Tetrahedron Letters 56 (2015) 3435–3437

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

A diastereoselective P450-catalyzed epoxidation reaction: *anti* versus *syn* reactivity

Adriana Ilie, Richard Lonsdale, Rubén Agudo, Manfred T. Reetz *

Department of Chemistry, Philipps-Universität Marburg, Hans-Meerwein Str., 35032 Marburg, Germany
Max-Planck-Institut für Kohlenforschung, Kaiser-Wilhelm-Platz 1, 45470 Mülheim an der Ruhr, Germany

ARTICLE INFO

Article history:
Received 4 December 2014
Revised 10 March 2015
Accepted 18 March 2015
Available online 21 March 2015

This Letter is dedicated to the memory of Harry Wasserman

Keywords:
Biocatalysis
P450 monooxygenase
Olefin epoxidation
Diastereoselectivity
Oxidation

ABSTRACT

The achiral cyclohexene derivative dimethyl *cis*-1,2,3,6-tetrahydrophthalate has been subjected to oxidation catalyzed by cytochrome P450 monooxygenase P450-BM3, leading to diastereoselective epoxidation rather than oxidative hydroxylation. This reaction occurs with 94% diastereoselectivity in favor of the *anti*-epoxide, in contrast to *m*-CPBA which delivers unselectively a 70:30 mixture of *anti*/*syn* diastereomers. The experimental results are nicely explained on a molecular level by docking experiments and molecular dynamics computations.

Cytochrome P450 monooxygenases (CYPs) have been used for a long time in order to perform regio- and stereoselective oxidative hydroxylation of organic compounds (RH → ROH), molecular oxygen O_2 serving as the oxidant under mild conditions.[1] The mechanism involves abstraction of a hydrogen atom by a catalytically active high-spin heme-Fe=O intermediate (so-called Compound I; Scheme 1) with formation of the respective R-radical followed by rapid C—O bond formation. Whenever wild-type (WT) CYPs are not regio- or stereoselective in the hydroxylation of a given substrate, protein engineering using rational design[2] or directed evolution[3] can be applied in order to manage the synthetic problem. These protein engineering techniques provide (bio)catalysts which are generally complementary to synthetic reagents or catalysts.[4]

Compounds containing olefinic double bonds are often hydroxylated at the allylic positions by CYP-catalysis, but they may also undergo epoxidation.[1,5] The reasons for the preference of one reaction type versus the other are not fully understood, but the specific pose of a given substrate in the CYP binding pocket is crucial in determining its oxidative 'fate'. The mechanism of epoxidation of alkenes by CYPs has not been studied as intensively as oxidative hydroxylation. A hydroperoxo–Fe-heme complex has been postulated in some cases, but Compound I is generally believed to be the catalytically active species.[5,6] A concerted process is generally favored based on stereochemical results, although in rare cases a two-step radical mechanism may be involved. Irrespective of which mechanism is preferred, the stereochemistry of epoxide formation will be determined by the face of the double bond that is placed closest to the heme species.

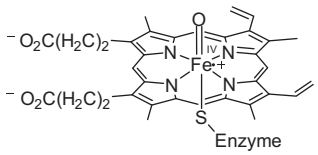
When prochiral olefins are epoxidized, enantioselectivity is relevant, while in the case of chiral olefins, the control of diastereoselectivity constitutes the challenge (in addition to the regioselectivity problem). A special example is the P450cam-catalyzed epoxidation of chiral 5,6-dehydrocamphor which results in the selective formation of 5,6-*exo*-epoxycamphor.[5b]

In the present study we report a different type of diastereoselectivity, namely *syn*/*anti*-selectivity in the epoxidation of an achiral substituted cyclohexene derivative. In this system it was possible to explain the origin of diastereoselectivity on a molecular level using docking and molecular dynamics (MD) computations. We employed P450-BM3 as the catalyst, a well-known self-sufficient CYP from *Bacillus megaterium*[1,7] which has been used very often in oxidative hydroxylation, but less so in epoxidation.[1,5]

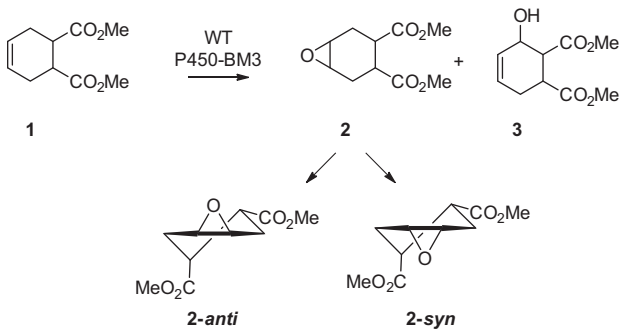
Commercially available dimethyl *cis*-1,2,3,6-tetrahydrophthalate (**1**) was chosen as the substrate (Scheme 2), which can be prepared by Diels–Alder reaction of butadiene and dimethyl maleate.[8] WT P450-BM3 proved to be 78% regioselective in favor of

* Corresponding author. Tel.: +49 (0)6421 28 25500; fax: +49 (0)6421 28 25620.
E-mail address: reetz@mpi-muelheim.mpg.de (M.T. Reetz).

http://dx.doi.org/10.1016/j.tetlet.2015.03.076
0040-4039/



Scheme 1. Compound I [Ferryl (Fe^{IV})-oxo-π porphyrin cation radical].



Scheme 2. Oxidation products resulting from WT P450-BM3 catalyzed oxidation of substrate **1**.[10]

epoxidation leading to **2**, while allylic hydroxylation with formation of **3** in essentially racemic form occurred to a smaller extent (22%) as revealed by GC analysis. Oxidative demethylation was not observed. Importantly, 94% diastereoselectivity favoring the *anti*-configuration **2-*anti*** was observed (Fig. 1, top). This compound constitutes an intermediate in the synthesis of effective inhibitors of glycosidases.[9] It was prepared as a mixture of *anti*/*syn*-diastereomers by *m*-CPBA-mediated epoxidation, which had to be separated by column chromatography.[9] Upon repeating this reaction, we indeed obtained a 70:30 mixture of **2-*anti*** and **2-*syn***, respectively (Fig. 1, bottom).

Why does compound **1** undergo epoxidation to a greater extent than oxidative hydroxylation, and why is *anti*-selectivity the preferred mode of attack? In order to address these questions, induced fit docking and MD simulations were performed. Guided by the crystal structure of P450-BM3 (PDB code 1JPZ),[11] substrate **1** was positioned in the active site using induced fit docking.[12] The pose which places the substrate closest to the heme has both the double bond and one of the allylic hydrogen atoms of **1** close to the heme (Fig. 2). In a previous QM/MM study of epoxidation and hydroxylation of alkenes by P450cam, a preference for epoxidation over allylic hydroxylation was observed when the two oxidation sites were equally accessible to Compound I.[13] If epoxidation is intrinsically preferred over hydroxylation, as is expected given that no hydroxylated product is observed upon reaction of substrate **1** with *m*-CPBA, this would explain the observation that the epoxide is the major product for the enzyme catalyzed reaction. A docking pose was observed in which the methyl group of one of the ester substituents is close to the heme. As no products are observed in which either of the two methyl groups undergo hydroxylation, this is not consistent with the observed reactivity. It is possible that hydrogen abstraction is not energetically feasible from this position, as it would lead to the formation of a primary radical intermediate.

Now we turn our attention to the stereoselectivity of the epoxide formation. As already pointed out, the species responsible for oxidation of alkenes by P450s is widely accepted to be Compound I.[1,5] When the Compd I oxygen atom is added to the model, the docking pose is consistent with the formation of **2-*anti***. No docking poses were observed in which oxidation would occur on the opposite side of the double bond, hence providing an explanation for the relatively low observed amount of **2-*syn***. To explore the conformational flexibility of this enzyme/substrate complex, two unrestrained MD simulations were performed starting from the above mentioned docking pose.[14] The substrate remains close to the observed docking pose during both of the two 26 ns

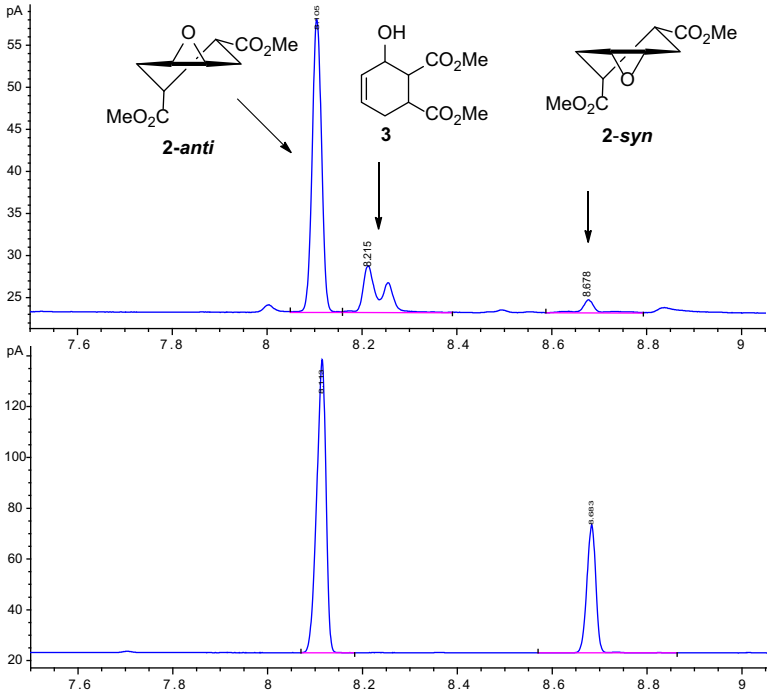


Figure 1. GC chromatograms of oxidation products using **1** as starting material. Top: oxidation products in the reaction catalyzed by WT P450-BM3 using resting cells; bottom: oxidation products in the transformation performed by chemical reaction using *m*-CPBA as oxidant. GC conditions: 30 m DB1, inner diameter 0.25 mm; pressure: 2 bar H_2; injector: 230 °C; oven: temperature gradient: from 50 to 300 °C with 12 °C/min FID detector: 350 °C. The peak assigned to substrate **1** was detected at retention time 6.81.

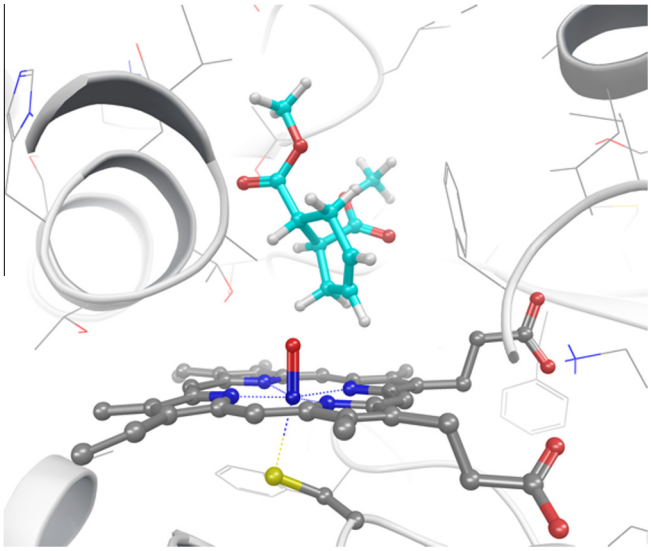


Figure 2. Induced fit docking pose of substrate **1** in the active site of WT P450-BM3. The docking pose places the substrate in an orientation in which oxidation will result in formation of **2-*anti***.

simulations. The active site of WT P450-BM3 is hydrophobic and, while no hydrogen bonding interactions are observed between the substrate and active site, the shape of the active site clearly influences the orientation in which the double bond can approach Compd I, to the extent that oxidation can only occur on one face of the double bond.

In summary, we have discovered that P450-BM3 is an efficient catalyst for the regio- and stereoselective epoxidation of the achiral cyclohexene derivative dimethyl *cis*-1,2,3,6-tetrahydrophthalate (**1**) with preferential formation of the *anti*-configured epoxide **2-*anti***. The observed *anti*-diastereoselectivity was explained on a molecular level by docking experiments and MD simulations, resulting in a reasonable model. The product is a useful intermediate in the synthesis of inhibitors of certain glycosidases.[9]

Acknowledgments

Financial support by the Max-Planck-Gesellschaft and the Arthur C. Cope Foundation is gratefully acknowledged.

References and notes

1. Recent reviews of CYPs: (a) Ortiz de Montellano, P. R. *Cytochrome P450: Structure, Mechanism, and Biochemistry*, 3rd ed.; Springer: Berlin, 2005; (b) Isin, E. M.; Guengerich, F. P. *Biochim. Biophys. Acta Gen. Subj.* **2007**, *1770*, 314–329; (c) Munro, A. W.; Girvan, H. M.; McLean, K. J. *Nat. Prod. Rep.* **2007**, *24*, 585–609; (d) Ortiz de Montellano, P. R. *Chem. Rev.* **2010**, *110*, 932–948; (e) Shaik, S.; Cohen, S.; Wang, Y.; Chen, H.; Kumar, D.; Thiel, W. *Chem. Rev.* **2010**, *110*, 949–1017; (f) Whitehouse, C. J. C.; Bell, S. G.; Wong, L.-L. *Chem. Soc. Rev.* **2012**, *41*, 1218–1260; (g) Lewis, J. C.; Coelho, P. S.; Arnold, F. H. *Chem. Soc. Rev.* **2011**, *40*, 2003–2021; (h) Holtmann, D.; Fraaije, M. W.; Arends, I. W. C. E.; Opperman, D. J.; Hollmann, F. *Chem. Commun.* **2014**, 13180–13200; (i) Bernhardt, R.; Urlacher, V. B. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 6185–6203.
2. Recent examples of rational design of P450 monooxygenases: (a) Sowden, R. J.; Yasmin, S.; Rees, N. H.; Bell, S. G.; Wong, L.-L. *Org. Biomol. Chem.* **2005**, *3*, 57–64; (b) Seifert, A.; Antonovici, M.; Hauer, B.; Pleiss, J. *ChemBioChem* **2011**, *12*, 1346–1351; (c) Nguyen, K. T.; Virus, C.; Günnewich, N.; Hannemann, F.; Bernhardt, R. *ChemBioChem* **2012**, *13*, 1161–1166; (d) Venkataraman, H.; de Beer, S. B. A.; van Bergen, L. A. H.; van Essen, N.; Geerke, D. P.; Vermeulen, N. P. E.; Commandeur, J. N. M. *ChemBioChem* **2012**, *13*, 520–523.
3. Review of directed evolution of P450 monooxygenases: Roiban, G.-D.; Reetz, M. T. *Chem. Commun.* **2015**. http://dx.doi.org/10.1039/c4cc09218j.
4. (a) Reetz, M. T. *J. Am. Chem. Soc.* **2013**, *135*, 12480–12496; (b) Nestl, B. M.; Hammer, S. C.; Nebel, B. A.; Hauer, B. *Angew. Chem., Int. Ed.* **2014**, *53*, 3070–3095; (c) Clouthier, C. M.; Pelletier, J. N. *Chem. Soc. Rev.* **2012**, *41*, 1585–1605.
5. Examples of P450-catalyzed olefin epoxidation: (a) Vaz, A. D. N.; McGinnity, D. F.; Coon, M. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3555–3560; (b) Gelb, M. H.; Malkonen, P.; Sligar, S. G. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 853–858; (c) Kolev, J. N.; Zaengle, J. M.; Ravikumar, R.; Fasan, R. *ChemBioChem* **2014**, *15*, 1001–1010; (d) Jin, S.; Makris, T. M.; Bryson, T. A.; Sligar, S. G.; Dawson, J. H. *J. Am. Chem. Soc.* **2003**, *125*, 3406–3407; (e) Li, A.; Wu, S.; Adams, J. P.; Snajdrova, R.; Li, Z. *Chem. Commun.* **2014**, 8771–8774; (f) Farinas, E. T.; Alcalde, M.; Arnold, F. *Tetrahedron* **2004**, *60*, 525–528; (g) Miller, V. P.; Freutel, J. A.; Ortiz de Montellano, P. R. *Arch. Biochem. Biophys.* **1992**, *298*, 697–702; (h) Roiban, G.-D.; Agudo, R.; Reetz, M. T. *Tetrahedron* **2013**, *69*, 5306–5311.
6. Meunier, B.; de Visser, S. P.; Shaik, S. *Chem. Rev.* **2004**, *104*, 3947–3980.
7. (a) Narhi, L. O.; Fulco, A. J. *J. Biol. Chem.* **1986**, *261*, 7160–7169; (b) Munro, A. W.; Leys, D. J.; McLean, K. J.; Marshall, K. R.; Ost, T. W. B.; Daff, S.; Miles, C. S.; Chapman, S. K.; Lysek, D. A.; Moser, C. C.; Page, C. C.; Dutton, P. L. *Trends BioChem. Sci.* **2002**, *27*, 250–257; (c) Clodfelter, K. H.; Waxman, D. J.; Vajda, S. *Biochemistry* **2006**, *45*, 9393–9407.
8. Petrow, S. *Sb. Statei Obshch. Khim.* **1953**, 853–855.
9. Brazdova, B.; Tan, N. S.; Samoshina, N. M.; Samoshin, V. V. *Carbohydr. Res.* **2009**, *344*, 311–321.
10. *Experimental procedure for the P450-BM3 catalyzed epoxidation of substrate* **1**: An Erlenmeyer flask (100 mL) containing LB (20 mL) and kan (50 μg/mL) was inoculated with a colony of WT P450 and incubated 6 h at 37 °C with gentle shaking. The pre-culture was further inoculated into TB (400 mL) containing kan (50 μg/mL) and allowed to grow at 37 °C until O.D. of 0.8–0.9 at 600 nm was reached. IPTG was added to a final concentration of 0.2 mM and the culture grown at 30 °C during 16 h with gentle agitation. Cells were pelleted by centrifugation (15 min, 5000 r.p.m. at 4 °C). The supernatant was discarded and the pellet was resuspended in 50 mL of reaction buffer [phosphate buffer (pH 7.4, 100 mM), NADP+ (300 μM) and glucose (100 mM)]. Resuspended cells were transferred to a 250 mL Erlenmeyer flask, and starting material (200 μL, 1.16 mmol) was added. Reaction was carried out at 25 °C for 20 h with mild agitation. The reaction mixture was extracted with ethyl acetate (400 mL) and the resulting colorless oil was loaded on chromatographic column (EA/PE 1:1). Spectroscopic data of the *anti* stereoisomer are in agreement with published data.[9] yield 23% (58 mg) *anti*-**2**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.20–1.96 (m, 4H), 2.76 (t, ^3J = 6.2 Hz, 2H), 3.07 (s, 2H), 3.53 (d, ^3J = 2.6 Hz, 6H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.19, 51.69, 51.23, 37.39, 24.55. HRMS (EI) calcd for $C_{10}H_{14}O_5$ [*M*]$^+$: 214.0834; found: 214.0893.
11. Haines, D. C.; Tomchick, D. R.; Machius, M.; Peterson, J. A. *Biochemistry* **2001**, *40*, 13456–13465.
12. (a) *Schrödinger Suite 2014-2 Induced Fit Docking protocol; Glide version 6.3*; Schrödinger, LLC: New York, NY, 2013. Prime version 3.6, Schrödinger, LLC, New York, NY, 2014; (b) Farid, R.; Day, T.; Friesner, R. A.; Pearlstein, R. A. *Bioorg. Med. Chem.* **2006**, *14*, 3160–3173; (c) Sherman, W.; Day, T.; Jacobson, M. P.; Friesner, R. A.; Farid, R. *J. Med. Chem.* **2006**, *49*, 534–553; (d) Sherman, W.; Beard, H. S.; Farid, R. *Chem. Biol. Drug Des.* **2006**, *67*, 83–84.
13. (a) Lonsdale, R.; Harvey, J. N.; Mulholland, A. J. *J. Phys. Chem. Lett.* **2010**, *1*, 3232–3237; (b) Lonsdale, R.; Harvey, J. N.; Mulholland, A. J. *J. Phys. Chem. B* **2010**, *114*, 1156–1162.
14. MD simulations were performed using AMBER14,[15] using the same settings and minimization/equilibration protocol outlined in Ref. 16.
15. Case, D. A.; Babin, V.; Berryman, J. T.; Betz, R. M.; Cai, Q.; Cerutti, D. S.; Cheatham, T. E., III; Darden, T. A.; Duke, R. E.; Gohlke, H.; Goetz, A. W.; Gusarov, S.; Homeyer, N.; Janowski, P.; Kaus, J.; Kolossváry, I.; Kovalenko, A.; Lee, T. S.; LeGrand, S.; Luchko, T.; Luo, R.; Madej, B.; Merz, K. M.; Paesani, F.; Roe, D. R.; Roitberg, A.; Sagui, C.; Salomon-Ferrer, R.; Seabra, G.; Simmerling, C. L.; Smith, W.; Swails, J.; Walker, R. C.; Wang, J.; Wolf, R. M.; Wu, X.; Kollman, P. A. *AMBER 14*; University of California: San Francisco, 2014.
16. Roiban, G.-D.; Agudo, R.; Ilie, A.; Lonsdale, R.; Reetz, M. T. *Chem. Commun.* **2014**, 14310–14313.