Highly Enantioselective Epoxidation of Disubstituted Alkenes with Hydrogen Peroxide Catalyzed by Chloroperoxidase

Eric J. Allain and Lowell P. Hager*

Department of Biochemistry
University of Illinois
Urbana, Illinois 61801

Li Deng and Eric N. Jacobsen*

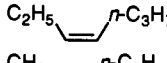
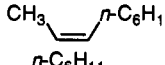
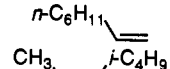
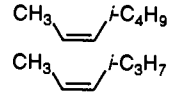
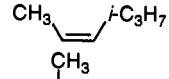
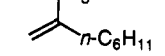
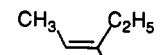
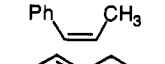
Department of Chemistry
University of Illinois
Urbana, Illinois 61801

Received January 19, 1993

The search for practical methods for enantioselective epoxidation of unfunctionalized olefins continues to present important challenges in the field of catalysis.[1] While recently developed synthetic catalysts now provide a viable method for the epoxidation of various types of conjugated olefins,[2] olefins bearing only aliphatic substituents are poor substrates for these catalysts and other synthetic catalysts as well.[3] In the realm of biological catalysis, several epoxidation methods that employ purified enzymes or whole cells have been reported,[4] but useful levels of enantioselectivity have been obtained in very few cases using purified enzymes, and as yet no enzymatic epoxidation methods have made their way into the repertoire of synthetic organic chemists.

Among enzymes that have been shown to catalyze olefin epoxidation, chloroperoxidase (CPO) is among the most well known and readily available.[5] CPO has been shown to be a versatile enzyme: in addition to catalyzing a variety of peroxidative halogenation reactions,[5a] CPO catalyzes the classical one-electron oxidations typical of plant peroxidases.[6] CPO also possesses a potent catalase activity,[9] and it mimics the P-450 cytochromes in catalyzing epoxidation and *N*-demethylation reactions.[7] Compared to the P-450 cytochromes, CPO may be considered a better candidate as a practical epoxidation catalyst since it utilizes H_2O_2 whereas the P-450 enzymes utilize molecular oxygen and require a regenerable reducing reagent, usually NADH. Although epoxidations with CPO were first reported a decade ago[8] and subsequently studied mechanistically in some detail,[9] enantioselectivity in such reactions has not yet been addressed in the literature.[10] We describe herein that under controlled conditions chloroperoxidase is highly effective for the enantioselective catalytic epoxidation of a variety of simple olefins by hydrogen peroxide and that as such it appears to represent the first example of a synthetically useful enzyme for olefin epoxidation.

Table I. Asymmetric Epoxidation Reactions Catalyzed by CPO[a]

entry	substrate	ee (%)	epoxide config	substrate conv (%)	epoxide yield (%)
1		96	2*R*,3*S*[b]	100	78
2		92	2*R*,3*S*[c]	96	82
3		97	2*R*,3*S*[c]	17	12
4			no reaction		
5			no reaction		
6		94	2*R*,3*S*[c]	53	33
7[d]		66	2*R*,3*S*[c]	28	n.d.[j]
8		74	n.d.[j]	10	n.d.[j]
9		81	n.d.[j]	≈50[e]	n.d.[j]
10[d]		96	1*S*,2*R*[f]	73	67[g]
11[h]		97[i]	1*R*,2*R*[i]	100	85[g]

[a] Reactions were run, and ees and yields were determined as described in ref 9 unless otherwise noted, using 0.03–0.12 mol % of the enzyme. [b] Epoxide configuration assigned by correlation to (*R*)-(−)-2-heptanol. [c] Epoxide absolute configuration tentatively assigned by analogy to entry 1. [d] Reaction run in the absence of acetone as cosolvent. [e] Accurate measurement not achieved due to overlapping GC signals from solvent. [f] Epoxide configuration assigned by correlation to pseudoephedrine: Witkop, B.; Foltz, C. M. *J. Am. Chem. Soc.* **1957**, *79*, 197. [g] Isolated yield. [h] Trans diol isolated as secondary product due to uncatalyzed epoxide hydrolysis. [i] Of trans diol. Absolute configuration assigned by correlation to (1*R*,2*S*)-(+)-dihydronaphthalene oxide. [j] Not determined.

Incubation of *cis*-β-methylstyrene with CPO at room temperature in the presence of H_2O_2 in citrate buffer (pH = 5) resulted in the stereospecific formation of the corresponding cis epoxide. Other common terminal oxidants (e.g., TBHP and PhIO) were found to be ineffective for CPO-catalyzed epoxidations. If excess H_2O_2 was initially present in the buffered enzyme solution when substrate was added, alkene conversion proceeded to only <5% before the enzyme was completely inactivated, but enantioselectivity in epoxide formation was high (92% ee). Given the known tendency of CPO to undergo irreversible decomposition in the presence of H_2O_2,[7] conditions were developed under which the concentration of the terminal oxidant was maintained low during the course of epoxidation. When H_2O_2 was provided to the system via syringe pump in a continuous and slow addition (2–10 μL/min), up to 70% substrate conversion was attained using 0.046 mol % of the enzyme, and enantioselectivity remained high. Complete substrate conversion could be achieved with the

(1) For leading references, see: (a) Bolm, C. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 403. (b) Jacobsen, E. N. In *Catalytic Asymmetric Synthesis*; Ojima, I., Ed.; VCH: New York, in press.

(2) (a) Jacobsen, E. N.; Zhang, W.; Muci, A. R.; Ecker, J. R.; Deng, L. *J. Am. Chem. Soc.* **1991**, *113*, 7063. (b) Lee, N. H.; Jacobsen, E. N. *Tetrahedron Lett.* **1991**, *32*, 6533.

(3) (a) Colletti, S. L.; Halterman, R. L. *Tetrahedron Lett.* **1992**, *33*, 1005. (b) Sinigalia, R.; Michelin, R. A.; Pinna, F.; Strukul, G. *Organometallics* **1987**, *6*, 728.

(4) (a) Ortiz de Montellano, P. R.; Fruetel, J. A.; Collins, J. R.; Camper, D. L.; Loew, G. H. *J. Am. Chem. Soc.* **1991**, *113*, 3195. (b) Boyd, D. R.; Sharma, N. D.; Smith, A. E. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2767. (c) Schurig, V.; Wistuba, D. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 796. (d) Takahasi, O.; Umezawa, J.; Furuhashi, K.; Takagi, M. *Tetrahedron Lett.* **1989**, *30*, 1583. (e) Ohta, H.; Tetsukawa, H. *J. Chem. Soc., Chem. Commun.* **1978**, 849. (f) Fu, H.; Newcomb, M.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 5878. (g) Colbert, J. E.; Katopodis, A. G.; May, S. W. *J. Am. Chem. Soc.* **1990**, *112*, 3993. (h) Katopodis, A. G.; Wimalasena, K.; Lee, J.; May, S. W. *J. Am. Chem. Soc.* **1984**, *106*, 7928.

(5) (a) Hager, L. P.; Morris, D. R.; Brown, F. S.; Eberwein, H. *J. Biol. Chem.* **1966**, *241*, 1769. (b) Morris, D. R.; Hager, L. P. *J. Biol. Chem.* **1966**, *241*, 3582. (c) Blanke, S. R.; Yi, S.; Hager, L. P. *Biotechnol. Lett.* **1989**, *11*, 769. Chloroperoxidase has been cloned: (d) Fang, G. H.; Kenigsberg, P.; Axley, M. J.; Nuell, M.; Hager, L. P. *Nucleic Acids Res.* **1986**, *14*, 8061. (e) Nuell, M.; Fang, G. H.; Axley, M. J.; Kenigsberg, P.; Hager, L. P. *J. Bacteriol.* **1988**, *170*, 1007.

(6) Thomas, J. A.; Morris, D. R.; Hager, L. P. *J. Biol. Chem.* **1970**, *245*, 3129.

(7) Kedderis, G. L.; Hallenberg, P. F. *Arch. Biochem. Biophys.* **1984**, *233*, 315.

(8) (a) McCarthy, M. B.; White, R. E. *J. Biol. Chem.* **1983**, *258*, 9153. (b) Greigert, J.; DeWitt, S. K.; Neidleman, S. L.; Lee, T. D.; Dalietos, D. J.; Moreland, M. *Biochem. Biophys. Res. Commun.* **1983**, *116*, 82.

(9) Ortiz de Montellano, P. R.; Choe, Y. S.; DePillis, G.; Catalano, C. E. *J. Biol. Chem.* **1987**, *262*, 11641.

(10) Asymmetric oxidation of sulfides catalyzed by CPO has been discovered recently: (a) Collonna, S.; Gaggero, N.; Casella, L.; Carrea, G.; Pasta, P. *Tetrahedron: Asymmetry.* **1992**, *3*, 95. (b) Fu, H.; Kondo, H.; Ichikawa, Y.; Look, G. C.; Wong, C.-H. *J. Org. Chem.* **1992**, *57*, 7265.

0002-7863/93/1515-4415$04.00/0

use of higher initial enzyme concentration (0.138 mol %), and the enantioselectivity in epoxidation was also measurably improved under these conditions (up to 97% ee).

Although the protocol described above proved satisfactory for epoxidation of *cis*-β-methylstyrene, most other alkenes benefited from the use of acetone as a cosolvent to improve the reactivity as well as the selectivity of the enzyme. For example, *cis*-2-heptene was oxidized to the corresponding epoxide with 60% conversion and 90% ee in citrate buffer, while under the same conditions substrate conversion was complete and epoxide was generated in 96% ee in acetone/citrate buffer medium.[11]

A variety of olefins were screened for epoxidation using this modified protocol, and particularly good results were obtained with cis-disubstituted alkenes bearing alkyl substituents (Table I, entries 1–7). Whereas trans olefins were found to be very unreactive substrates, as is generally the case in epoxidation reactions mediated by heme proteins and their synthetic models,[12] cis olefins were oxidized stereospecifically to the corresponding cis epoxides with very high enantioselectivity. The enzyme tolerated branching on the alkyl substituents, although enantioselectivities were dependent on the position of the branch (entries 7, 8). Certain 1,1-disubstituted and trisubstituted olefins (e.g., entries 9–10, Table I) were also accepted as substrates by CPO with moderate to good enantioselectivity in epoxidation. No reaction was observed with C_9 olefins (e.g., entry 5, Table I), presumably due to size restrictions in the active site, and terminal alkenes were also found to be generally unreactive. In addition to *cis*-β-methylstyrene (entry 10), other *cis*-disubstituted aryl-substituted olefins were effectively epoxidized. Dihydronaphthalene was oxidized cleanly to afford the corresponding trans diol (entry 11) due to an uncatalyzed and highly selective hydrolytic ring-opening of the acid-sensitive epoxide.

Compared with previously reported enzymatic and microbial epoxidation methods, the CPO-based system appears to accept a broader range of substrates and to effect epoxidation with generally much higher enantioselectivities. Perhaps most important, this method is highly complementary to existing asymmetric epoxidation protocols involving either synthetic or biological catalysts, which exhibit very poor selectivity with most of the substrates in Table I.[1b] Synthetic elaboration of the newly accessible enantiomerically enriched epoxides, application of CPO to enantioselective oxidations of other substrates including functionalized alkenes, and examination of the effects of active-site modification on epoxidation selectivity constitute some of the current areas of focus in these laboratories.

Acknowledgment. This work was supported by B.R.D.C. (Grant No. 2-4-055 to L.P.H.) and by the National Institutes of Health (GM-43214 to E.N.J.). L.D. is the recipient of a University of Illinois graduate fellowship. E.N.J. gratefully acknowledges awards from the National Science Foundation (PYI program), the David and Lucille Packard Foundation, the Sloan Foundation, the Camille and Henry Dreyfus Teacher-Scholar Program, the Lilly Grantee Program, American Cyanamid, Merck, and Pfizer.

Supplementary Material Available: Chromatographic analyses of all racemic and enantiomerically enriched epoxides (10 pages). Ordering information is given on any current masthead page.

(11) General procedure: chloroperoxidase was obtained and its concentration assayed by the published procedure,[5b] and all other reagents and solvents were used as received from commercial suppliers. Acetone (15 mL) was added to citrate buffer (0.1 M, 45 mL, pH = 5), resulting in a clear solution, and *cis*-2-heptene (Wiley, 49 mg, 0.5 mmol) was added to this solution. The mixture was stirred vigorously at room temperature for 1–2 min, after which the CPO solution (2 mL, 4.8 mg/mL, 0.046 mol %) was added. Stirring was sustained as undiluted 30% H_2O_2 was added at a rate of 2 μL/min via a syringe pump, and the progress of the reaction was followed by GC. After 2 h of continuous addition, disappearance of olefin was complete and epoxide was present as the only detectable product. The aqueous solution was extracted with ether/pentane (1:1 v/v, 2 × 50 mL), and the combined organic phases were dried over Na_2SO_4. The epoxide was not isolated due to its volatility, but the yield was determined to be 82% by GC analysis using dodecane as a quantitative internal standard. The ee of the epoxide was determined to be 96% by capillary GC (Cyclodex-B, J&W Scientific).

(12) See, for example: Groves, J. T.; Nemo, T. E. *J. Am. Chem. Soc.* **1983**, *105*, 5791.