Enantioselective Sulfoxidation Catalyzed by Vanadium Haloperoxidases

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Vanadium haloperoxidases catalyze the oxidation of halides by hydrogen peroxide to produce hypohalous acid. We demonstrate that these enzymes also slowly mediate the enantioselective oxidation of organic sulfides (methyl phenyl sulfide, methyl *p*-tolyl sulfide, and 1-methoxy-4 (methylthio)benzene) to the corresponding sulfoxides (turnover frequency 1 min^{-1}). The vanadium bromoperoxidase from the brown seaweed *Ascophyllum nodosum* converts methyl phenyl sulfide to the (*R*)-enantiomer of the sulfoxide (55% yield and 85% enantiomeric excess (ee)). At low peroxide concentrations a selectivity of 91% can be attained. The enzyme catalyzes the selective sulfoxidation reaction over a broad pH range with an optimum around pH 5−6 and remains completely functional during the reaction. When the vanadium bromoperoxidase from the red seaweed *Corallina pilulifera* is used the (*S*)-enantiomer (18% yield and 55% ee) is formed. In contrast, the vanadium chloroperoxidase from the fungus *Curvularia inaequalis* catalyzes the production of a racemic mixture (54% yield), which seems to be an intrinsic characteristic of this enzyme.

Introduction

Vanadium haloperoxidases are enzymes, which are thought to be responsible for the production of a variety of halogenated compounds, ranging from simple volatile halohydrocarbons[1] to relatively complicated chiral structures.[2] In the presence of hydrogen peroxide and halides these enzymes produce hypohalous acid as a reactive intermediate, which may subsequently react with organic compounds.[3] In the absence of suitable organic acceptors oxygen evolution can be observed due to the catalyzed halide-assisted disproportionation of hydrogen peroxide.[4] During catalysis the vanadium metal resides in the highest oxidation state as vanadium(V).

The vanadium-containing bromoperoxidases (VBPO's) were originally discovered in marine algae,[5] and vanadium chloroperoxidases (VCPO's) are found in a number of dematiaceous hyphomycetes.[6] In particular the enzyme from the fungus *Curvularia inaequalis* has been studied in detail,[7] and its X-ray structure has been determined.[8] High stability is one of the characteristic features shared by all the vanadium peroxidases. The enzymes are capable of withstanding temperatures up to 70 °C, remain completely functional in the presence of several organic solvents and are not susceptible toward oxidative inactivation.[9,10]

During the two-electron oxidation of halides catalyzed by vanadium peroxidases an active enzyme−peroxo-intermediate is formed[11] followed by nucleophilic attack of the halide on the side-on coordinated peroxide and subsequently halide oxidation. Prior to the discovery of vanadium peroxidases it was established that dioxovanadium (VO^{2+}) was capable of producing iodine in the presence of iodide and hydrogen peroxide in acidic solution.[12] Further investigations showed that both the monoperoxo- and diperoxovanadium(V) species ($VO(O_2)^+$ and $VO(O_2)_2^-$ are formed and that both species are needed for halide oxidation activity.[13] Several vanadium(V) monoperoxocomplexes of tridentate Schiff-base ligands were reported to exhibit bromoperoxidase-like activity.[14] In addition, a *N*-(2-hydroxyethyl)iminodiacetic acid oxoperoxovanadium-(V) complex was also observed to oxidize bromide under relatively acidic conditions.[15] In this compound the vanadium-(V) is the center of a pentagonal bipyramidal geometry, a single

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terminal oxygen is coordinated in the axial position, peroxide resides in the equatorial plane side-on bound and the additional four positions are occupied by a tetradentate ligand.

Inorganic vanadium(V) peroxo-compounds have also been reported to mediate oxygen-transfer reactions including the oxidation of sulfides to sulfoxides and sulfones and the epoxidation of alkenes and allylic alcohols.[16] The oxidation reactions of a variety of organic sulfides in the presence of hydrogen peroxide or *tert*-butyl hydroperoxide catalyzed by vanadium(V) species to sulfoxides and sulfones in organic solvents have been studied.[17] Only Schiff-base ligated vanadium(V) complexes could convert methyl phenyl sulfide in a stereoselective manner yielding the (*R*)-enantiomer of the sulfoxide in small enantiomeric excess (8−14%).[18] These particular complexes were also shown to be suitable catalysts to perform stereoselective epoxidation reactions of alkenes.[19] Significant enantiomeric excesses (up to 50%) were found for vanadium(V)-catalyzed epoxidation reactions of allylic alcohols using chiral hydroxamic acids as ligands.[20]

Heme peroxidases are capable of promoting enantioselective oxygen-transfer reactions[21] and the heme chloroperoxidase from *Caldariomyces fumago* promotes the enantioselective sulfoxidation of methyl phenyl sulfide, a frequently studied substrate for selective oxidation reactions, yielding the (*R*)-enantiomer with 99% ee. However, it is well-known that the prosthetic group of the heme peroxidases degrades due to the presence of hydrogen peroxide and therefore inactivation of the heme peroxidases during catalysis occurs.[22] In contrast with the heme peroxidases, which are capable of catalyzing the one-electron oxidation of organic compounds and of monooxygenase-like enantioselective oxygen-transfer reactions, the vanadium haloperoxidases have been reported to catalyze only peroxidative halogenation reactions and are unable to oxidize the classical heme peroxidase substrates.[23]

In view of this and the fact that vanadium(V) complexes catalyze sulfoxidation reactions, we investigated whether the vanadium enzymes also catalyze selective oxygen-transfer reactions. Recently, it was discovered that a vanadium bromoperoxidase from the alga *Corallina officinalis* oxidizes some aromatic bicyclic sulfides stereoselectively to the corresponding (*S*)-sulfoxides with high enantiomeric excess.[24] However, this enzyme did not seem to be capable of converting methyl *p*-tolyl sulfide, which is a commonly used substrate in asymmetric sulfoxidation studies.

We now present evidence for the involvement of vanadium haloperoxidases as biocatalysts in enantioselective sulfoxidation reactions. The activities of three different vanadium peroxidases, the chloroperoxidase from the fungus *C. inaequalis*, the bromoperoxidase from the seaweed *C. pilulifera*, and, in particular, the bromoperoxidase from the seaweed *Ascophyllum nodosum* in the sulfoxidation of methyl phenyl sulfide were investigated.

Experimental Section

The oxygen-transfer activity of three different vanadium haloperoxidases was studied using methyl phenyl sulfide as a substrate. The VBPO's originate from the seaweeds *A. nodosum*[5] and *C. pilulifera*.[25] The recombinant VCPO was obtained from a developed *Saccharomyces cerevisiae* expression system,[26] as the recombinant VCPO (r-VCPO) can be obtained in large quantities and behaves kinetically very similar to the native enzyme from the fungus *C. inaequalis*.[27] The enzyme was activated by the addition of vanadate as described.[26]

Methyl phenyl sulfide (1 mM) was incubated in the presence of H_2O_2 (2 mM) and enzyme (1 μM) in 100 mM sodium acetate (pH 5.0) and sodium citrate/NaOH (pH 6.2) buffers at 25 °C in 1 mL sealed glass vials. These reaction conditions were used for all experiments unless stated otherwise. As controls experiments were carried out in the absence of enzyme or in the presence of vanadate (10 μM). After 20 h the peroxide in the reaction mixture was quenched with sulfite and acetophenone (0.5 mM) was added as an internal standard. The enantiomeric excess was determined as follows; the mixture was extracted with CH_2Cl_2, evaporated to 20 μL, and dissolved in 1 mL of hexane/2-propanol (4:1). A 20 μL sample was used for HPLC analysis on a Diacel chiral OD column (0.46 cm × 25 cm) equipped with a Pharmacia LKB-HPLC pump 2248 and a LKB Bromma 2140 rapid spectral detector connected to a personal computer. The Borwin program was used to evaluate peak areas. The column was eluted with hexane/2-propanol (4:1) at a flow rate of 0.5 mL/min. The retention times for the *R* and *S* isomers were 14 and 17 min, respectively. The HPLC effluent was monitored at 254 nm. A sulfoxide standard curve was constructed from the peak areas of sulfoxide/acetophenone mixtures, which had been treated in the same way as above. The yields were determined separately on a Waters RPLC column using a mixture of methanol/water (3:2) as eluent.

The halide-free buffer was prepared by preincubating a 100 mM sodium acetate buffer (pH 5.0) with a low concentration of recombinant VCPO (5 nM), H_2O_2 (2 mM), and phenol red (100 μM) as a scavenger for HOBr and HOCl. After 2 days the buffer was filtered through active carbon, which was washed with H_2O purified by a Millipore system, to remove the dye.

The formation of the methyl phenyl sulfoxide was monitored continuously on a HP diode array spectrophotometer from the absorbance decrease at 290 nm in a cuvette (4 mL) containing 100 mM sodium acetate buffer (pH 5.0), methyl phenyl sulfide (1 mM), H_2O_2 (2 mM), and enzyme (1 μM) with a capillary opening and no headspace to prevent evaporation of substrate and product. A $\Delta\epsilon$ of 0.44 mM^{-1} cm^{-1} at 290 nm was determined from the difference in absorbance between 1 mm methylphenylsulfide and 1 mM of the corresponding sulfoxide. This extinction coefficient was used to determine the concentration of sulfoxide produced. The enantioselectivity and the consumption of H_2O_2 were determined independently in a series of 1 mL glass vials with sealed caps at 25 °C containing 100 mM sodium acetate buffer (pH 5.0), methyl phenyl sulfide (1 mM), H_2O_2 (2 mM), and enzyme (1 μM). After 1, 3, 6, 12, 22, 24, and 29.5 h the incubations were quenched, extracted with CH_2Cl_2, and treated as described, and the enantioselectivity (ee) was determined. Before addition of sulfite, 100 μL samples were taken to determine the H_2O_2 concentration. The H_2O_2 concentration was measured in an assay system containing ABTS [2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid][28] (1 mM) and lactoperoxidase (100 nM) in 100 mM sodium acetate buffer (pH 5.0) at 414 nm on a Varian Cary 17 spectrophotometer. The reaction was started by addition of 10 μL of sample. Haloperoxidase activity was

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Table 1. Yield and Enantioselectivity (ee) of the Sulfoxidation of Methyl Phenyl Sulfide by Vanadium Haloperoxidases

		pH 5.0		pH 6.2	
haloperoxidase	sulfide	yield (%)[a]	ee (%)	yield (%)[a]	ee (%)
VBPO *A. nodosum*	methyl phenyl	52	83 (*R*)	55	85 (*R*)
	methyl *p*-tolyl	18	82 (*R*)	33	62 (*R*)
	methyl 4-methoxy benzene	n.d.	78 (*R*)	n.d.	60 (*R*)
VBPO *C. pilulifera*	methyl phenyl	45	30 (*S*)	18	55 (*S*)
rVCPO	methyl phenyl	56	racemic	54	racemic
no enzyme	methyl phenyl	7	racemic	7	racemic

[a] Yield is defined as mole of sulfoxide/mole of methyl phenyl sulfide; n.d., not determined.

monitored in an assay containing 10 μL of sample, monochlorodimedone (50 μM), and KBr (100 mM) in 100 mM sodium citrate buffer (pH 6.0) at 290 nm on a Zeiss spectrophotometer. The reaction was started by addition of H_2O_2 (2 mM).

Results

Experiments on the enzyme-mediated asymmetric oxidation of organic sulfides were carried out using methyl phenyl sulfide as a substrate, hydrogen peroxide and three different vanadium haloperoxidases; a VBPO originating from the brown seaweed *A. nodosum*, a VBPO isolated from the red seaweed *C. pilulifera*, and the r-VCPO from the fungus *C. inaequalis*. The results are presented in Table 1. VBPO from *A. nodosum* converts the organic sulfide to the (*R*)-enantiomer of the sulfoxide under slightly acidic conditions with 52% yield and an enantiomeric excess (ee) of 85% after 20 h. In contrast, the (*S*)-enantiomer of the sulfoxide was observed to be produced when VBPO from *C. pilulifera* was used to oxidize methyl phenyl sulfide. In this case at more neutral pH the yield decreases, but there is a significant increase in the ee.

When recombinant VCPO was used in the catalyzed oxidation of the organic sulfide a racemic mixture resulted. As the affinity of VCPO for halides is very high (the K_m value for bromide equals 10 μM, and that for chloride 0.3 mM,[7] respectively), this may be explained by formation of HOBr or HOCl, which may aspecifically form the racemic sulfoxide. Therefore a halide-free buffer was prepared (Experimental Section) and while using this buffer we still found a racemic mixture (yield 55% at pH 5.0). It is likely that the formation of a racemic product is an intrinsic characteristic of this enzyme.

In the absence of enzyme approximately 7% of the methyl phenyl sulfide is converted into a racemic mixture. Vanadate, which is the prosthetic group of these enzymes, is known to dissociate from the active site under acidic conditions.[29] To check whether traces of vanadate are responsible for some of the observations, the effect of 10 μM of vanadate on the sulfoxidation reaction was studied. The yield of the sulfoxide was similar to that of the uncatalyzed reaction.

During these studies we noticed that some preparations of vanadium BPO from *A. nodosum*, although pure according to SDS gel electrophoresis, showed a much lower yield (40%) and ee (45%). In particular, highly colored enzyme preparations were catalytically less efficient. When an additional purification step was incorporated in the isolation procedure of the vanadium BPO from *A. nodosum*[30] following the final DEAE ion-exchange step using a Mono Q column (a strong anion-exchanger) on a FPLC system, the brown component could be removed. This resulted in an uncolored vanadium bromoperoxidase preparation, which produces the sulfoxides with an increased enantiomeric excess. Probably the brown component is responsible for the relatively low stereoselectivity of the sulfoxidation catalyzed by some preparations.

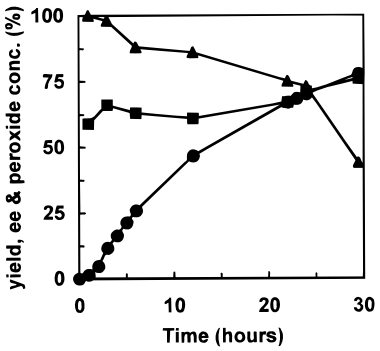

Figure 1. Methyl phenyl sulfide sulfoxidation by VBPO from *A. nodosum* at pH 5. The formation of sulfoxide (●) was monitored continuously on an HP diode array spectrophotometer at 290 nm, the consumption of H_2O_2 (▲) was determined with the ABTS assay, and the enantioselectivity (■) of the sulfoxidation was analyzed with chiral HPLC as described in the Experimental Section.

To establish this assumption, we examined the enantioselectivity of three separate sulfoxidation reactions using the following enzyme preparations: a highly colored vanadium BPO preparation obtained after the DEAE column purification,[30] the same preparation after an additional purification on a Mono Q column using a FPLC system (essentially colorless), and an enzyme preparation consisting of an equimolar mixture of these two enzyme batches. In each experiment the same amount of vanadium BPO was used for the experiments. The colored and colorless vanadium BPO converted methyl phenyl sulfide in to the (*R*)-enantiomer with 32% and 65% ee, respectively, after 20 h. The combined enzyme preparation produced the (*R*)-enantiomer with an enantiomeric excess of only 33%. Consequently the sulfoxidation activity of the uncolored vanadium BPO is influenced strongly by the presence of the brown component. Colorless enzyme preparations were used for further studies.

The time-dependence of the conversion of methyl phenyl sulfide to the (*R*)-sulfoxide by VBPO from *A. nodosum* at pH 5.0 was determined. Three independent experiments were simultaneously conducted: the formation of the sulfoxide was monitored at 290 nm, the yield and ee were determined by HPLC analysis, and the consumption of H_2O_2 during the incubation was followed by taking samples and measuring the lactoperoxidase-catalyzed oxidation of ABTS at 414 nm. From Figure 1 it can be deduced that the reaction is not completed in 30 h; however, this is not due to substrate depletion or enzyme inactivation, as the enzyme was found to remain completely active during the reaction. At the start of the conversion the enantioselectivity of the reaction was 56% ee but gradually started to increase as the reaction proceeded to 78% ee after 30 h. We assume that the low ee at the beginning of the reaction

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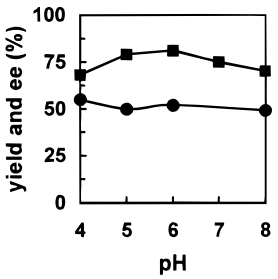

Figure 2. The pH-dependence of the VBPO sulfoxidation activity. The yield (●) was determined by RPLC analysis, and the selectivity (■) was analyzed with chiral HPLC as described in the Experimental Section.

is due to the contribution of the uncatalyzed reaction between methyl phenyl sulfide and H_2O_2. This reaction is only significant when both substrates are present in relatively high concentration.

The influence of the pH and the addition of several organic solvents on the oxidation reaction were studied. Figure 2 shows that the catalyzed sulfoxidation of methyl phenyl sulfide does not exhibit a pronounced pH optimum. The highest enantiomeric excess (80%) is obtained at slightly acidic pH (5−6) and the yield is hardly affected by pH.

The pH had a more distinct influence on stereoselectivity when the conversion of methyl *p*-tolyl sulfide and 1-methoxy-4-(methylthio)benzene by vanadium BPO to the corresponding (*R*)-sulfoxides at pH 5 and 6 was studied. The (*R*)-enantiomers were formed with 82% and 78% ee, respectively, at pH 5, whereas 64% and 60% ee were found when reactions were carried out at pH 6 (Table 1).

An attempt to determine the K_m of vanadium BPO for methyl phenyl sulfide failed as the solubility of this hydrophobic substrate in water is quite low (1.7 mM). It was established that the initial rate of the sulfoxidation reaction is 30 μM/h when 0.5 mM sulfide was present at an enzyme concentration of 1 μM. When 1 mM methyl phenyl sulfide was used the rate of reaction increased to 45 μM/h, whereas a conversion rate of 60 μM/hour was found when the sulfide concentration was raised to 1.5 mM. From this it can be concluded that the K_m for methyl phenyl sulfide exceeds 1.5 mM and it is therefore not possible to perform the sulfoxidation reactions under optimal kinetic conditions.

To increase the solubility of methyl phenyl sulfide the addition of cosolvents seemed useful. Therefore the influence of various organic solvents on the yield and selectivity of the catalyzed sulfoxidation reaction was studied. However, the addition of *tert*-butyl alcohol (25%) decreased the selectivity of the reaction from 83% to 52% ee and the yield dropped to 18% after incubation for 20 h at pH 5 (not shown). When 10% of this cosolvent was added the selectivity was found to be 51% without appreciable effect on the yield as compared to the catalyzed reaction in aqueous buffer. A similar, more dramatic negative effect on both yield and selectivity was observed in the presence of 25% methanol, ethanol or 2-propanol, where approximately 20% of the sulfoxide was obtained with only 34% ee. The addition of DMF, THF, and DMSO (25%) also decreased the yield and selectivity of the reaction. We also explored the effect of glycerol (25%) on enzymatic sulfoxidation. However the yield (28%) and selectivity (34% ee) were again negatively influenced by the addition of a cosolvent. Only the presence of high salt concentration (0.1 M Na_2SO_4) did not influence the yield and selectivity of the enzymatic production of the sulfoxide.

The selectivity of the catalytic sulfide conversion could be increased when the H_2O_2 concentration was lowered. The concentration of methyl phenyl sulfide in these experiments (data not shown) was increased to 1.5 mM in order to increase the rate of conversion and consequently enlarge the production of the sulfoxide. The yield was determined on a spectrophotometer at 290 nm ($\Delta\epsilon = 0.44$ mM^{-1} cm^{-1}). As the initial hydrogen peroxide concentration was lowered from 2 to 0.25 mM at pH 5 the selectivity of the enzymatic sulfoxide formation increased from 75% ee to 91% ee (not shown). The yield was observed to decrease under these reaction conditions.

Discussion

We have demonstrated that vanadium haloperoxidases are capable of catalyzing the conversion of an organic sulfide to an organic sulfoxide in the presence of hydrogen peroxide. We have shown that the vanadium BPO from the red seaweed *C. pilulifera* selectively oxidizes methyl phenyl sulfide to the (*S*)-enantiomer which is consistent with the recently reported selective conversion of aromatic sulfides to the (*S*)-sulfoxides by the vanadium BPO from the seaweed *C. officinalis*.[24] However, the latter did not seem capable of accepting methyl *p*-tolyl sulfide, a methyl phenyl sulfide derivative, as a substrate for the sulfoxidation reaction. In addition, we have established that the vanadium BPO from the brown seaweed *A. nodosum* produces the (*R*)-enantiomer of the sulfoxide and the recombinant vanadium CPO originally from the fungus *C. inaequalis* mediates the formation of a racemic mixture. No sulfones were produced by the enzymes, which is consistent with previous data.[24]

It is not yet clear why VBPO from *C. pilulifera* catalyzes the formation of the (*S*)-enantiomer of the sulfoxide whereas the VBPO from *A. nodosum* promotes the production of the (*R*)-enantiomer and VCPO mediates the synthesis of a racemic mixture. The crystal structure of the vanadium CPO has been solved,[8] but a structure of a vanadium BPO is not yet known; therefore it is not possible to compare the active site of the enzymes on a structural basis. We expect to be able to compare the structures of the enzymes soon as the vanadium BPO from the brown seaweed has been crystallized, and its structure is being analyzed at the moment.[31]

Previously, it was observed that some vanadium BPO preparations from *A. nodosum* were brown after purification, although these preparations were established by gel electrophoresis to be pure. The color was difficult to remove but its presence did not interfere with kinetic studies. An additional purification step including a Mono Q column on a FPLC system enabled us to separate the unknown brown component from the enzyme. We have found that the specificity of the sulfoxidation reaction depends on the purity of the enzyme preparation. Highly colored preparations give rise to products with relatively low enantioselectivity (45% ee), whereas colorless preparations mediate the sulfoxidation reaction yielding the sulfoxide with high selectivity (85% ee).

In the studies using colored enzyme preparations an initial rapid decrease in the H_2O_2 concentration was observed at the beginning of the sulfoxidation reactions, which was not accompanied by a corresponding decrease in sulfide concentration. The formation of oxygen bubbles was observed, when the reaction was started.[32] Because no halides were present, several vanadium bromoperoxidase and chloroperoxidase preparations were tested for catalase activity activity using a 100 mM sodium

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citrate buffer (pH 5.0), enzyme (1 μM), H_2O_2 (2 mM), and a dioxygen electrode. We found however that the catalase activity seemed to depend on the enzyme preparation used (data not shown). There seems to be a connection between the purity of the preparation and the catalase activity of the enzyme preparation, because colored preparations exhibit catalase activity whereas colorless preparations do not mediate the decomposition of hydrogen peroxide. Vanadium CPO preparations also did not show catalase activity. Therefore we conclude that the disproportionation of hydrogen peroxide is not an intrinsic characteristic of these enzymes, which is in line with studies conducted by Everett and Butler.[33] Attempts are now being made to isolate the brown component and identify it by mass spectroscopy. We believe that, since the bromoperoxidases are isolated from seaweed, the component presents a polyphenolic structure. As of yet we do not know where this compound binds or how it affects the sulfoxidation and/or catalase activity of the enzyme, but it may bind near the active site and obstruct the selective conversion of organic sulfides.

In contrast to the steep pH optimum at pH 6.5 for the enzymatic bromide oxidation,[30] the sulfoxidation activity of the vanadium bromoperoxidase from the brown seaweed exhibits a rather broad pH-profile over the range of pH 4 to 8, which is consistent with the results of recent investigations by Allenmark et al.[24] There is no distinct pH optimum for the conversion of methyl phenyl sulfide although the selectivity of the reaction was found to have an optimum with an ee of approximately 80% between pH 5 and 6. Similar specificity was found for the catalyzed sulfoxidation of methyl *p*-tolyl sulfide and 1-methoxy-4-(methylthio)benzene at pH 5, but a decrease in specificity was observed (60% ee) at pH 6. Further studies were therefore conducted at pH 5.

It is clear that the enantioselective sulfoxidation is a relatively slow process requiring several hours for completion. From Figure 1 a turnover (mole of sulfoxide/mole of enzyme) of 750 can be calculated. Turnovers of 57 and 29 were found for lactoperoxidase and horseradish peroxidase, respectively, whereas a turnover of 6.3×10^4 was determined for heme chloroperoxidase from *C. fumago*.[22,34−36] For heme peroxidases it is also well documented that the oxygen-transfer reactions are slow processes.[34−36] However, these enzymes are inactivated by H_2O_2 during catalysis,[22] whereas the activity of VBPO is not affected as we have found also in this study. It is therefore possible to calculate a lower-limit of a turnover frequency from our data. A value of 1 min^{-1} is found for the vanadium BPO from *A. nodosum*. This is slow compared to the brominating activity of this enzyme (166 s^{-1}).[37]

Attempts have been made to establish the K_m of vanadium BPO for methyl phenyl sulfide at pH 5, however the solubility of organic sulfides in aqueous buffers is low and it was therefore impossible to use higher concentrations than 1.5 mM of sulfide under these conditions. The presence of an organic solvent increases the solubility of organic compounds in water, consequently the effect of several solvents, including methanol, ethanol, 2-propanol, *tert*-butyl alcohol, DMSO, DMF, and THF on the enzymatic sulfoxide formation was investigated. Previously, it was established that the presence of 40% methanol, ethanol, or 2-propanol does not affect the halogenation activity of the vanadium haloperoxidases. Surprisingly, the presence of 25% of these and other cosolvents was observed to reduce both the yield and enantioselectivity of the enzymatic sulfoxidation reaction considerably.

Recently three Schiff-base vanadium(V) peroxocomplexes have been synthesized which are capable of mediating selective sulfoxidation reactions with relatively high selectivity (78% ee).[38] Earlier investigations already reported selective conversions of methyl phenyl sulfide with 70% ee catalyzed by similar Schiff-base ligated vanadium(V) peroxocomplexes.[39] The vanadium haloperoxidases are more versatile since ee values of 91% can be reached. That bromoperoxidases catalyze sulfoxidation reactions in an enantioselective manner confirms the work of Tschirret-Guth and Butler[3] that the bromoperoxidases have a specific binding site for organic substrates at or near the active site. We assume, however, that this selective sulfoxidation reaction does not proceed via the formation of the highly reactive hypohalous acid, which stays bound to the vanadium and then oxidizes the organic sulfide in a selective manner induced by the environment of the active site. First, halides are not present during the incubation and, second, the presence of bromide completely canceled the selectivity of the reaction. We believe that the reaction proceeds via a direct oxygen-transfer step from the peroxo-coordinated enzyme intermediate[11] to the organic sulfide present in the active site of the vanadium enzyme. This is similar to what has been proposed for the vanadium peroxocomplexes, which catalyze the oxidation of sulfides to sulfoxides via a two-electron direct oxygen transfer pathway.[40]

At the moment attempts are being made to further optimize the selective sulfoxidation reactions catalyzed by vanadium haloperoxidases. We now examine the possibility of creating enantioselective vanadium enzymes by site-directed mutagenesis of the VCPO.

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