) is a promising biocatalyst for industrial reactions owing to its broad substrate spectrum and the excellent stereoselectivity. However, the low stability of BVMOs in general and especially for CHMO is an obstacle for their exploitation in industry. [2,3] This unmet need is the main objective of this study.

EXPERIMENTS

Cyclohexanone monooxygenase (CHMO) originating from *Acinetobacter* NCIB 9871 as a prototype BVMO was chosen for this study. To improve the thermostability of $CHMO_{Acinteo}$, we have used a data-driven protein design method that requires fewer homologous sequences than the traditional consensus approach and utilizes structural information to limit the number of variants created. [4] After choosing the residues for mutagenesis, site-directed mutagenesis method has been performed to implement the mutations. The library of mutants has been examined for their characteristics, especially the thermal stability, melting temperature, and activity. After creating the first library with single point mutations, the best variants were determined. To boost the effect of the mutation, the best single point mutations were combined and a new series of mutants created which contained more than one mutation. We also did implement the literature known mutation in our new library. The overview of the experimental part can be seen in figure 1.

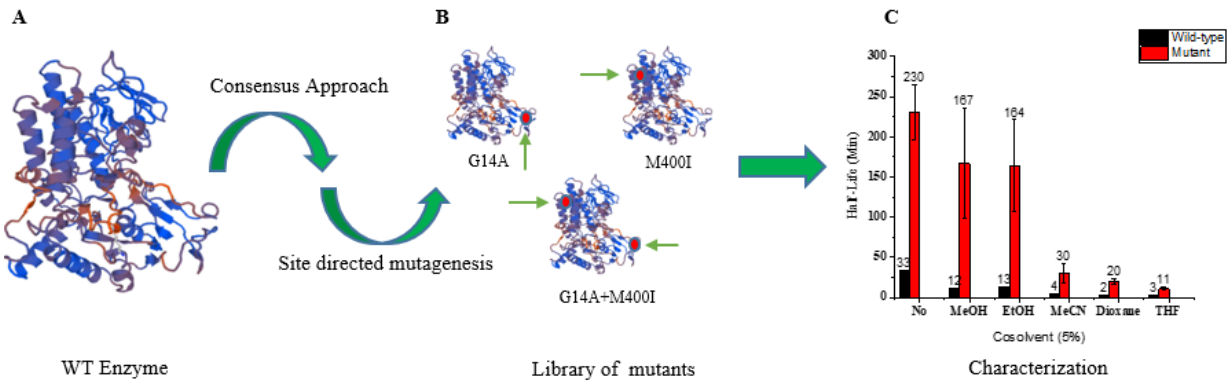

Figure 1. Overview of the study. A; the enzyme of interest was chosen. B; the predicted mutations based on the consensus approach have been implemented in the enzyme sequence, the variants with the combination of mutations created. C; the enzyme characteristic like organic solvent tolerance, half-life time and activity have been determined.

RESULTS AND DISCUSSION

The combination of single point mutations gave rise to a library of 14 mutants. Then, activity, melting temperature, and thermostability for all mutants have been evaluated. We have found several single point mutants with increased activity and thermodynamic stability. After the combination of improved variants, we ended up with a mutant (S2), composed of 7 single point mutations, which showed about 40% higher activity, 2-fold enhanced thermostability and 2°C increase in the melting temperature than the wild-type. S2 as the best variant was used for another round of mutation. The literature known mutations were combined with S2 and lead us to 6 new variants. These new variants examined for their characteristic. There was an increase in melting temperature and thermal stability in all mutants. The new mutants showed either the same or even higher activity in comparison to wildtype. S8 showed the highest improvement in the case of melting temperature by 5 °C and S7 showed the highest thermal stability by more than 8 fold improvement.

CONCLUSION

We successfully prepared a semi-rational library of CHMO mutants. The improvement in activity, thermostability and also melting temperature achieved with the used method. We can conclude that the consensus approach would be an efficient method to predict the mutations and can be used for further studies in the family of BVMOs. This study also can help us to determine the residues which can be important for the stability of this class of enzyme.

REFERENCES

[1] H. Leisch, K. Morley, P. C. K. Lau. Chemical Reviews, 4165-4222, 2011.

[2] U. Bornscheuer, G. Huisman, R. Kazlauskas, S. Lutz, J. Moore, K. Robins. Nature, 185– 194, 2012.

[3] L. Goncalves, D. Kracher, S. Milker, F. Rudroff, M. J. Fink, R. Ludwig, A. Bommarius, M. Mihovilovic. Advanced Synthesis & Catalysis, 2121-2131, 2017.

[4] K. M. Polizzi, J. F. Chaparro-Riggers, E. Vazquez-Figueroa,A.S. Bommarius. Biotechnology Journal , 531-536, 2006.