Asymmetric Sulfoxidation Catalyzed by a Vanadium-Containing Bromoperoxidase

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A vanadium-containing bromoperoxidase (VBrPO) from the alga *Corallina officinalis* has been shown to catalyze the stereoselective oxidation of some aromatic bicyclic sulfides to the corresponding (*S*)-sulfoxides in high (up to 91%) ee. Hydrogen peroxide was found to have a large effect on the catalyzed reaction, most likely due to an inhibition of VBrPO. High optical and chemical yields were found to be favored by a continuous slow addition of hydrogen peroxide to keep a low excess. The reaction gives no overoxidation to sulfone, and its stereochemistry is the opposite as compared to that previously found with the heme-containing chloroperoxidase (CPO) from *Caldariomyces fumago*.

Introduction

Biocatalytic methods have been demonstrated to be excellent alternatives to chemical procedures for a wide range of asymmetric organic reactions. One example is the asymmetric oxidation of prochiral sulfides to optically active sulfoxides, which has been performed by many different techniques.[1] Of the various biocatalysts examined,[2] chloroperoxidase from the fungus *Caldariomyces fumago* (CPO)[3] has been shown to be one of the more successful enzymes in terms of the enantioselectivity of sulfoxidation[4,5] as well as epoxidation.[6] Chloroperoxidase belongs to the class of haloperoxidases characterized by the presence of an iron heme unit in the active site.

Another unique class of haloperoxidases with a vanadium-dependent catalytic activity has recently been discovered. Several vanadium-containing bromoperoxidases (VBrPO) from marine algae have been characterized.[7] They all catalyze halide-assisted biotransformations, such as bromination of organic substrates (e.g. monochlorodimedone)[8] and hydrobromination,[9] by utilizing hydrogen peroxide as oxygen source. As with heme haloperoxidases, disproportionation of hydrogen peroxide to give molecular oxygen has been observed, albeit with the difference that this only occurs in the presence of halide ions yielding singlet oxygen and is consequently not classified as a classical catalase activity.[10] Kinetic investigations have indicated that halogenation and dioxygen formation catalyzed by VBrPO proceed through a common intermediate. A bi-bi ping-pong mechanism preceding this intermediate, with binding of hydrogen peroxide prior to halide, has been suggested.[7]

The VBrPOs characterized thus far all resemble each other in amino acid composition, vanadium content, isoelectric point, etc.[7] Each consists of a characteristic albeit different number of subunits, which all have a size of ca. 65 kDa. Maximum activity is achieved with one vanadium atom per subunit. During isolation the vanadium atom/subunit ratio falls to about 0.4, and full activity has to be restored by dialysis in an orthovanadate buffer.[11] The vanadium site is believed to be a distorted octahedron with the vanadium atom coordinated by a single terminal oxygen, two equatorial nitrogens, and a further three light-atom ligands.[7] The vanadium atom has been shown to be in its highest oxidation state (+5) both in the resting state of the enzyme and throughout catalytic turnover. Consequently hydrogen peroxide will not oxidize the vanadium ion but is rather thought to coordinate in a bidentate manner.

An interesting feature of these enzymes is their remarkably high stability toward organic solvents, chelating agents, and elevated temperatures, properties appreciated in a synthetic application.[8c]

The aim of this work was to explore whether these VBrPOs are able to catalyze oxidation of sulfides and further whether any resultant enantioselectivity would be found, suggesting that the oxidation takes place within an active site.

Only halide-assisted oxidations have been observed thus far, and the VBrPOs have been claimed to be

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(1) (a) Bolm, C.; Bienewald, F. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2640−2642. (b) Brunel, J.-M.; Diter, P.; Duetsch, M.; Kagan, H. B. *J. Org. Chem.* **1995**, *60*, 8086−8088. (c) Palucki, M.; Hanson, P.; Jacobsen, E. N. *Tetrahedron Lett.* **1992**, *33*, 7111−7114.

(2) (a) Blée, E.; Schuber, F. *Biochemistry* **1989**, *28*, 4962−4967. (b) Katapodis, A. G.; Smith, Jr., H. A.; May, W. S. *J. Am. Chem. Soc.* **1988**, *110*, 897−899. (c) Colonna, S.; Gaggero, N.; Pasta, P.; Ottolina, G. *Chem. Commun.* **1996**, 2303−2307. (d) Takata, T.; Yamazaki, M.; Fujimori, K.; Kim, Y. H.; Iyanagi, T.; Oae, S. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 2300−2310.

(3) Morris, D. R.; Hager, L. P. *J. Biol. Chem.* **1966**, *241*, 1763−1768.

(4) (a) Colonna, S.; Gaggero, N.; Manfredi, A.; Casella, L.; Gullotti, M.; Carrea, G.; Pasta, P. *Biochemistry* **1990**, *29*, 10465−10468. (b) Fu, H.; Kondo, H.; Ichikawa, Y.; Look, G. C.; Wong, C.-H. *J. Org. Chem.* **1992**, *57*, 7265−7270. (c) Colonna, S.; Gaggero, N.; Casella, L.; Carrea, G.; Pasta, P. *Tetrahedron: Asymmetry* **1992**, *3*, 95−106.

(5) (a) Allenmark, S. G.; Andersson, M. *Tetrahedron: Asymmetry* **1996**, *7*, 1089−1094. (b) Allenmark, S. G.; Andersson, M. *Chirality*, in press.

(6) (a) Zaks, A.; Dodds, D. R. *J. Am. Chem. Soc.* **1995**, *117*, 10419−10424. (b) Colonna, S.; Gaggero, N.; Casella, L.; Carrea, G.; Pasta, P. *Tetrahedron: Asymmetry* **1993**, *4*, 1325−1330. (c) Allain, E. J.; Hager, L. P.; Deng, L.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1993**, *115*, 4415−4416.

(7) Butler, A.; Walker, J. V. *Chem. Rev. (Washington, D.C.)* **1993**, *93*, 1937−1944.

(8) (a) Wever, R.; Plat, H.; de Boer, E. *Biochim. Biophys. Acta* **1985**, *830*, 181−186. (b) Soedjak, H. S.; Butler, A. *Biochim. Biophys. Acta* **1991**, *1079*, 1−7. (c) Sheffield, D. J.; Harry, T.; Smith, A. J.; Rogers, L. J. *Phytochemistry* **1993**, *32*, 21−26.

(9) Coughlin, P.; Roberts, S.; Rush, C.; Willetts, A. *Biotechnol. Lett.* **1993**, *15*, 907−912.

(10) (a) Everett, R. R.; Butler, A. *Inorg. Chem.* **1989**, *28*, 393−395. (b) Everett, R. R.; Kanofsky, J. R.; Butler, A. *J. Biol. Chem.* **1990**, *265*, 4908−4914.

(11) Yu, H.; Whittaker, J. W. *Biochem. Biophys. Res. Commun.* **1989**, *160*, 87−92.

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Table 1. Results Obtained from Initial Attempts to Asymmetrically Oxidize Sulfides by VBrPO at pH 6.5 and 25 °C

substrate	time (h)	VBrPO (units)	H_2O_2[a]	yield[b] (%)	ee (%)
1	2	1	A	2.0	–
1	2	5	B	1.7 (1.2)	–
2	2	1	A	13 (8.2)	37
2	6	1	A	40 (25)	39
2	2	5	A	20 (5.9)	65
2	2	5	B	31 (4.4)	84
2	2	5	C	42 (2.5)	87

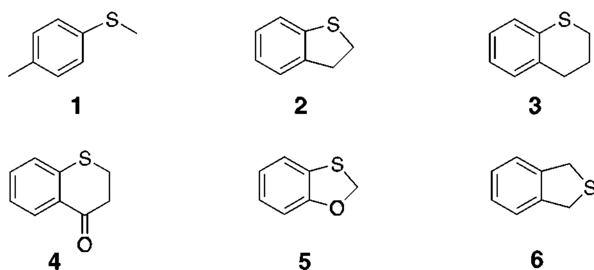
[a] In method A 2 equiv of hydrogen peroxide were added at the beginning of the reaction, whereas in method B the hydrogen peroxide was added in equal aliquots every 5 min and in method C continuous addition was used. [b] Values within brackets correspond to yield obtained in the absence of enzyme.

completely inactive in the absence of halide since attempted oxidations of guaiacol and pyrogallol, which are typical peroxidase substrates, have been reported to be unsuccessful.[12]

In the present investigation VBrPO from the red alga *Corallina officinalis* was used.[11,13] A series of sulfides, previously studied together with CPO,[5] were used as substrates. Below we describe for the first time the use of VBrPO as an efficient catalyst for asymmetric sulfoxidation and compare its catalytic and stereoselective properties with that of CPO.

Results and Discussion

Initial Studies. The first attempt to use VBrPO as a catalyst in asymmetric oxidation of sulfides was carried out with methyl *p*-tolyl sulfide (**1**), one of the most frequently used substrates in studies of asymmetric sulfoxidation. The experimental conditions applied were based on a modification of the method previously used with CPO. The reaction was carried out at pH 6.5 since this is the pH-optimum for the bromination of monochlorodimedone.[8c] The oxidation of **1**, however, was not catalyzed by VBrPO, Table 1.



Indene has been found to be a good substrate for VBrPO-catalyzed hydrobromination[14] and 2-substituted indoles have been suggested to bind into an active site of a vanadium bromoperoxidase.[15] The possibility that VBrPO would be able to catalyze sulfoxidation of 2,3-dihydrobenzothiophene (**2**), a sulfide structurally related to indene and indole, was therefore examined. This proved to be a successful approach, and the first results showing VBrPO to be a potent catalyst in asymmetric oxidation of sulfides were obtained, Table 1. Previously,

(12) Soedjak, H. S.; Butler, A. *Biochemistry* **1990**, *29*, 7974–7981.
(13) Rush, C.; Willetts, A.; Davies, G.; Dauter, Z.; Watson, H.; Littlechild, J. *FEBS Lett.* **1995**, *359*, 244–246.
(14) Mullins, G.; Willetts, A., unpublished results.
(15) Tschirret-Guth, R. A.; Butler, A. *J. Am. Chem. Soc.* **1994**, *116*, 411–412.

Table 2. Results Obtained in VBrPO-Catalyzed Oxidation of 2 at pH 6.5 and 25 °C after 2 h Reaction

scale[a]	temp (°C)	peroxide	yield[b] (%)	ee (%)
1	25	H_2O_2[c]	11.5 (0.2)	87
1/5	25	H_2O_2[c]	16 (<0.1)	72
2/5	25	H_2O_2[c]	16	70
1/5[d]	25	H_2O_2[c]	16	72
1/5	40	H_2O_2[c]	74 (22)	74
1/5	25	tBuOOH[e]	6.3 (2.3)	<1

[a] This denotion of scale is relative to 25 μmol of **2** and 5 units of VBrPO in 3 mL of buffer. [b] Values within brackets correspond to yields obtained in the absence of enzyme. [c] Two equivalents of hydrogen peroxide were added continuously during the 2 h reaction time. [d] The stirring rate was decreased from 700 to 200 rpm. [e] Two equivalents of *tert*-butyl hydrogen peroxide were used, and the reaction was carried out for 5 h.

2 has been shown to be oxidized by CPO to the corresponding (*R*)-sulfoxide in 99% ee and quantitative yield.[5a] In contrast, the (*S*)-configuration was obtained with VBrPO. The mode of addition of hydrogen peroxide was found to be important since both yield and ee increased when the hydrogen peroxide was added progressively throughout the reaction. This phenomenon has been observed previously in CPO-catalyzed sulfoxidation, where it is caused by a competing catalase activity resulting in a hydrogen peroxide decomposition into molecular oxygen and water.

Since **2** shows poor solubility in the aqueous buffer, the possibility of using different cosolvents was examined. The VBrPO activity has been shown to be unaffected by the presence of different cosolvents, e.g. ≤40% of alcohols.[13] A reaction mixture containing 20% ethanol, however, gave a reduced activity in the oxidation of **2**. On the other hand, the use of a minimum volume of 1-propanol to dissolve **2** prior to addition, resulting in a final concentration of 0.6% in the reaction mixture, slightly increased the chemical and optical yield. This modification, together with an automatized, continuous addition of hydrogen peroxide, resulted in reproducible results as long as VBrPO from the same preparation was used. The scale of the reaction was reduced to 1/5 to decrease the consumption of VBrPO, resulting in an increased yield and decreased ee, the rate enhancement most likely being caused by a more efficient mixing, leading to increased contact between the enzyme and the suspended sulfide, Table 2.

tert-Butyl hydrogen peroxide was shown to be a poor oxygen source in VBrPO-catalyzed sulfoxidation, Table 2. Likewise, alkyl hydrogen peroxides have proved to be inefficient for the VBrPO-catalyzed generation of a brominating intermediate, whereas different peracids have been successfully used.[8b,12]

Influence of pH and Temperature. The pH-dependence of yield and ee in VBrPO-catalyzed oxidation of **2** was determined. A relatively flat pH-profile was obtained over the range pH 4–8, which contrasted with the more narrow pH-profile of halogenation reactions which have an optimum at pH 6.5.[8c] For consistency, a pH of 6.5 was kept throughout this investigation as no significant improvement would be expected at a different pH value within the range 5.5–7.0.

A temperature increase to 40 °C resulted in a much higher yield while the ee was not significantly affected, Table 2. It seems likely, though, that the large extent of uncatalyzed oxidation will prevent any improvement in ee.

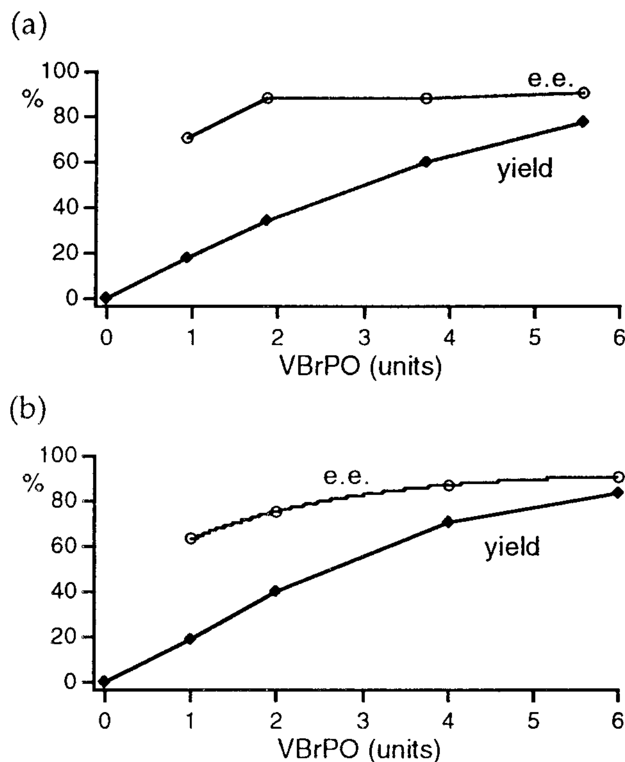


Figure 1. Influence of the enzyme concentration on yield and ee in the VBrPO-catalyzed oxidation of **2** for 2 h (a) and **3** for 16 h (b), respectively at 25.0 °C.

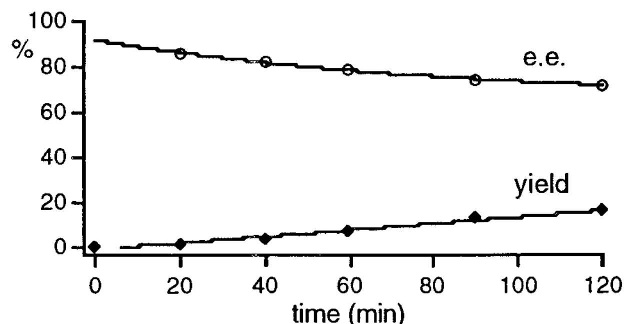


Figure 2. Development of yield and ee during the oxidation of **2** with 1 unit of VBrPO. The hydrogen peroxide was added at a rate corresponding to an addition of 2 equiv after 2 h.

Variation of the Enzyme/Substrate Ratio. The effect of changing the enzyme/substrate ratio on yield and ee is shown in Figure 1. A yield of 76% and an ee of 91% was achieved by using 5.6 units of VBrPO, and the profile in Figure 1 shows that the yield might be further increased although a relatively large amount of catalyst would have to be used. The curve shape is as expected from Michaelis–Menten kinetics.[5b] The constant rate of product formation from **2** found during the first 2 h at low enzyme concentration is shown in Figure 2.

Effects of Hydrogen Peroxide. Hydrogen peroxide was found to have a significant effect on both yield and ee in the VBrPO-catalyzed oxidation of **2**. This was clearly demonstrated in an approach designed to obtain a high ee together with a high yield by prolonging the reaction time. In this way the hydrogen peroxide can be added at a slower rate and its concentration kept at a lower level during the reaction. This proved to be an excellent method since oxidation of **2** during 16 h resulted in 98% yield and 90% ee, Figure 3.

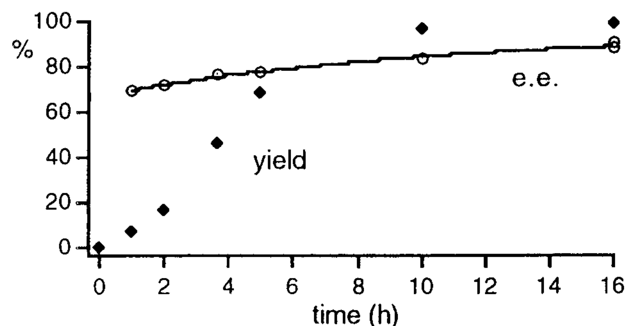


Figure 3. Yield and ee as a function of the reaction time in the oxidation of **2**. The hydrogen peroxide was added at a rate corresponding to a total addition of 2 equiv at the end of each reaction.

Previous investigations have shown that, in contrast to CPO, disproportionation of hydrogen peroxide to molecular oxygen and water by VBrPO only occurs in the presence of halide.[10] Prior to our investigation, however, catalytic turnover has only been observed in the presence of halide. With a suitable secondary substrate (e.g. monochlorodimedone) present, the catalyzed bromination has been found to totally dominate over any oxygen formation.[10b] The possibility that VBrPO could promote dismutation of hydrogen peroxide during catalytic turnover with sulfides was therefore studied, but no oxygen could be detected in the presence of **2**. However, by exchanging the sulfide for an equal concentration of bromide, significant O_2 release was observed. Interestingly, this was inhibited completely by the presence of **2** in the bromide-containing reaction mixture. Together these results might indicate that the catalysis of sulfoxidation predominates over the formation of a brominating intermediate, but alternatively could reflect a reaction between the sulfide and the brominating intermediate.

The lack of oxygen formation in the catalyzed sulfoxidation reaction ruled out the disproportionation of hydrogen peroxide to account for the disfavorable effect of high levels of hydrogen peroxide on the reaction. An inhibition of vanadium bromoperoxidase catalysis by hydrogen peroxide has been suggested earlier[16] and should be the most likely cause here as well. The rate of product formation, illustrated by Figure 3, shows a distinct sigmoidal curvature which can be explained by an inhibition by hydrogen peroxide over the shorter reaction times.

Another remarkable feature of the hydrogen peroxide influence on VBrPO is demonstrated in Figure 2. The ee continuously decreases during the progress of the reaction. The uncatalyzed reaction was found to be negligible (<0.1%, Table 2) over the same 2 h period and consequently cannot account for this phenomenon. Instead, an inhibition by hydrogen peroxide could account for the decrease in stereoselectivity, an assumption supported by the data shown in Figure 3. The prolonged reaction time would rather decrease the ee due to a higher extent of uncatalyzed reaction; however, the opposite is observed. A higher concentration of hydrogen peroxide will be present when hydrogen peroxide is added faster over shorter reaction times, thus exacerbating any inhibitory effects on the enzyme.

(16) (a) Soedjak, H. S.; Walker, J. V.; Butler, A. *Biochemistry* **1995**, *34*, 12689–12696. (b) Everett, R. R.; Soedjak, H. S.; Butler, A. *J. Biol. Chem.* **1990**, *265*, 15671–15679.

Table 3. Results Obtained after 16 h Reaction in VBrPO-Catalyzed Asymmetric Oxidation of Sulfides at pH 6.5 and 25 °C

substrate	VBrPO (units)	H_2O_2[a] (equiv)	yield[b] (%)	ee (%)	abs config
1	1	2	6 (6)	0	–
2	1	2	99	89	*S*
2	1	1.1	99 (38)	90	*S*
3	1	2	25 (12)	49	*S*
3	1	1.1	19 (6.1)	63	*S*
3	6	1.1	84	91	*S*
4	1	1.1	1.3 (0)	–	–
5	1	1.1	25 (9.0)	38	*R*
6	1	1.1	99 (58)	–	–

[a] The continuous addition of hydrogen peroxide was completed after 16 h reaction time. [b] Values within brackets correspond to yields obtained in the absence of enzyme.

Substrate Recognition. A series of sulfides were oxidized, using the optimized experimental conditions, to give an initial view of the capacity of VBrPO as an asymmetric catalyst, Table 3. Again compound **1** appeared as a nonsubstrate as the uncatalyzed reaction completely dominated. The steric tolerance of the active site was explored by increasing the size of the sulfide from the five-membered ring in **2** to a six-membered ring, as in thiochroman **3**. However, the oxidation of **3** was less effectively catalyzed, and some further experiments to improve the outcome of the reaction were carried out. Decreasing the excess of hydrogen peroxide reduced the contribution from the uncatalyzed reaction leading to a decreased yield and an increased ee. By increasing the VBrPO/substrate ratio both yield and ee were increased (Table 3 and Figure 1b).

The oxidation of thiochroman-4-one (**4**) was not catalyzed by VBrPO (Table 3) although the reason for this nonsubstrate behavior remains to be elucidated.

1,3-Benzoxathiole (**5**), a structural analogue of **2**, was oxidized by VBrPO with the same stereopreference shown for **2** and **3** (Table 3). The designation of (*R*)-configuration is only a consequence of the Cahn–Ingold–Prelog nomenclature. Although no further attempts to improve the yield and ee have been performed, this initial result is of interest since **5** has been shown to act as a competitive inhibitor of CPO on attempted oxidation.[5b]

1,3-Dihydrobenzo[*c*]thiophene (**6**), the symmetric analogue of **2**, was oxidized in quantitative yield. This indicates that further substrate variations may be tolerated by the enzyme.

Experimental Section

General Procedures. The continuous addition of hydrogen peroxide was carried out by use of a syringe pump (Sage Instruments Model 352; 100 μL or 250 μL syringes). Oxygen evolution was measured by a Clark-electrode linked to an oxygraph and O_2-electrode control box (Hansa Tech Instruments). Methyl *p*-tolyl sulfide and the corresponding (*R*)- and (*S*)-sulfoxides were obtained from Aldrich. The synthesis and characterization of **2**–**6** and the corresponding sulfoxides have been reported previously.[5] The concentration of hydrogen peroxide in the buffer solutions was determined spectrophotometrically at 240 nm (ϵ = 43.6 M^{-1} cm^{-1}).[8c] For further details regarding instrumentation and general procedures, see refs 5a,b.

Enzyme Preparation and Activity Measurement. The alga *C. officinalis* was collected from the intertidal zone at Exmouth, UK. Washed biomass (450 g) was mascerated in 1 L of chilled 50 mM Tris-sulfate buffer, pH 8.0. Following centrifugation (3000*g*, 20 min at 4 °C), the protein precipitated after treatment with 60% ammonium sulfate and was centrifuged out of solution (3000*g*, 20 min at 4 °C) and resuspended in minimum volume of 50 mM Tris-sulfate buffer, pH 8.0. Following overnight dialysis at 4 °C against 2 L of the same buffer containing 1 mM sodium orthovanadate, the sample was centrifuged (10000*g*, 20 min at 4 °C) and any precipitated material discarded. The dialysate was frozen at −15 °C and lyophilized until completely desiccated. No significant loss in enzyme activity (total units extracted from source material) occurred throughout these procedures, or on subsequent storage of the freeze-dried preparation at 4 °C for periods up to 6 months.

The purified enzyme was dissolved in 50 mM Tris-sulfate buffer, pH 8.0 and stored at 4 °C. The enzyme stock solutions had a specific activity in the range of 135–175 units/mL, determined spectrophotometrically at 25 °C by the monochlorodimedone assay using the extinction coefficient of monochlorodimedone of 19.9 mM^{-1} cm^{-1} at 290 nm.[13] A 50 mM phosphate buffer, pH 6.0 was used.

Enzymatic Oxidation. The initial oxidation reactions were performed by mixing sulfide (25 μmol) and 5 units of VBrPO (stock solution) in 50 mM phosphate buffer, pH 6.5. The mixture was stirred and thermostated at 25.0 °C. The hydrogen peroxide (50 μmol) was dissolved in the buffer used (0.160 M) and added either as one aliquot to initiate the reaction, stepwise as a series of equal small portions every 5 min, or continuously using an auto injector. The total volume of the reaction mixture was 3 mL. The reaction was quenched after the requisite reaction time (2 h) with 1 mL of a saturated sodium sulfite solution and extracted with 3 × 2 mL of dichloromethane. The organic phase was dried ($MgSO_4$), filtered, and then analyzed by enantioselective gas chromatography.[5]

All reactions, except for those referred to in Table 1, were carried out with 0.5–0.6% of 1-propanol.

The buffers used for the pH-dependence study were either 50 mM phosphate buffer (pH 6.5–8.0) or 50 mM citrate–phosphate buffer (pH 4.0–6.3).

By an overall volume reduction the reaction was scaled down to 1/5 and extraction by 3 × 1 mL dichloromethane was used in the workup procedure. Reactions referred to in Table 3 and all figures were carried out at this reduced scale.

In the experiment with *tert*-butyl hydrogen peroxide, 10 μmol was added as a single aliquot to the reaction mixture.

The reaction represented in Figure 2 was studied at a rate of hydrogen peroxide addition of 1 equiv/h, whereas at each reaction time given in Figure 3, 2 equiv has been added.

An estimation of the background oxidation in the absence of enzyme under the same reaction conditions was performed in most cases.

Measurement of Oxygen Evolution. The Clark-electrode was calibrated using dithionite-containing water (zero) and air-saturated water (0.247 mM O_2).[16a] The reaction buffer was sparged with nitrogen gas prior to use to minimize the O_2 content, and the background rate of O_2 formation was measured and compensated for. The assay was initiated by addition of hydrogen peroxide in one aliquot. The total volume was 1.2 mL and consisted of 8 mM sulfide, 0.33 units of VBrPO, and 8 mM hydrogen peroxide in 50 mM phosphate buffer, pH 6.5. Corresponding reactions were carried out with 9 mM KBr with or without sulfide present.

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