Retracing the evolutionary emergence of stereoselectivity in borneol-type dehydrogenases

AUTHORS

Jasmin ZUSON / INSTITUTE OF MOLECULAR BIOTECHNOLOGY, TU GRAZ, PETERSGASSE 14, GRAZ

Andrea M. CHÁNIQUE / INSTITUTE FOR BIOLOGICAL AND MEDICAL ENGINEERING, PONTIFICAL CATHOLIC UNIVERSITY OF CHILE, VICUÑAMACKENNA 4860, SANTIAGO

Katarína KAVČIAKOVÁ / INSTITUTE OF MOLECULAR BIOTECHNOLOGY, TU GRAZ, PETERSGASSE 14, GRAZ

Bernhard LOLL / FREIE UNIVERSITÄT BERLIN, INSTITUTE FOR CHEMISTRY AND BIOCHEMISTRY, LABORATORY OF STRUCTURAL BIOCHEMISTRY, TAKUSTRASSE 3, BERLIN

Daniel KRACHER / INSTITUTE OF MOLECULAR BIOTECHNOLOGY, TU GRAZ, PETERSGASSE 14, GRAZ

Robert KOURIST / INSTITUTE OF MOLECULAR BIOTECHNOLOGY, TU GRAZ, PETERSGASSE 14, GRAZ

PURPOSE OF THE ABSTRACT

The exceptional stereoselectivity of enzymes is a key feature for industrial applications, and elucidating the structural basis thereof is a critical step toward their optimization. Even though directed evolution and rational design are successful methods for the improvement of stereoselectivity [1], it remains unclear how Nature evolved stereoselectivity within enzyme classes. Enzymes within the family of plant borneol dehydrogenases (BDHs), although closely related, display striking differences in enantiospecificity towards borneol enantiomers [2, 3]. In this context, ancestral sequence reconstruction (ASR) has emerged as a powerful approach to infer the evolutionary history of BDH enzymes and identify key mutations contributing to their stepwise diversification. Following Nature's footsteps, we conducted a comprehensive analysis of the effect of the active site and peripheral mutations on BDH selectivity, toward rationalizing natural enzyme evolution. To this end, ASR was employed to create common ancestors of selective and non-selective dehydrogenases in order to retrace the emergence of selectivity in BDHs via a plausible natural evolutionary pathway to the enantiospecific SrBDH1 [2] (Figure 1). Ancestral enzymes were recombinantly produced, biochemically characterized, and their structures predicted. Consecutively, guided by ASR and de-novo protein structure prediction, selected residues were substituted to further deepen the insights into the structure-function relation in BDHs. This work elucidates the importance of the structural and evolutionary changes in enzymes and exemplifies how the application of computationally-guided enzyme engineering tools and structural analysis leads to a deeper understanding of natural evolutionary principles in less investigated enzyme classes.
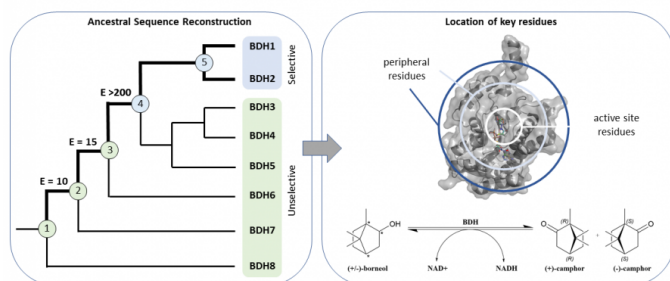
FIGURES

FIGURE 1

Ancestral sequence reconstruction as guide for enzyme engineering.

Ancestral sequence reconstruction is thereby utilized to identify peripheral and active site mutations in BDH ancestors leading to changes in enantioselectivity.

FIGURE 2

KEYWORDS

Ancestral Sequence Reconstruction | borneol-type dehydrogenases | camphor | Enantioselectivity

---

BIBLIOGRAPHY

[1] u.t. bornscheuer, g.w. huisman, r.j. kazlauskas, s. lutz, j.c. moore, k. robins, nature. 2012, 485, 185-194.

[2] a.m. chnique, n. dimos, i. drienovsk, e. calderini, m.p. pantn, c.p.o. helmer, m. hofer, v. sieber, l.p. parra, b. loll, r. kourist, chemcatchem. 2021, 13, 2262-2277.

[3] r. croteau, c.l. hooper, m. felton, arch. biochem. 2012, 188, 182-193.