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Novel applications of chloroperoxidase: enantioselective oxidation of racemic epoxyalcohols

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Abstract

The CPO-catalysed enantioselective oxidations of racemic glycidol and *cis*-2,3-epoxyhexanol to the corresponding aldehydes were studied using *tert*-butyl hydroperoxide in the 9:9:1 and 18:1 mixtures of hexane:ethyl acetate:buffer and hexane:buffer, respectively. Temperature and pH (100 mM citrate buffer, pH 4.0–7.0) effects on enantioselectivity in the terms of ee for the less reactive alcohol enantiomers with conversion were studied.

1. Introduction

Chloroperoxidase from *Caldariomyces fumago* (CPO, EC 1.11.1.10) is one of the most promising of the heme peroxidase enzymes for synthetic applications.[1–4] The enzyme shows broad substrate specificity catalysing various halide-requiring as well as halide-independent oxidation reactions using hydrogen peroxide as the natural source of oxygen without the need of cofactors. The studies for the CPO-catalysed oxidation of primary alcohols to the corresponding aldehydes belong to a class of interesting halide-independent reactions which are proposed to proceed from an alcohol to the aldehyde through a radical intermediate (Scheme 1).[5–8] According to our previous work, two-phase systems consisting of an organic solvent and a buffer (pH 5.0) lead to fast oxidations with nearly complete consumption of an alcohol.[8] The enzyme was shown to be active and selective even in organic solvents saturated with a buffer. In these reactions the nature of an oxidant is important, *tert*-butyl hydroperoxide being most favourable: it is partitioned between the two phases and the produced *tert*-butyl alcohol exerts a stabilizing effect[9] on CPO.

Optically active 2,3-epoxyalcohols are important synthons for the synthesis of biologically or pharmaceutically important compounds. The Sharpless asymmetric epoxidation of allylic alcohols is among the

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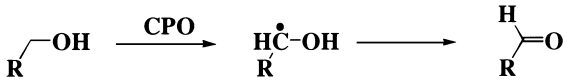


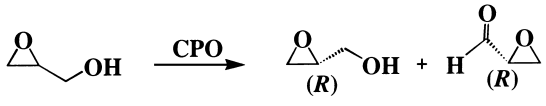
Scheme 1.

most versatile methods for the preparation of optically active epoxyalcohols.[10] The lipase-catalysed resolution of a racemic alcohol is an alternative to the Sharpless reaction. Thus, the hydrolysis of acylated 2,3-epoxypropanol (glycidol) in aqueous solutions and the acylation of glycidol and 3-substituted glycidols in organic solvents have been studied in the presence of porcine pancreatic lipase.[11,12] Enantioselectivity in these reactions is relatively low (*E* 1–30). In addition to this, the equilibrium nature of the reactions tends to degrade the enantiopurity of the remaining enantiomer, thereby further lowering the efficiency of the resolution. On the other hand, it is firmly established that CPO shows high enantioselectivity for the epoxidation of olefins.[2–4,13–16] Unfortunately, the enzyme does not catalyse the formation of epoxyalcohols from allylic alcohols.[8] Rather the reaction leads to the formation of the aldehyde. The aim of the present work has been to exploit the aldehyde formation for studying the factors which influence the enantioselectivity of the CPO-catalysed oxidation of racemic glycidol and *cis*-2,3-epoxyhexanol in the formation of the less reactive alcohol as one enantiomer and the aldehyde as a new product (Schemes 2 and 3). The reactions have been performed with *tert*-butyl hydroperoxide as an oxidant in two-phasic solvent systems at relatively low water contents (Schemes 2 and 3). Both pH and temperature effects on enantioselectivity have been studied.

2. Results and discussion

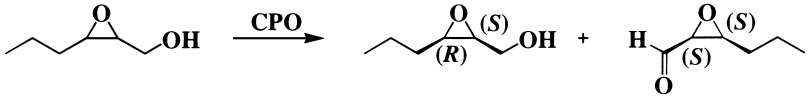
2.1. Resolution of glycidol

Encouraged by the slight enantioselectivity previously observed for the oxidation of 2,3-epoxyhexanols,[8] and because of our general interest in the preparation of enantiopure glycerol-based C_3-synthons,[17–19] the CPO-catalysed kinetic resolution of racemic glycidol has been studied (Scheme 2). The solvent system 9:9:1 of hexane:ethyl acetate:buffer proved favourable in order to keep glycidol mainly dissolved in the organic phase. The CPO-catalysed oxidation of glycidol in the solvent system (100 mM citrate buffer, pH 5.0, room temperature) gave only 67% ee for the less reactive (*R*)-glycidol at 56% conversion (Table 1, row 2). It was not possible to follow the formation of the aldehyde by GLC analysis and, in addition, the aldehyde was expected to be practically racemic, as was found to be the case for the oxidation of *cis*-2,3-epoxyhexanol.[8] Therefore attention was concentrated on obtaining the highly enantiopure (*R*)-glycidol as the less reactive enantiomer from the enantioselective oxidation.



Scheme 2.

High enantioselectivity is typical of various types of CPO-catalysed halide-independent oxidation reactions.[2–4,13–16] Accordingly, highly enantioselective oxidation of glycidol by CPO was expected



Scheme 3.

Table 1
Effect of pH and temperature for the oxidation of glycidol (0.4 M) with *tert*-butyl hydroperoxide in the presence of CPO in hexane:EtOAc:water (9:9:1)

Row	Temperature/°C	pH	Time/h	Conversion/%	ee_{OH}[a]/%
1	25	4.0	24	44	65
2	25	5.0	24	56	67
3	25	5.5	24	21	22
4	25	6.0	24	11	9
5	25	6.5	24	5	3
6	25	7.0	24	4	2
7	5	4.0	24	42	50
8	5		48	55	82
9	5	5.0	24	25	38
10	5		54	52	96
11	5	5.5	24	24	36
12	5		54	53	95
13	5	6.0	24	26	36
14	5		54	56	98
15	5	6.5	24	21	37
16	5		54	54	99
17	5	7.0	24	20	34
18	5		54	42	72
19	5		72	47	79

[a]For the unreacted (*R*)-glycidol according to the chiral GLC method

although not observed (Table 1, row 2) in the present two-phasic system. The lack of high enantioselectivity may be caused by purely chemical reasons. Thus, the oxirane ring in glycidol easily opens to glycerol, e.g., under mildly acidic conditions, and hence the observed conversion (56%) can include a considerable contribution from the chemical ring opening, leading to seemingly low enantioselectivity as measured against the observed enantiopurity (ee 67%) of the less reactive alcohol enantiomer. When the disappearance of glycidol was followed under the reaction conditions in the absence of CPO or in the absence of both CPO and the oxidant conversions of the order of 40% were observed at room temperature. In order to try to suppress the chemical reaction the CPO-catalysed oxidation of glycidol was performed

in solvent systems at various pH (4.0 to 7.0) for the buffer. However, this was not a method of enhancing the efficiency of the resolution (Table 1, rows 1–6). Rather, a clear dependence of conversion on pH was observed, the conversion being highest near pH 5.0 at room temperature.

A commonly used method of enhancing enzymatic enantioselectivity is to perform reactions at low temperatures. This strategy is based on the equation $E_1^{T_1} = E_2^{T_2}$ where E_1 and E_2 are the enantiomer ratios of enzymatic transformations at temperatures T_1 and T_2, respectively.[20] Enantioselectivity of enzymatic resolutions are commonly discussed in terms of E values. Due to the chemical ring opening of the oxirane ring and to the amount of the alcohol in the water phase the calculation of the E values is impossible in the present work. Thus, the values of ee_{OH} close to the theoretical 50% conversion are used as the measure of enantioselectivity. The CPO-catalysed oxidation of racemic glycidol was carried out at 5°C. In hexane:ethyl acetate:buffer (9:9:1; 100 mM citrate buffer, pH 5.0) the ee enhancement from ee=67% at room temperature (Table 1, row 2) to 96% at 5°C (row 10) was observed for the less reactive (*R*)-glycidol. Interestingly, at 5°C, pH changes over the range 5.0–6.5 had practically no effect on conversion and on the corresponding ee value of the alcohol (rows 9–16). There is a drop in ee connected to rate retardations at longer reaction times at pH 7.0 (rows 17–19). Evidently, high pH affects the enzymatic stability. For the oxidation of alcohols, the pH optimum of CPO seems somewhat indefinite at the moment because various results have been obtained depending on the substrate. Thus, pH-independent conversions were observed over the examined pH range 3.0–6.0 in the case of allyl alcohol.[5] For the oxidation of 5-hydroxymethylfurfural, on the other hand, optimum activity was obtained at pH 5.[7] The present results show that, at least for the oxidation of glycidol, the pH dependence of CPO is temperature dependent.

In addition to the observed enantioselectivity enhancement at 5°C, reduced chemical opening of the oxirane ring (only 13% within 24 hours in the absence of CPO) was observed.

2.2. *Resolution of* cis-*2,3-epoxyhexanol*

In the previous work, enantioselective oxidation of *cis*-2,3-epoxyhexanol in the two-phasic system (4:1) of hexane:citrate buffer (100 mM, pH 5.0) produced enantiomerically enriched resolution products, (2*S*,3*R*)-epoxyhexanol (ee 42%) and (2*S*,3*S*)-epoxyhexanal (ee 40%), at 50% conversion (Scheme 3).[8] Thus, it was of great interest to study the effect of pH on enantioselectivity for the resolution of racemic *cis*-2,3-epoxyhexanol by CPO in the solvent system hexane:buffer (18:1; 100 mM citrate buffer) at 5°C. The results are shown in Table 2. In the present case, the efficiency of the kinetic resolution in the terms of ee with the conversion is enhanced with increasing pH. Thus, high enantiomeric excesses for (2*S*,3*R*)-epoxyhexanol were achieved at 50% conversion by performing the resolution at 5°C at pH 6.5 (row 8). For the resolution of glycidol, the effects of pH and temperature were inextricably included in the observed enantioselectivity. The present results for the resolution of *cis*-2,3-epoxyhexanol show that the decrease of temperature and the increase of pH up to 6.5 really work together. Due to the electron releasing propyl group at C-3 of the substrate, the oxirane ring of *cis*-2,3-epoxyhexanol is more sensitive to acid-catalysed ring-opening than that of glycidol.

In accordance with the previous work, there is a decrease in the ee values of the produced (2*S*,3*S*)-epoxyhexanal with time.[8] The drop in ee is not affected by the pH of the buffer and is evidently connected to the free radical mechanism of Scheme 1.

Table 2
Oxidation of *cis*-2,3-epoxyhexanol (0.2 M) by CPO in hexane:water (18:1) at 5°C

Row	pH	Time/h	Conversion/%	ee_{CHO}[a]/%	ee_{OH}[b]/%
1	5.0	10	39	40	57
2		24	51	25	82
3		48	76	10	93
4	6.0	10	23	40	58
5		24	55	24	87
6		48	70	-	>95
7	6.5	10	16	38	58
8		48	50	-	>95

[a]For the produced (2*S*,3*S*)-epoxyhexanal; chiral GLC. [b]For the unreacted (2*S*,3*R*)-epoxyhexanol; chiral GLC.

3. Conclusions

The CPO-catalysed oxidations of racemic glycidol and *cis*-2,3-epoxyhexanol are shown to proceed in a highly enantioselective manner provided that the reactions are performed at low temperatures such as 5°C and at appropriate pH over the range 5.0–6.5. The preferred pH is connected to the nature of the substrate. The present results show that pH is more critical to the stability of CPO at room temperature (inactivation is clear already at pH 5.5 or higher) compared to the stability at lower temperatures.

4. Experimental

4.1. Materials and methods

Chloroperoxidase from *Caldariomyces fumago* (CPO, EC 1.11.1.10) with a specific activity of 1300–1400 IU/mg was obtained as a solution (5.5 mg protein/ml) from Chirazyme (Urban, IL, USA). Racemic and (*R*)-glycidol as well as *tert*-butyl hydroperoxide (70% (w/w) in water) were obtained from Aldrich. The racemate was distilled before use. Racemic *cis*-2,3-epoxyhexanol was prepared from the corresponding allylic alcohol as previously described.[21,22] The solvents were purchased from Lab-Scan.

The GLC analysis was performed on a Perkin–Elmer 8500 gas chromatograph equipped with a flame ionization detector and a CP-Chirasil-Dex CB (25 m) column. Good base-line separation for the ee determination was recorded for the enantomers of glycidol at isothermal conditions (55°C). For the enantiomers of *cis*-2,3-epoxyhexanol and those of the hexanal the enantiomers were separated using a temperature program 75°C (21 min) to 120°C (10 min) with the ramp of 25°C/min using N_2 as a carrier gas.

^{1}H NMR (TMS as internal standard) and ^{13}C NMR spectra were measured on a Lambda GX 400 spectrometer in $CDCl_3$.

4.2. Enantioselective oxidation of glycidol

A freshly distilled glycidol (56 μl, 0.85 mmol) was added into a 4 ml vial containing hexane (450 μl), ethyl acetate (450 μl) and a citrate buffer (100 mM; pH 4.0–7.0; 200 μl). The mixture was stirred for 10 min followed by the addition of CPO (0.65 mg). The stirring was continued for an hour at room temperature (25°C) or at 5°C. The reaction was initiated by adding *tert*-butyl hydroperoxide (1.0 mmol in seven portions within 24 hours). The progress of the reaction against decane as an internal standard and the enantiomeric excess values for the prevailing alcohol enantiomer were determined by taking samples from the reaction mixture and analysing them by the chiral GLC method. The absolute configuration of the less reactive (*R*)-glycidol was determined by using commercial enantiomers as reference for gas chromatograms. The presence of the aldehyde in the reaction mixture was confirmed using spectroscopic methods directly on the sample and substracting the glycidol from the spectra. ^{13}C NMR: 171.04 (C-1); 43.72 (C-2) and 50.58 (C-3). ^{1}H NMR: 2.97–2.98 and 3.02–3.05 (m, 2H, 3-H); 3.26–3.30 (m, 1H, 2-H) and 8.89 and 8.87 (d, 1H, J=6.4 Hz, 1-H).

These data can be compared to the spectroscopic data for glycidol. ^{13}C NMR: 44.23 (C-1); 61.91 (C-2) and 52.23 (C-3). ^{1}H NMR: 2.73–2.93 (m, 2H, 3-H); 3.07–3.13 (m, 1H, 2-H); 3.51–3.56 and 3.83–3.88 (m, 2H, 1-H) and 4.93 (m, 1H, OH).

4.3. Enantioselective oxidation of cis-2,3-epoxyhexanol

cis-2,3-Epoxyhexanol (50 μl, 0.43 mmol) was added into a 4 ml vial containing hexane (1.8 ml) and a citrate buffer (100 mM; pH 5.0–6.5; 200 μl). The mixture was stirred for 10 min followed by the addition of CPO (0.65 mg). The stirring was continued for an hour at 5°C. The reaction was initiated by adding *tert*-butyl hydroperoxide (0.58 mmol in 4 portions within 10 hours). The progress of the reaction and enantiomeric excess values were determined as decribed above. The absolute configuration of less reactive starting material and the produced aldehyde were assigned in the previous work.[8] The spectroscopic data below were previously determined for the separated resolution products.

(2*S*,3*R*)-Epoxyhexanol: ^{13}C NMR: 60.85 (C-1); 57.08 (C-2); 56.79 (C-3); 29.86 (C-4); 19.86 (C-5) and 13.82 (C-6). ^{1}H NMR: 0.95 (t, 3H, 6-H); 1.5–1.6 (m, 4H, 5-H and 4-H); 2.3 (br., 1H, OH).

(2*S*,3*S*)-Epoxyhexanal: ^{13}C NMR: 199.18 (C-1); 57.82 (C-2); 56.00 (C-3); 30.02 (C-4); 26.34 (C-5) and 13.66 (C-6). ^{1}H NMR: 0.95 (t, 3H, 6-H); 1.45–1.75 (m, 4H, 4-H and 5-H); 3.25 (m, 1H, 3-H); 3.31 (m, 1H, 2-H); 9.44 and 9.33 (d, 1H, J=5.2 Hz, 1-H).

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