



HAL
open science

Tailoring chemoenzymatic oxidation via in situ peracids

Rebecca N Re, Johanna C Proessdorf, James J La Clair, Maeva Subileau,
Michael D Burkart

► To cite this version:

Rebecca N Re, Johanna C Proessdorf, James J La Clair, Maeva Subileau, Michael D Burkart. Tailoring chemoenzymatic oxidation via in situ peracids. ACS NATIONAL MEETING: FALL 2019 SAN DIEGO, Aug 2019, San Diego, United States. ACS NATIONAL MEETING: FALL 2019 SAN DIEGO, <https://scimeetings.acs.org/search?query=&eventName=ACS+Fall+2019&sort=Recently+Added&start=1>. hal-04771150

HAL Id: hal-04771150

<https://hal.science/hal-04771150v1>

Submitted on 7 Nov 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Tailoring chemoenzymatic oxidation via *in situ* peracids

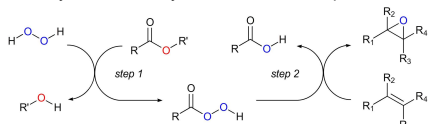


Rebecca N. Re,¹ Johanna C. Proessdorf,¹ James J. La Clair,¹ Maeva Subileau,^{1,2} and Michael D. Burkart¹

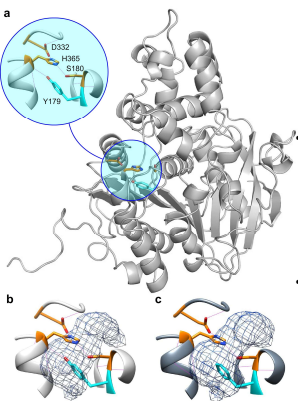
1. University of California, San Diego, Department of Chemistry and Biochemistry; 2. UMR IATE, Montpellier SupAgro, INRA, Université Montpellier, CIRAD

Introduction

- Epoxides are often present in commodity chemicals and pharmaceuticals and often act as chemical intermediates since they are easily transformed into other functional groups.
- While limited by the harsh conditions needed to install epoxides, recent attention has turned to using enzymatic and chemoenzymatic methods to incorporate them.
- Lipases have been used as tools to prepare peracids *in situ* by intercepting conventional hydrolysis with H₂O₂ to translate their activity to the epoxidation of olefins.¹
- Current chemoenzymatic methods are often implemented in harsh conditions with high H₂O₂ concentrations in toluene in order to displace the reaction equilibrium towards perhydrolysis rather than hydrolysis.²
- Lipases/acyltransferases with higher selectivity for H₂O₂ over H₂O as the nucleophilic acceptor can be used to increase the efficiency of chemoenzymatic oxidation in aqueous medium.



Methods

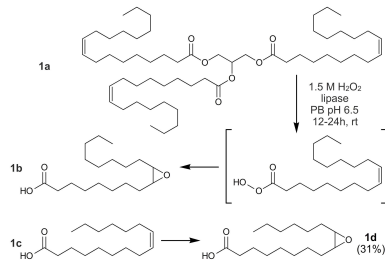


- The enzyme used in this study, CpLIP2 from *Candida parapsilosis*, displays preferences for nucleophiles other than H₂O such as alcohols, H₂O₂ and amines.³
- CpLIP2 Y179F is used due to its potential to catalyze perhydrolysis over hydrolysis more efficiently than the wt enzyme.⁴
- Y179F mutant is also known to enhance the enzyme stability by increasing its resistance to alcohol.⁵
- Addition of an extra ester or acid as a co-catalyst was found to increase conversion of olefin to epoxide with H₂O₂ in this two-phase enzymatic system.

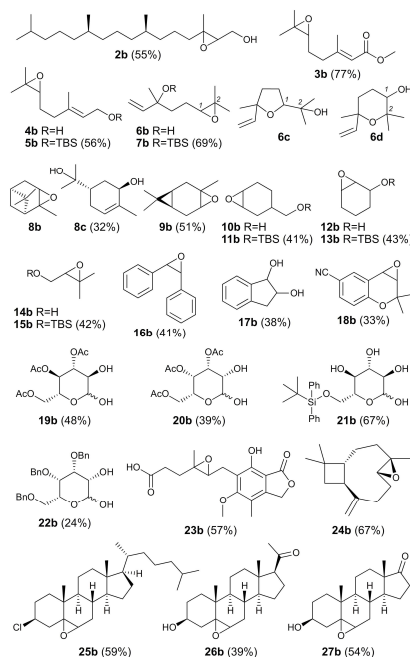
Above: a) Structure of CpLIP2 with active site highlighted. b) Close up of wtCpLIP2 active site pocket. c) Close up of CpLIP2 Y179F active site pocket.

Results

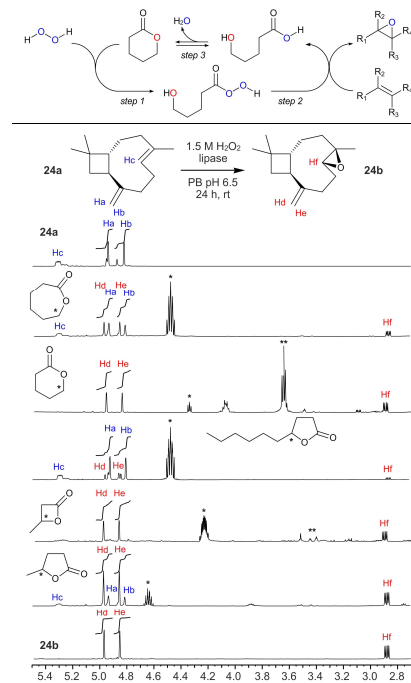
- With CpLIP2 showing particular efficiency for unsaturated fatty acid monoesters and triglycerides, auto-epoxidation of triolein was initially screened with an optimized concentration of H₂O₂ (4.6% v:v) and CpLIP2 Y179F without inhibiting enzyme activity at pH 6.5 over 24h at rt.



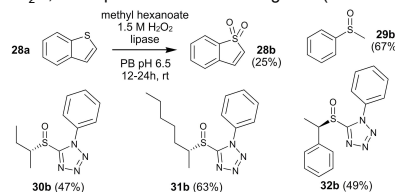
Above: Oxidation of triolein (**1a**) undergoes release of corresponding peroleic acid (brackets) towards epoxyoleic acid (**1b**) as one component of a complex product mixture. Auto-epoxidation of palmitoleic acid (**1c**) under the same conditions yielded epoxyalmitoleic acid (**1d**).



Below: NMR analyses of the relative efficacy of lactones as peracid surrogates, showing the recycling of acid to lactone as a means to reduce the co-catalyst requirement to 300 mM.



- Addition of a co-catalytic ester, methyl hexanoate, was found to increase conversion of olefin to epoxide with epoxyalmitoleic acid (**1d**) formed in 71% yield.
- The reaction was screened on a variety of olefins and sulfides using 100mM substrate, 1.5 M H₂O₂, 500mM methyl hexanoate, 100 mM phosphate buffer at pH 6.5, H₂O, and CpLIP2 Y179F at 0.4 mg mL⁻¹ (0.008 mM).



Left and above: Epoxide products **2b-27b** and rearranged epoxides **6c, 6d**, and **8c** were prepared by oxidation of alkenes **2a-27a**. Sulfone **28b** and sulfoxides **29b-32b** from sulfides **28a-32a** were also prepared using this chemoenzymatic method.

Conclusions

- This mild reaction can be used to epoxidize olefins and glycols as well as oxidize sulfides to their respective sulfoxides.
- The oxidation method uses low enzymatic loading (0.4 mg mL⁻¹) in aqueous medium with low concentrations of H₂O₂ (< 5% v:v).
- It presents a greener and cost-effective advantage to utilize this reaction since the reaction is conducted without organic solvent.
- The simple preparation of peracids from ester and lactone co-catalysts *in situ* offers an effective route to peracids not easily obtained.
- Mutagenesis of CpLIP2 proves to be a viable approach for the rational engineering of the substrate pocket.
- Preliminary results suggest that mutations can change the selectivity toward the co-ester and the subsequent final epoxidation yield.

Screen optimized conditions on a variety of olefins and sulfides

Identify enzyme mutant that efficiently catalyzes chemoenzymatic oxidation

Determine best co-catalyst oxidant for each substrate and engineered enzyme

Acknowledgements

We would like to thank the Training Program in Marine Biotechnology (NIH T32 GM067550) for their support, as well as the Montpellier SupAgro scientific council (International Mobility Fellowship) and US Department of Energy grant EE0008246 for their funding of this work.



NIGMS



References

- F. Björkling, S.E. Godtfredsen and O. Kirk, *J. Chem. Soc., Chem. Commun.*, 1990, 1301
- U. Törnvall, C. Orellana Coca Akerman and R. Hatti-Kaul, D. Adlercreutz, *Enz. Microb. Technol.*, 2007, **40**, 447
- P. Saitthai, J. Lecomte, E. Dubreucq and V. Tanrattanakul, *eXPRESS Polymer Lett.*, 2013, **7**, 910.
- A. H. Jan, A. H. Deniau, M. Subileau and E. Dubreucq, *Chembiochem*, 2018, **19**, 1839
- M. Subileau, A.-H. Jan, H. Nozac'h, M. Pérez-Gordo, V. Perrier and E. Dubreucq, *Biochim. Biophys. Acta, Proteins Proteomics*, 2015, **1854**, 1400