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Chloroperoxidase catalyzed oxidations in *t*-butyl alcohol/water mixtures

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Abstract

Chloroperoxidase catalyzed oxidations of sulfides and indoles were performed in *t*-butyl alcohol/water mixtures at ambient temperature. *t*-Butyl alcohol/water (50:50, v/v) proved to be a good solvent system for performing synthetic oxidations catalyzed by chloroperoxidase. The sulfoxidation of alkyl aryl sulfides and related compounds catalyzed by chloroperoxidase in *t*-butyl alcohol/water mixtures (50:50, v/v) was compared to the sulfoxidation in water. In both solvent systems, complete enantioselectivity to the *R*-sulfoxide (ee = 99%) was observed with hydrogen peroxide as oxidant when the size of the alkyl moiety was smaller than propyl. The uncatalyzed, racemic sulfoxidation did not proceed under these conditions. This is in contrast to literature data on sulfoxidation in water, where enantioselectivities were lower due to this uncatalyzed reaction. Reactions in water generally proceed faster than reactions in the cosolvent system except for substrates which dissolve poorly in water or for solid substrates for which diffusion becomes an important limiting factor in water. The lower activity in *t*-butyl alcohol/water for sulfoxidation and for indole oxidation is mainly due to an increase of the K_m value (thermodynamically controlled). Also a decrease of k_{cat} (catalytic turnover frequency) is observed, probably caused by a change in structure of the enzyme.

Keywords: Chloroperoxidase; *t*-Butyl alcohol/water; Indoles oxidation; Sulfides oxidation

1. Introduction

The current interest in catalytic oxidative transformations is induced by two major issues in industry: the first one is the replacement of oxidations which use a stoichiometric amount of heavy metal salts by catalytic processes using hydrogen peroxide or oxygen as the oxidant. A second major issue is the need for high chemo-, regio- or enantioselectivities in order to improve chemical yields, to minimize waste streams and to avoid enantiomeric ballast.

Peroxidases are potentially suitable biocatalysts for meeting these two goals. Peroxidases are a class of enzymes which use hydrogen peroxide as the oxidant without the need for a cofactor. Moreover, they are relatively stable and can accommodate a broad range of substrates. The most versatile peroxidase, chloroperoxidase from *Caldariomyces fumago* (CPO), is known to catalyze enantioselective sulfoxidations [1–3], epoxidations [4,5], benzylic hydroxylations [6,7] and indole oxidation to oxindole [8,9].

Practical application of CPO in organic synthesis entails the use of organic solvents be-

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cause the solubility of organic reactants in water is too low. Although there have been earlier reports regarding the use of CPO in aqueous cosolvent mixtures [1,4,5,10], most of the solvents used have some major drawbacks. Methanol and dimethylsulfoxide are both reported to be substrates for CPO [11,12], acetonitrile is unacceptable due to its toxicity and acetone has been reported to lower the enantiomeric excess in sulfoxidation reactions [1] and is known to form acetone peroxide complexes with hydrogen peroxide. We have previously reported on the use of *t*-butyl alcohol as a cosolvent for CPO catalyzed oxidations [13]. Owing to its bulky structure *t*-butyl alcohol is not a substrate for CPO. Moreover, it is environmentally acceptable and is completely water miscible. In this paper, we present the results obtained with CPO catalyzed oxidation reactions, particularly sulfoxidation and indole oxidation, using *t*-butyl alcohol as a cosolvent and including reactions at a synthetically useful scale. Furthermore, the influence of *t*-butyl alcohol on the rate and the selectivity of the reactions is discussed.

2. Experimental

2.1. Materials and analytical methods

Chloroperoxidase from *Caldariomyces fumago* was isolated and purified as described in the literature [13]. The enzyme preparation contained 6100 U/ml according to the method of Morris and Hager [14], with a purity of $R_z = 1.3$ and an enzyme concentration of 115 μM. Enzyme concentrations were determined using a molar extinction coefficient of 91 200 M^{-1} cm^{-1} at 400 nm [14].

Hydrogen peroxide 35% was obtained from Merck. Methyl phenyl sulfide was obtained from Janssen Chimica. Ethyl phenyl sulfide, methyl *p*-tolyl sulfide, methyl *p*-methoxyphenyl sulfide, methyl *p*-bromophenyl sulfide and methyl *p*-nitrophenyl sulfide were obtained from Aldrich. Methyl *p*-bromophenyl sulfide and methyl *p*-nitrophenyl sulfide were recrystallized from 60% acetic acid prior to use. Methyl *p*-chlorophenyl sulfide, 1-chloro-4-ethylthiobenzene, propyl phenyl sulfide and methyl *m*-methoxyphenyl sulfide were synthesized from the corresponding thiophenols according to the method of Herriot and Picker [15]. Methyl *o*-methoxyphenyl sulfide, methyl *m*-chlorophenyl sulfide, methyl *m*-bromophenyl sulfide, 2-methylthio-thiophene and 2-methylthio-thiazole were donated by Prof. Dr. Brandsma of the University of Utrecht, and were distilled before use.

2.1.1. HPLC analysis

A Chiralcel OD column (Baker, 25 × 0.46 cm) was used for monitoring sulfoxidation reactions. Eluents (flow 0.5 ml/min) used: hexane/2-propanol (75:25, v/v) for methyl *p*-nitrophenyl sulfide and hexane/2-propanol (85:15, v/v) for all other sulfides. Detection was performed using a Shimadzu SPD-6a UV spectrophotometer at 220 nm. Samples were treated with excess Na_2SO_3 to remove H_2O_2. Subsequently the samples were diluted with eluent, 1,3,5-trimethoxybenzene was added as internal standard and the samples were dried over $MgSO_4$. After filtration through a 0.4 μm membrane filter the samples were analyzed by chiral HPLC.

A Novapak C_{18} column (Waters, 8 × 10 mm 4 μm) contained in a Waters RCM 8 × 10 compression unit was used for analyzing indole and sulfide oxidations. Eluent (flow 1.0 ml/min) used for sulfide derivatives: methanol/water (60:40, v/v), eluent used for indole derivatives: methanol/water (50:50, v/v). Detection was performed using a Shimadzu SPD-6a UV spectrophotometer at 220 nm and an Erma ERC-7510 RI detector. *t*-Butyl alcohol was used as internal standard. Samples were diluted with eluent saturated with Na_2SO_3. After filtration through a 0.4 μm membrane filter, the samples were analyzed by reversed phase HPLC.

NMR spectra were recorded on a Varian

VXR-400S spectrometer, using TMS as an internal standard and $CDCl_3$ as the solvent. UV measurements were performed on a Cary 3 spectrophotometer from Varian.

2.2. General oxidation procedure

Sulfide oxidation: At room temperature 1.25 mmol of sulfide was dissolved in 25 ml of solvent (0.1 M aqueous acetate buffer pH 5 or *t*-butyl alcohol/0.1 M aqueous acetate buffer pH 5 (50:50, v/v)). 610 U chloroperoxidase was added to the reaction mixture, followed by 5 min of stirring. The reaction was started by the continuous addition of 1.66 M H_2O_2 in buffer (0.1 M acetate, pH 5) at a rate of 1 eq./2 h. In total 1.1 eq. of H_2O_2 was added and the reaction was quenched after 2.5 h by the addition of an excess of Na_2SO_3. The reactions in *t*-butyl alcohol/water mixtures were monitored by removing aliquots which were analyzed by chiral HPLC. The products of the reactions in 0.1 M aqueous acetate buffer pH 5 were analyzed after 2.5 h by adding 25 ml of *t*-butyl alcohol to the reaction mixture for homogenization. Subsequently a sample was taken and analyzed by chiral HPLC. Sulfoxides were isolated by addition of 25 ml water to the reaction mixture and saturation of the solution with NaCl. The aqueous solution was extracted with 3×40 ml of $CHCl_3$ and the collected organic layers were dried over $MgSO_4$. After evaporation of the $CHCl_3$ in vacuo the sulfoxide was obtained. If necessary, the sulfoxide was purified by column chromatography (stationary phase: silica; eluent methanol/toluene (10:90, v/v)). The structures of the sulfoxides were confirmed by ^{1}H- and ^{13}C-NMR. The enantiomeric purity of the sulfoxides was determined by chiral HPLC [13]. Reactions concerning the influence of the amount of *t*-butyl alcohol and the mode of addition on oxidation of methyl phenyl sulfide were performed as described in [13]. Reactions with indole derivatives were performed as described in [9].

2.3. Blank reaction according to the method of Colonna et al. [2]

10 mM sulfide was dissolved in 42 ml 0.1 M citrate buffer pH 5. The reaction mixture was stirred for 5 min. Subsequently 1 eq. H_2O_2 (0.2 M) was added in 13 aliquots of 330 μl at 5 min intervals and the reaction was continued for 5 min. After addition of excess Na_2SO_3, *t*-butyl alcohol was added for homogenization and samples were analyzed by chiral HPLC.

2.4. Reaction rates

Reaction rates of methyl phenyl sulfide and methyl *p*-bromophenyl sulfide were determined in buffer pH 5, 0.1 M and in *t*-butyl alcohol water. In buffer, reaction rates were determined by spectrophotometric measurement of the decrease of sulfide by UV. The difference of molar extinction coefficient between the sulfides and the corresponding sulfoxides were as follows: methyl phenyl sulfide $\epsilon = 7.3 \times 10^2$ M^{-1} cm^{-1} at 280 nm and methyl *p*-bromophenyl sulfide $\epsilon = 6.7 \times 10^3$ M^{-1} cm^{-1} at 259 nm. Reaction rates were determined in a 0.3–3 ml cuvet at 50–400 μM sulfide, 400 μM H_2O_2. Reactions were started by addition of 0.3–1.2 U CPO. All experiments were carried out in triplicate.

The kinetic constants in *t*-butyl alcohol/water (50:50, v/v) solution were determined in a 5 ml flask at 10–100 mM sulfide, 400 μM H_2O_2 and reactions were started by the addition of 1.8–3.0 U CPO. For 1 min, every 20 s a sample was taken which was added to a methanol/water solution (60:40, v/v) containing Na_2SO_3 to quench the reaction. The samples were analyzed on reversed phase HPLC. All experiments were carried out in duplicate. Initial reaction rates were calculated and kinetic parameters were determined using the Scientist program.

2.5. Solubility of sulfides in buffer

Solubilities of methyl phenyl sulfide and methyl *p*-bromophenyl sulfide were determined

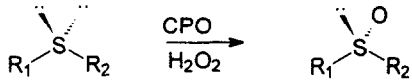

Fig. 1. Oxidation of sulfides by CPO.

by dissolving the corresponding sulfide in 0.1 M acetate buffer pH 5. From the saturated solution, a sample was taken and *t*-butyl alcohol was added as internal standard. The solubility of the sulfide was determined by analyzing the sample on reversed phase HPLC. The sample was compared to a standard solution of sulfide in *t*-butyl alcohol/water (50:50, v/v).

3. Results and discussion

3.1. Oxidation of alkyl aryl sulfides and related compounds

Kobayashi et al. [16] was the first one to report the enantioselective oxidation of methyl phenyl sulfides by CPO and hydrogen peroxide (depicted in Fig. 1), however, the obtained enantiomeric excess was low (13%). A more detailed study on the enantioselective oxidation of sulfides was carried out by Colonna et al. [1,2]. The best results were obtained with hydrogen peroxide as the oxidant. Hydrogen peroxide however, can also spontaneously oxidize sulfides to racemic sulfoxides. Colonna et al. observed substantial uncatalyzed oxidation of the sulfides in blank reactions (10–30%), therefore many of the obtained sulfoxides in the enzymatic procedure were not completely enantiopure. A subsequent study by the group of Colonna [17] demonstrated that chloride had a negative influence on the stereochemical outcome of the reaction probably due to the formation of hypochlorite. Fu et al. [3] investigated the chloroperoxidase catalyzed oxidation of *p*-substituted alkyl phenyl sulfides by hydrogen peroxide or racemic alkyl hydroperoxides as the oxidant in buffer. Slow addition of the hydrogen peroxide to the reaction mixture resulted in nearly enantiopure sulfoxides (ee = 97–99%).

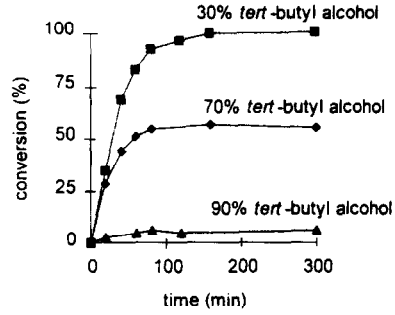

Fig. 2. Effect of amount of *t*-butyl alcohol on sulfoxidation of methyl phenyl sulfide. 1 Eq. H_2O_2 /h continuously.

When racemic alkyl hydroperoxides were used as the oxidant optically active alcohols and alkyl hydroperoxides were obtained (ee up to 89%).

In our study the influence of the *t*-butyl alcohol content on the oxidation of methyl phenyl sulfide by CPO was initially investigated [13]. The activity of CPO was measured for the sulfoxidation of methyl phenyl sulfide at 10 mM concentration and with hydrogen peroxide as the oxidant. CPO retained its activity in *t*-butyl alcohol/water mixtures up to 70% cosolvent (v/v, Fig. 2). However, the reactivity decreased with increasing amounts of cosolvent. The final conversion which was obtained was also very much dependent on the mode of addition of hydrogen peroxide (Fig. 3).

This is due to inactivation of CPO by hydrogen peroxide, hence, the hydrogen peroxide concentration in the reaction mixture should be kept as low as possible. Optimal conditions are achieved when the rate of hydrogen peroxide

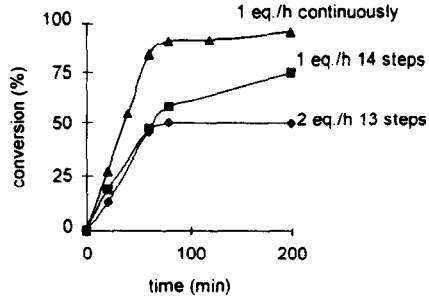

Fig. 3. Effect of mode of addition on sulfoxidation of methyl phenyl sulfide in *t*-butyl alcohol/water (50:50, v/v).

addition equals the initial reaction rate and hydrogen peroxide is added continuously.

Since organic solvents can influence both the reaction rate [18] and selectivity [19] of enzymatic reactions, we investigated the influence of the composition of *t*-butyl alcohol/water mixtures on the rate and enantioselectivity of the sulfoxidation of a number of sulfides by CPO. The results were compared to the oxidation in aqueous buffer. Furthermore, some new heteroaromatic sulfides and *m*/*p*-substituted phenyl methyl sulfides were oxidized to the corresponding sulfoxides. The experiments were carried out at 50 mM sulfide concentration. Reactions performed in *t*-butyl alcohol/water mixtures (50:50, v/v) were homogeneous whereas the reactions carried out in buffer were heterogeneous. The results are compiled in Table 1.

Sulfoxidation in *t*-butyl alcohol mixtures is facile (Table 1), although the final conversion of the sulfide is generally somewhat lower than in aqueous buffer. The observed conversions are final conversions since addition of more H_2O_2 did not increase the conversion. Reactions which did not reach completion stopped due to inactivation of CPO by an excess of H_2O_2 (Fig. 4). Moreover, as shown in Fig. 3 the mode of addition of the oxidant is crucial for obtaining high conversions. The oxidant concentration should always be rate limiting, thus preventing accumulation of H_2O_2 and inactivation of the catalyst.

The lower conversions to the sulfoxides in the cosolvent system are caused by a lower k_{cat}/K_m value in *t*-butyl alcohol/water mixtures (Table 2) compared to aqueous buffer. These lower k_{cat}/K_m values can be caused by several factors [18]:

(a) Better stabilization of the ground state of the sulfides in the cosolvent system than in water. This will result in a higher K_m value for the sulfides.

(b) Destabilization of the enzyme–substrate complex causing an increase in K_m.

(c) Solvent induced changes in the secondary

Table 1
Oxidation of sulfides by CPO and H_2O_2 [a]

Sulfide		*t*-butyl alcohol/buffer (50:50, v/v)		Buffer	
R_1	R_2	Conversion (%)	ee (%)	Conversion (%)	ee (%)
C_6H_5	CH_3	73	99	100	99
C_6H_5	CH_2CH_3	52	99	83	99
C_6H_5	$CH_2CH_2CH_3$	1	60	3	27
p-CH_3-C_6H_4	CH_3	66	99	83	99
p-OCH_3-C_6H_4	CH_3	50	99	53	99
m-OCH_3-C_6H_4	CH_3	19	99	37	99
o-OCH_3-C_6H_4	CH_3	2	99	3	99
p-NO_2-C_6H_4	CH_3	17	99	19	99
p-Cl-C_6H_4	CH_3	73	99	78	99
m-Cl-C_6H_4	CH_3	50	99	59	99
p-Br-C_6H_4	CH_3	46	99	15	99
m-Br-C_6H_4	CH_3	22	99	11	99
p-Cl-C_6H_4	CH_2CH_3	49	99	33	99
	CH_3	91	99	100	99
	CH_3	80	99	100	99

[a] 50 mM sulfide, 25 ml solvent, 610 U CPO, 1 Eq. H_2O_2/2 h.

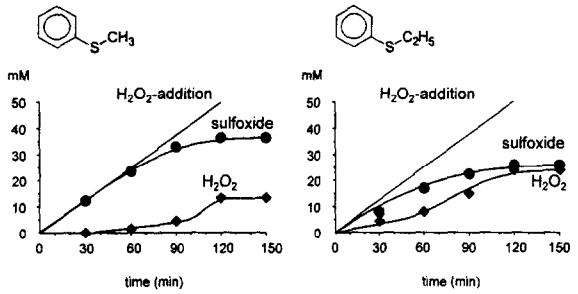

Fig. 4. Effect of $[H_2O_2]$ on sulfoxidation in *t*-butyl alcohol/water (50:50, v/v), 50 mM sulfide, 1 eq. H_2O_2 /2 h.

and tertiary structure of CPO in *t*-butyl alcohol/water mixtures leading to a decrease in the catalytic turnover frequency (k_{cat}).

(d) Penetration of the cosolvent in the active site of CPO, which can decrease the local polarity in the active site of CPO. This can have an effect on k_{cat}, on K_m and on the reaction selectivity.

For some sulfides, a higher conversion to the corresponding sulfoxides was obtained in cosolvent mixtures than in pure buffer. We ascribe this effect to the low solubility of the corresponding sulfides in water. Methyl *p*-bromophenyl sulfide is nearly 10 times less soluble in buffer (0.1 M acetate, pH 5) than methyl phenyl sulfide (Table 2). The reaction rate at sulfide saturation in water is lower than the reaction rate at 50 mM sulfide concentration in *t*-butyl alcohol/water mixtures for methyl *p*-bromophenyl sulfide (Table 2). At saturation the reaction rate in buffer is slightly higher than the rate of hydrogen peroxide addition (reaction rate is 95 mM/h at 400 μM H_2O_2 vs. 25 mM/h H_2O_2 addition). As chloroperoxidase already is inactivated at low concentrations of H_2O_2 [20] and as some diffusion limitation probably occurs during reaction because methyl *p*-bromophenyl sulfide is solid, the reaction rate may be expected to quickly drop below the critical value of 25 mM/h leading to further deactivation of chloroperoxidase due to accumulation of hydrogen peroxide. The reaction rate in *t*-butyl alcohol/water mixtures is somewhat higher at the start (120 mM/h), but during the course of the reaction the reaction rate declines due to lowering of the sulfide concentration and some enzyme inactivation. Again, the reaction ceases when the reaction rate is lower than the rate of hydrogen peroxide addition of 25 mM/h.

It is clear from Table 1 that *t*-butyl alcohol as cosolvent does not negatively influence the stereochemical outcome of the reaction. For all tested sulfides, with the exception of propyl phenyl sulfide, essentially complete enantioselectivity to the corresponding *R*-sulfoxide was observed in water as well as in *t*-butyl alcohol/water. A plausible reason for the observed low enantiomeric excess of propyl phenyl sulfide is its size. From the crystal structure of chloroperoxidase [21], it has been shown that chloroperoxidase has a small opening above the heme which allows access of substrates to the iron–oxo complex. The size of propyl phenyl sulfide is presumably too large for this opening, therefore the enzymatic oxidation of the sulfide

Table 2
Reaction rates and solubilities of sulfides

Sulfide		Solvent	k_{cat}/K_m ($s^{-1}mM^{-1}$)	K_m (mM)	Solubility (mM)	$V_{50\,mM}$ [a] (s^{-1})
R_1	R_2					
C_6H_5	CH_3	H_2O	730	0.9	2.4	480
		H_2O/*t*-BuOH (50:50)	20	29	> 50	367
p-Br-C_6H_4	CH_3	H_2O	193	≫ 0.3 [b]	0.3	58
		H_2O/*t*-BuOH (50:50)	3	55	> 50	71

[a] Turnover frequency when 50 mM of sulfide is present in the reaction mixture. The actual sulfide concentration in aqueous solution is the saturated concentration.

[b] The K_m value could not be determined as the enzyme could not be saturated with the sulfide in the solubility range.

Table 3
Comparison of blank experiments for methyl *o*-methoxyphenyl sulfide [a]

	Conv. (%)	ee (%)	Conv. [2] (%)	ee (%)
Blank	< 1	0	23	0
CPO	3.3	99	24	27

[a] Experiments performed as described in [2].

is very slow. The non-catalyzed racemic oxidation becomes competitive, leading to a lower enantioselectivity of the obtained sulfoxide.

Blank experiments without enzyme present showed less then 1% spontaneous oxidation to the racemic sulfoxide after 2.5 h. This contrasts with results of Colonna et al. [2], who observed substantial oxidation in blank experiments (up to 33%) leading to a lower enantiomeric purity of the obtained sulfoxides in enzymatic reactions. As our experiments were performed under different conditions, we repeated the blank reaction with methyl *o*-methoxyphenyl sulfide using the method of Colonna et al. [2] (Table 3).

We observed much less blank reaction compared to the results reported by Colonna et al. (Table 3), although the reaction conditions were the same. This may be due to the presence of adventitious impurities catalyzing the spontaneous racemic oxidation of the sulfide to the sulfoxide. It is known for example [22] that catalytic amounts of acid or metal oxide enhance the oxidation of sulfides to sulfoxides.

3.2. Indole oxidation

In addition to sulfoxidation, we investigated another selective reaction catalyzed by CPO in *t*-butyl alcohol/water mixtures (50:50, v/v), namely the oxidation of substituted indoles to oxindoles (Fig. 5, [9]). Substituted oxindoles are interesting compounds for pharmaceutical applications, for example 5-chloro-oxindole is an intermediate for the synthesis of Tenidap ((1-carbamoyl-5-chloro-3-[hydroxy(2-thienyl)methylene]indol-2-(3*H*)-one) [23]), an anti-inflammatory agent.

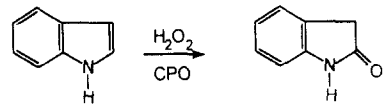

Fig. 5. Oxidation of indole to oxindole.

For substituted indoles which showed sufficient activity [9], preparative scale experiments were carried out on a 50 mM scale in 25 ml *t*-butyl alcohol/water (50:50, v/v). The amount of CPO was varied, depending on the reactivity of the substituted indole (Table 4).

t-Butyl alcohol/water proved again to be a good system for performing synthetic oxidations with CPO. When the amount of enzyme was adjusted to the reactivity of the indole [9], complete conversion to the corresponding oxindoles could be obtained. In the case of 4-chloro-oxindole, the product was contaminated with starting material, because the oxidation stopped before completion.

To study the influence of *t*-butyl alcohol on the catalytic efficiency of CPO, indole oxidation was taken as the standard reaction. Kinetic parameters were determined for indole in water and compared to *t*-butyl alcohol/water (30:70 and 50:50, v/v). The kinetic parameters were determined at 400 μM H_2O_2 concentration. Samples were taken every 20 s during 1 min and analyzed on reversed phase HPLC. Oxindole production was linear during this period of time. Contrary to the results of Corbett and Chipko [8] we could determine a K_m value for the oxidation of indole in water. The difference between the two studies is most likely caused

Table 4
Preparative scale synthesis of oxindole derivatives [9]

Indole derivative	CPO (kU)	Yield (%)	Purity (%)
Indole	1	96	96
7-Aza-indole	2	97	99
4-Cl	6	70	76
5-Cl	2	99	99
5-Br	3	86	95
5-CH_3	6	92	94
5-OCH_3	6	93	95
6-Cl	2	96	99

Table 5
Kinetic parameters for indole oxidation with various cosolvent amounts [a]

t-butyl alcohol/ water (v/v)	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m (s^{-1} mM^{-1})
0:100	740	3.3	225
30:70	580	12	48
50:50	250	22	11

[a] 20°C, 400 μM H_2O_2.

by the difference in the hydrogen peroxide concentration which is used to determine the kinetic parameters. Corbett and Chipko used an oxidant concentration of 4 mM. At such a high hydrogen peroxide concentration other reactions interfere with the oxidation of indole [8,24].

As can be seem from Table 5, the cosolvent effects both k_{cat} and K_m. A lower k_{cat} value is probably caused by structural changes of CPO induced by the cosolvent, thus changing the structure of the active site and the catalytic turnover frequency. The increase of the K_m value is thermodynamically expected as the ground state of indole is stabilized in cosolvent mixtures. The same effects were observed for methyl phenyl sulfide for which the K_m value was increased from 0.9 mM in aqueous solution to 29 mM in *t*-butyl alcohol water mixtures (50:50, v/v). However, the influence of the medium on k_{cat} was less pronounced for methyl phenyl sulfide: a decrease from 650 1/s in aqueous buffer to 580 1/s in *t*-butyl alcohol/water mixtures (50:50, v/v) was observed compared to a decrease by a factor of nearly 3 in the case of indole.

4. Conclusions

t-Butyl alcohol/water provides an excellent solvent system for performing synthetic oxidations with CPO and hydrogen peroxide as the oxidant. Reactions remain highly (enantio)selective in this cosolvent system. *R*-sulfoxides are obtained in high optical and chemical yield. Substituted indoles are oxidized to the corresponding oxindoles in almost quantitative yield. Generally the reaction rate in *t*-butyl alcohol/water is lower than in aqueous buffer. However, the problems caused by the low solubility of substrates and the diffusion limitation, which are inherent to reactions in water, can be circumvented. The lower reactivity in the cosolvent system is mainly due to the higher K_m value of the substrates in this solvent system as compared to water.

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