Highly Enantioselective Epoxidation of 1,1-Disubstituted Alkenes Catalyzed by Chloroperoxidase

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In the past several years, substantial progress has been made in developing catalysts for the enantioselective epoxidation of a broad range of nonactivated olefins.[1] Among catalysts shown to effect highly enantioselective epoxidation of unfunctionalized olefins, manganosalen derivatives,[2] chiral metalloporphyrins,[3] and various biocatalysts[4] have received much attention. Although catalysts in the salen and porphyrin classes were initially limited mainly to use with *cis*-disubstituted olefins,[1a,b] the substrate repertoire for salen catalysts has recently been expanded to included *trans*-disubstituted olefins,[2e] trisubstituted olefins,[2f] and styrene,[2g] and highly enantioselective epoxidation of several substituted styrenes has also been achieved using chiral iron porphyrins.[3d] However, for the remaining structural classes of alkenes, and for aliphatic olefins in general, effective enantioselective epoxidation catalysts have yet to be developed. In this work, we report the identification of 1,1-disubstituted aromatic and aliphatic olefins as substrates for highly enantioselective epoxidation by chloroperoxidase.

Chloroperoxidase (CPO) from the fungus *Caldariomyces fumago* is an effective catalyst for the enantioselective epoxidation of *cis*-2-alkenes, giving high turnovers in concert with high enantiomeric excess (ee) for a range of aliphatic and aromatic olefins in this class.[4e] Early attempts to use the enzyme for the enantioselective epoxidation of other classes of alkenes gave disappointing results. In particular, aliphatic terminal olefins and *trans*-disubstituted olefins gave low yields of epoxide,[4e] while substituted styrenes gave only moderate enantioselectivities.[4c] We have recently shown that chloroperoxidase reacts with a number of aliphatic terminal olefins to give an inactive derivative in which the active-site heme is N-alkylated,[4f] thus accounting for the poor yields obtained with these substrates. In addition, the epoxidation enantioselectivity is much lower for monosubstituted alkenes than for closely related *cis*-2-alkenes.[4f]

We have been interested in investigating the factors controlling the emergence of the heme N-alkylation reaction and the reduction in epoxidation enantioselectivity with CPO for olefins lacking a *cis*-methyl substituent on the double bond. Given the likely role played by steric factors in facioselective positioning of the alkene within the enzyme active site, and the postulation on theoretical grounds that steric effects exert control over heme N-alkylation in a related cytochrome P-450 system,[5] we reasoned that repositioning the methyl substituent on the double bond might offer insights into steric control of both heme N-alkylation and reaction enantioselectivity. In the process of this work, we have identified 2-methyl-1-alkenes as a general class of substrates for high turnover, highly enantioselective epoxidation by CPO.

Epoxidation of several monosubstituted olefins with CPO (Table 1, entries 1, 4, 6, 8, 10) under conditions similar to those employed previously for *cis*-2-alkenes[4e,6] gave low catalytic turnovers (mol of epoxide/mol of enzyme), with poor to moderate enantioselectivities. While the highest turnover and enantioselectivity were obtained with styrene, in accordance with a previously published report,[4c] the ee was only moderate. Enzymatic oxidation of the remaining four monosubstituted olefins led to the formation of the green enzyme species similar to that previously reported for allylbenzene,[4f] accompanied by low yields of epoxide with inferior enantioselectivity (10−46%).

By contrast, epoxidation of matched 2-methylalkenes (Table 1, entries 2, 5, 7, 9, 11) showed a dramatic increase in both turnover and enantioselectivity. For the matched pair allyl and methallyl propionate, an increase in catalytic turnovers of 3 orders of magnitude could be observed as a consequence of substitution of the double bond. Further, while the epoxidation of allyl propionate with CPO leads rapidly to formation of an inactive green enzyme derivative, we were not able to detect formation of such a species during the epoxidation of methallyl propionate. At the same time, the epoxidation enantioselectivity increased from 24% with allyl propionate to 94% with methallyl propionate. Similar increases in both turnover and enantioselectivity were observed for each pair of matched olefins, except for styrene, in which substitution of the double bond led to a *decrease* in the turnover number accompanied by an increase in enantioselectivity.

In order to test the generality of the CPO epoxidation with 1,1-disubstituted olefins, we tested α-ethylstyrene and 2-ethylheptene (Table 1, entries 3 and 12). High epoxidation enantioselectivity was observed in the former case, but the catalytic turnover declined for both to a level similar to those observed for monosubstituted alkenes. For ethyl-substituted terminal

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(6) General procedure: chloroperoxidase was purified from culture medium of *C. fumago* using standard methods. Stock enzyme concentrations were determined using a molar absorptivity of 87 400 M^{-1} cm^{-1} at 400 nm in 25 mM potassium acetate, pH 5.2. Epoxidation reactions: to a vigorously stirred solution of 2.6 mg of CPO in 3 mL of 10 mM sodium citrate, pH 5.5, containing 500 ppm Antifoam A (Sigma), was added 1 mL of a stock alkene solution in acetone (2−100 mg/mL), and addition of hydrogen peroxide (2.5 M, 0.5 mL/h) was commenced immediately. After 45 min, the reaction was neutralized with 0.5 g of K_2CO_3 and extracted with 2 mL of pentane. Quantitative gas chromatography was carried out with decane as internal standard on a Varian 3300 GC equipped with a J&W CDX-B capillary column. Peak areas relative to decane were corrected using relative sensitivity factors previously determined with authentic standards. Chiral GLC analysis of the reaction products was carried out using an Astec Chiraldex G-TA column (40 m × 0.25 mm). Separation conditions are given in the supplementary material. Olefin concentrations were adjusted to allow for maximum catalytic turnover (mol of epoxide/mol of enzyme) at low conversion, and the reaction yields are therefore not optimized.

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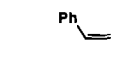
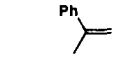
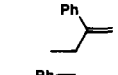
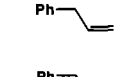
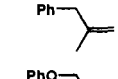
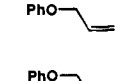
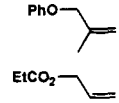
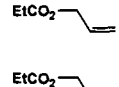
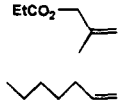
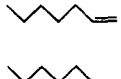
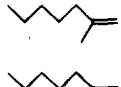
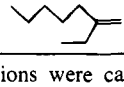
Table 1. Enantioselective Epoxidation of Monosubstituted and 1,1-Disubstituted Olefins Catalyzed by Chloroperoxidase[a]

Entry	Substrate	Major Product	ee (%)	Yield (%)[b]	Turnovers[c]
1[d]			49	89	900
2[e]			89	55	440
3			81	1	26
4[f,g]			37	7	77
5[e]			70	41	3400
6[f,h]			46	1	28
7[i]			89	22	1700
8[f,h]			24	12	5
9[i]			94	34	4200
10[f,e]			10	2	34
11[e]			95	23	1700
12			n.d.[j]	4	25

[a] Reactions were carried out and yields and ee's determined as detailed in note 6. [b] Not optimized; see note 6. [c] Mol of epoxide/mol of CPO. [d] Configuration of major product determined by correlation with authentic (*R*)-styrene oxide (Aldrich). [e] Configuration of major product determined by Sharpless asymmetric dihydroxylation (ref 9) of corresponding alkene followed by ring closure and comparison of known optical rotations. [f] Green enzyme derivative forms during epoxidation reaction. [g] Configuration of major product determined by correlation with (1*S*,2*S*)-phenylpropylene oxide (Aldrich). [h] Configuration of major product determined by correlation with (*S*)-glycidol (Sigma). [i] Configuration of major product determined by correlation with (*S*)-methylglycidol (Aldrich). [j] Not determined.

olefins, it appears that the greater steric size begins to limit access of the olefin to the active site. Steric exclusion of the olefin from the active site is expected to promote catalase activity when peroxide is added to the enzyme reaction, leading to oxidative destruction of the catalytic heme in a formally alkene-independent process, and hence to reduction of epoxidation turnovers. Conversely, addition of facile olefin substrates to the reaction should act to protect the enzyme from autoxidative inactivation. It appears probable that this effect accounts for an almost linear increase in turnover number observed when the initial concentrations of facile substrates such as methallyl propionate are increased. For example, with an initial concentration of methallyl propionate of 10 mg/mL, 1400 turnovers to epoxide were recorded, while an initial concentration of 25 mg/mL yielded 4200 turnovers with the same quantity of enzyme. In both cases, the enzyme was fully inactivated at the end of the reaction.

The turnover numbers recorded in Table 1 for olefins which give green enzyme derivatives (entries 4, 6, 8, 10) may underestimate the partition numbers for heme N-alkylation (mol of epoxide/mol of alkylated enzyme) because of the existence of multiple inactivation pathways for the enzyme, in particular because of the catalase reaction. We reinvestigated the epoxidation of allyl propionate under conditions favoring rapid formation of the N-alkylated enzyme, in order to obtain a better estimate of the partition number. Treatment of CPO (8.7 mg, 206 nmol) with allyl propionate (35.6 mg, 1510 equiv) dispersed in 3 mL of citrate buffer gave complete formation of a green enzyme complex with absorption maxima at 314, 419, 551 (sh), 608, and 651 nm upon addition of 13.6 μmol H_2O_2 (66 equiv) over 1 min. GLC analysis of the pentane/acetone extract demonstrated the formation of 0.33 mg of epoxide (12.4 equiv) and recovery of 33.2 mg (1410 equiv) of unchanged olefin. The excess oxidant was probably used by catalase activity of the enzyme. A partition number of 12 is an order of magnitude lower than has been previously reported for any cytochrome P-450 or model system[7] and suggests that heme N-alkylation is more facile than has been previously recognized. Steric inhibition of heme N-alkylation, due to the inability of a hindered substrate to approach sufficiently close to the heme face to form a covalent bond, may account for at least some of the differences in turnover numbers between different monosubstituted olefins in Table 1. It appears reasonable to suggest that it also accounts for the absence of detectable N-alkylation with 1,1-disubstituted olefins.

For those products whose absolute stereochemistry has been determined, some surprising results have emerged. In previous work with chloroperoxidase,[4e] tentative assignments of absolute stereochemistry were made by analogy within a related series. Independent determinations for 10 epoxides in Table 1 show that the products cannot readily be fit to a single stereochemical pattern; configurations are inverted for three of the five matched pairs. Furthermore, three of the epoxides result from a facioselectivity opposite to that of the other seven, though each of these has a length of seven atoms. The most salient regularity is that all the methyl-substituted alkenes exhibit the same facioselectivity. It will be of great interest to investigate the active site features underlying this subtle stereocontrol using the recently determined crystal structure of CPO.[8]

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Supplementary Material Available: Chiral gas chromatographic analyses of racemic and enantiomerically enriched epoxides (11 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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