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Bromination of phenols in bromoperoxidase-catalyzed oxidations

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ABSTRACT

Phenol and *ortho*-substituted derivatives furnish products of selective *para*-bromination, if treated with sodium bromide, hydrogen peroxide, and the vanadate(V)-dependent bromoperoxidase I from the brown alga *Ascophyllum nodosum*. Relative rates of bromination in morpholine-4-ethane sulfonic acid (MES)-buffered aqueous *tert*-butanol (pH 6.2) increase by a factor 32, as the *ortho*-substituent in a phenol changes from F via Cl, OCH_3, $C(CH_3)_3$, and H to CH_3. The polar effect in phenol bromination by the enzymatic method, according to a Hammett-correlation ($\rho=-3$), compares to reactivity of molecular bromine under identical conditions ($\rho=-2$). Hypobromous acid is not able to electrophilically substitute bromine for hydrogen at pH 6.2 in aqueous *tert*-butanol. The tribromide anion behaves in MES-buffered aqueous *tert*-butanol as electrophile ($\rho\sim-3$), showing a similar polar effect in phenol bromination as molecular bromine.

1. Introduction

Bromide, dissolved in ocean water ($c_{Br^-}\sim1$ mM) or deposited in minerals, is the major resource for production of molecular bromine, the most important reagent for synthesis of organobromines.[1–3] In an oxidative environment, such as the marine boundary layer or in hydrogen peroxide-producing compartments of living cells, bromide is rapidly oxidized into hypobromous acid, molecular bromine, and tribromide, to mention the major products.[4] All products of peroxidative bromide oxidation are able to convert hydrocarbons into organobromines, for example, as part of natural product synthesis, although with different functional group selectivity.[5]

Naturally occurring organobromines show considerable structural diversity regarding site of bromosubstitution and carbon skeleton the halogen atom is attached to.[6] Simple bromoalkanes, such as bromomethane or bromopentanes, are similarly found in the environment as more complex metabolites, for example, bromosubstituted fatty acids, phenylpropanes, or amino acids. Probably the most complex structures arise from highly substituted, chiral, cyclic terpenes, and acetogenins.[7,8] Functional groups that appear to be particularly receptive for bromination in putative biogenic progenitors, proposed on the basis of purely structural arguments, are carbon–carbon double bonds of donor-substituted alkenes or arenes, and the aromatic core of π-excess heteroarenes.[9,10]

Biosynthetic pathways for organobromine formation are largely unexplored.[11] From biochemical experiments it is known, that hydrogen peroxide oxidizes bromide at pH 6–7, if catalyzed by vanadate(V)-dependent bromoperoxidases (V_{Br}POs).[12–14] According to the general mechanism of V_{Br}PO-catalyzed bromide oxidation (Scheme 1),[12,14] hydrogen peroxide binds first to vanadate(V), in a reversible proton-assisted step. The peroxido-loaded cofactor is the active form of the enzyme and able react with bromide in a second reversible reaction, to furnish a structurally uncharacterized but kinetically relevant intermediate. Oxygen atom transfer from the peroxido complex to bromide follows, possibly resulting in a short-lived intermediate,[15] which rapidly hydrolyzes, to regenerate by the end of the catalytic cycle the resting state of the enzyme. Hydrolysis of the assumed intermediate furthermore provides one molecule of hypobromous acid, which is considered to be the primary product of enzymatic bromide oxidation (Scheme 1, top).[10,16] In an aqueous solution of bromide, such as ocean water, hypobromous acid, rapidly equilibrates to provide a mixture of molecular bromine, tribromide (Scheme 1, bottom), and possibly further products.[3,12,17]

The chemical behavior of hypobromous acid, bromine, and tribromide toward functional groups relevant for explaining organobromine formation in nature is distinctively different.

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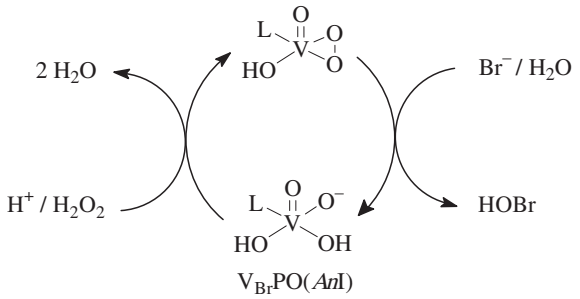
• *bromoperoxidase-catalyzed oxidation – primary product formation*



• *thermodynamic equilibration – secondary product formation*

$$HOBr + Br^- + H^+ \overset{K^1}{\rightleftharpoons} Br_2 + H_2O$$

$$Br_2 + Br^- \overset{K^2}{\rightleftharpoons} Br_3^-$$

Scheme 1. Proposed mechanism for bromide oxidation catalyzed by the vanadate(V)-dependent bromoperoxidase I ($V_{Br}PO$) from *Ascophyllum nodosum* (*An*) (top), and equilibria associated with secondary product formation in bromide-containing brines (bottom) [K^1=for example, 1.45×10^8 M^{-2} for H_2O at 20 °C (I=0.1 M, pH 2.6–3.8)[26] or 1.04×10^8 M^{-2} in H_2O at 25 °C (pH 1.5);[27] K^2=16.9 M^{-1} in H_2O at 25 °C[28]].[3]

Hypobromous acid bromohydroxylates alkenes to afford 2-bromoalcohols (bromohydrins). Unless activated by a strong Brønsted-acid, hypobromous acid is comparatively inert toward arenes and not able to electrophilically displace bromine for hydrogen. Tribromide, a weakly bound adduct between bromine and bromide, shows chemical reactivity at the borderline between nucleophilic and electrophilic,[18–20] and reacts with alkenes in polar aprotic solvents with different selectivity for vicinal dibromination than molecular bromine.[21–23] In protic solvents, such as water[24] or acetic acid,[25] tribromide seems to predominantly serve as safe-to handle substitute for bromine, liberating the halogen at a steady rate for converting, for example, phenol, 2-naphthol, aniline, and other strongly activated arenes into bromoderivatives with bromine-like selectivity.

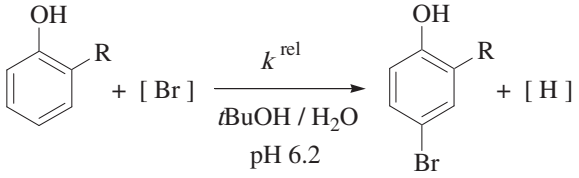
In bromoperoxidase chemistry, the chemical nature of the reagent that controls reactivity and selectivity for carbon–bromine bond formation, so far has not been determined. Endogeneous substrates that bind to the active site in vanadate(V)-dependent bromoperoxidases are bromide and hydrogen peroxide. A binding site for an organic substrate close to the vanadate co-factor has not yet been identified. This observation correlates with the lack in hydrocarbon specificity and regio- or stereoselectivity for carbon–bromine bond formation in bromoperoxidase-catalyzed oxidations.[3,10]

In a project dealing with biomimetic synthesis of brominated natural products, we encountered the problem to predict selectivity for preparing the correct starting material via bromoperoxidase-catalyzed oxidation. We therefore specified the chemical nature of the bromoelectrophile that is directly involved in carbon–bromine bond formation in enzymatic reactions. The major results from a combined synthetic and competition kinetic study on phenol bromination show that bromination in oxidations catalyzed by the vanadate(V)-dependent bromoperoxidase I from the brown alga *Ascophyllum nodosum* [$V_{Br}PO$(*An*I)] shows considerable parallels to the chemistry of molecular bromine in water. This information allowed us to derive a reaction model for explaining a striking difference between phenol- and anisol bromination.

2. Results and discussion

2.1. Concept

The strategy used in this study to reference reactivity and selectivity in bromoperoxidase-catalyzed oxidation combines a product study for determining individual reactivity of *ortho*-substituted phenols in enzymatic reactions with competition kinetics for determining polar substituent effects on rates in phenol bromination. The results from bromoperoxidase-catalyzed oxidations subsequently were compared to controls, using hypobromous acid, molecular bromine, and tetrabutylammonium tribromide as bromination reagents (Scheme 2).



[Br] = Br_2, HOBr, NBu_4Br_3, $NaBr / H_2O_2 / V_{Br}PO$(*An*I)

1, **2**	R
a	H
b	CH_3
c	$C(CH_3)_3$
d	OCH_3
e	Cl
f	F

Scheme 2. Summary of bromination reagents and indexing of phenols used in this study ([H]=e.g., H^+).

The approach in chemistry to quantify polar substituent effects on chemical reactivity is correlation analysis.[29,30] In the present study, we used the phenol competition system to determine relative rates of bromination in bromoperoxidase-catalyzed oxidations, and in non-enzymatic references (Scheme 2). Correlation of decadic logarithms of relative rate constants $\lg k^{rel}=\lg(k^R/k^H)$ with substituent constants[31] σ_m, according to equation 1, provides the reaction parameter ρ. Sign and magnitude of ρ characterize responsivity of polar substituent effects for accelerating or retarding the rate determining step, and helps to characterize the chemical nature of the reagent that is directly involved in the reactivity determining step.

$$\lg k^{rel} = \lg \frac{k^R}{k^H} = \rho \cdot \sigma_m \quad (1)$$

2.2. Bromoperoxidase isolation and preparation of bromination reagents

The bromoperoxidase used in this study is the isoenzyme I from the brown alga *A. nodosum* [$V_{Br}PO$(*An*I), EC 1.11.1.18, PDB-code 1QI9].[32,33] The enzyme was isolated from specimen collected in Roscoff, France, following an established freeze–drying, milling, and liquid–liquid partitioning process.[34] The crude bromoperoxidase fraction, which precipitated upon addition of acetone from the final extract of the extraction process, was dialyzed against sodium metavanadate for restoring bromoperoxidase activity of the