Evidence for Organic Substrate Binding to Vanadium Bromoperoxidase

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Vanadium-containing bromoperoxidases (V-BrPO) are unique enzymes isolated primarily from marine algae.1-4 Halogenated natural products, including chiral halogenated terpenes5 and indoles,6 are abundant in marine organisms, and the biosyntheses of these products are thought to be mediated by haloperoxidases. The role of haloperoxidase in direct halogenation or in production of diffusible oxidized bromine species (HOBr, Br2, Br3-) is a topic of much current interest.1-4 V-BrPO catalyzes the peroxidative halogenation (Cl-, Br-, I-) of organic substrates and the halide-assisted disproportionation of hydrogen peroxide, forming dioxygen (Scheme 1).7-9 Under conditions under which V-BrPO reactions are usually carried out (i.e., pH 6.5 (refs 1-4 and references therein)), oxidized bromine species (e.g., HOBr, Br2, Br3-, Enz-Br) cannot be detected because the reaction of these species with H2O2 or organic substrates is too fast. However, in the absence of an organic substrate, Br2 was detected under conditions (i.e., pH 5)10 which stabilized it with respect to formation of HOBr and Br2 or oxidation of H2O2. We now report the first evidence that V-BrPO can bind certain organic substrates (i.e., indoles) and that the active brominating moiety under these conditions is not an enzyme-released bromine species (e.g., HOBr, Br2, Br3-).

V-BrPO catalyzes the bromination of 2-methylindole, 2-phenylindole, and phenolsulphonephthalein (phenol red) to 3-bromo-2-methylindole,11 3-bromo-2-phenylindole,11 and 3',3''3,5''-tetrabromophenolsulphonephthalein (bromophenol blue),12 respectively. In a mixture of 2-methylindole and phenol red, V-BrPO preferentially brominates 2-methylindole, as shown by the lag phase in the appearance of bromophenol blue (Figure 1). The lag phase increases with increasing concentration of 2-methylindole, although the rate of bromination of phenol red remains independent of the 2-methylindole concentration. By comparison, the lag phase under these conditions, bromination of 2-methylindole and phenol red by HOBr (Figure 2). Under these conditions, bromination of 2-methylindole and phenol red occurs concurrently and an increase in the 2-methylindole concentration leads to a decrease in the appearance of bromophenol blue. This differential reactivity between V-BrPO and HOBr suggests that HOBr is not the active brominating species in the V-BrPO-catalyzed reactions of 2-methylindole, a situation which could arise by indole binding to V-BrPO (see below).

Further evidence that the enzyme-catalyzed bromination of indole is not mediated by enzyme-released HOBr comes from a comparison of the rate of V-BrPO-catalyzed bromide-assisted disproportionation of hydrogen peroxide (forming O2) in the presence and absence of 2-methylindole versus the rate of oxidation of H2O2 by HOBr (forming O2) in the presence and absence of 2-methylindole. The normalized rate of V-BrPO-catalyzed O2 disproportionation of H2O2 increases with increasing concentration of 2-methylindole, whereas the normalized rate of HOBr oxidation of H2O2 decreases with increasing concentration of 2-methylindole (Figure 3). This result is consistent with HOBr not being the active brominating species in the V-BrPO-catalyzed reactions of 2-methylindole.

Scheme 1

V-BrPO + Br- + H2O2 \rightarrow \text{"Br"}^+\text{-like intermediate} (eg., HOBr, Br2, Br3-, Enz-Br)

\[ \text{Br-Org} + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \text{Br}^- \]

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(10) de Boer, E.; Wever, R. J. Biol. Chem. 1988, 263, 12236-12332.
(11) 3-Bromo-2-methylindole and 3-bromo-2-phenylindole were identified by FD mass spectral analyses and 1H and 13C NMR. The absorbance changes between 2-methylindole and 3-bromo-2-phenylindole are too small to allow measurement of the rate of bromination of 2-methylindole spectrophotometrically.
formation in the presence and absence of 2-methylindole [i.e., \( \frac{v(O_2)}{v(O_2)_0} \), where \( v(O_2)_0 \) is the initial rate of \( O_2 \) formation in the absence of organic substrate] decreases as the concentration of 2-methylindole increases (Figure 3), showing that indole bromination is favored over \( H_2O_2 \) oxidation. To compare the enzymatic reaction to that using HOBr, hypobromite was added to the \( H_2O_2 \) solution by syringe pump at a rate adjusted to match the activity of V-BrPO. In distinct contrast to V-BrPO, the normalized rate of \( O_2 \) formation from the oxidation of \( H_2O_2 \) by HOBr in the presence and absence of 2-methylindole is constant \( \frac{v(O_2)}{v(O_2)_0} \approx 1 \) as the concentration of 2-methylindole is increased (Figure 3), indicating that HOBr preferentially oxidizes \( H_2O_2 \) and that enzyme reaction does not occur by released HOBr. The same kinetic behavior is observed for 2-phenylindole (data not shown).

Indole binding to V-BrPO is further indicated by quenching of the fluorescence of 2-phenylindole by V-BrPO (Figure 4). By contrast, bovine serum albumin, a protein of molecular weight similar to that of V-BrPO, did not quench the fluorescence of 2-phenylindole (Figure 4). Apo-V-BrPO also quenches the fluorescence of 2-phenylindole, indicating that indole binding does not require active-site bound vanadium.

In summary, these results show that the bromination reactivity of V-BrPO toward substituted indoles is not consistent with enzyme-released HOBr. These results are also the first demonstration that organic substrates can bind to V-BrPO from both competitive kinetic results and fluorescence quenching results. A mechanistic scheme for V-BrPO involving substrate binding is proposed in Scheme 2: V-BrPO binds \( H_2O_2 \) and Br\(^-\), leading to a putative "enzyme-bound" or "enzyme-trapped" brominating moiety, EBr, which in the absence of an indole releases HOBr (or other bromine species, e.g., Br\(_2\), Br\(_3\)). When indole is present, it binds to V-BrPO, preventing release of an oxidized bromine species and leading to indole bromination. The present studies do not address whether \( O_2 \) formation in the presence of the indole could arise from reaction of the substrate-bound-EBr species with \( H_2O_2 \) or only by reaction of the released species with \( H_2O_2 \). We are extending these studies to investigate how the nature of other organic substrates directs the reactivity of V-BrPO.

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