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HALONIUM ION-INDUCED BIOSYNTHESIS OF CHLORINATED MARINE METABOLITES

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Key Word Index—*Coralina* sp.; Rhodophyta; biological halogenation; bromoperoxidase; enzymatic bromochlorination; marine chlorination; role of bromonium ions and iodonium ions; seawater.

Abstract—Bromoperoxidases do not directly oxidize the chloride ion; nevertheless, in the presence of bromide ions, chloride ions and hydrogen peroxide, bromoperoxidases react with alkenes and alkynes to produce bromochloro-derivatives. This same reaction is catalysed when seawater is the source of chloride and bromide ions. This suggests that bromonium ion-induced biosynthesis of chlorinated metabolites occurs in marine environments. The role of iodonium ions in the biosynthesis of chlorinated metabolites is also discussed.

INTRODUCTION

A most striking feature of the marine natural products literature is the frequency with which halogenated compounds have been described. There are now over 700 marine halometabolites which have been isolated and characterized, primarily from the algae. In a recent study of marine plants, four out of every 100 lipid molecules extracted from the tissue contained either a chlorine or bromine atom [1].

The enzymatic incorporation of chlorine into organic metabolites is a well-known process in man [myeloperoxidase (EC 1.11.1.10) in leucocytes] and terrestrial fungi [chloroperoxidase (EC 1.11.1.10) in *Caldariomyces fumago*], but to date no enzyme capable of chlorination has been discovered in marine algae [2, 3]. How, then, do these marine organisms incorporate chlorine into halometabolites?

Bromoperoxidases (EC 1.11.1.7, enzymes that in the presence of hydrogen peroxide oxidize I^- and Br^-, but not Cl^-) are ubiquitous in marine algae. A survey in the Caribbean established the presence of 55 algae containing bromoperoxidase [4]. Characterization of these enzymes has subsequently been carried out on the red algae *Rhodomela larix* and *Bonnemaisonia hamifera* [5, 6], and on the green algae *Penicillus capitatus* and *Rhipocephalus phoenix* [7, 8].

Peroxidative bromination has been suggested to be responsible for the synthesis of a wide variety of marine biometabolites. Several biomimetic studies have been conducted which implicate an enzyme-formed bromonium ion in the synthesis of brominated phenols [9], in the synthesis of brominated ketones from 3-oxoalkanoic acids [6], and for initiating cyclization reactions [10]. We have recently shown that the bromoperoxidase from cow's milk (lactoperoxidase) can convert alkenes to α-, β-bromohydrins; alkynes to α-bromo-, α,α-dibromo- and α,α′-dibromoketones; and cyclopropanes to α,γ-bromohydrins (Table 1).

It has also been postulated that peroxidative bromination is responsible for the formation of at least some chlorometabolites. A biosynthetic sequence has been proposed that consists of addition of the bromonium ion (Br^+) to an olefinic bond, followed by addition of the chloride ion (Cl^-) to the intermediate, as shown in Fig. 1. Such a sequence has been proposed for the synthesis of non-isoprenoid and terpenoid compounds containing adjacent bromine and chlorine [10, 13, 14]. However, no experimental evidence has been obtained to test these assumptions.

RESULTS AND DISCUSSION

In earlier studies [15], we demonstrated that in the presence of molar amounts of bromide ion, the bromo-

Table 1. Bromoperoxidase reaction with alkenes, alkynes and cyclopropanes [11, 12]

$$\text{Substrate} + Br^- \text{ (mM)} \xrightarrow[H_2O_2]{\text{Lactoperoxidase}} \text{Products}$$

Substrate	Products
$R\text{–}CH{=}CH_2$	$R\text{–}CH(OH)\text{–}CH_2Br + R\text{–}CH(Br)\text{–}CH_2OH$
$R\text{–}CH_2C{\equiv}CH$	$R\text{–}CH_2C(=O)CH_2Br + R\text{–}CH_2C(=O)CHBr_2 + R\text{–}CH(Br)C(=O)CH_2Br$
R △	$R\text{–}CH(OH)CH_2CH_2Br$

R=H, Me, φ, etc.

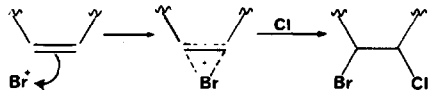

Fig. 1. Proposed bromonium ion-induced biosynthesis of bromochloro metabolites.

Table 2. Bromoperoxidase reaction forming α,β-dibrominated products from alkenes and alkynes [15]

$$\text{Substrate} + Br^- \text{ (M)} \xrightarrow[H_2O_2]{\text{Lactoperoxidase}} \text{Dibrominated product}$$

Substrate	Dibrominated product
R–CH=CH$_2$	R–CH(Br)–CH$_2$(Br)
R–C≡CH	R–C(Br)=CH(Br)

R=H, Me, CH_2OH, ϕ, etc.

peroxidase, lactoperoxidase, also forms α,β-dibrominated products from alkenes and alkynes (Table 2). For example, with allyl alcohol (R = CH_2OH) at 1 M Br^- over 90% of the total product was the dibrominated derivative. However, when the lactoperoxidase reaction was run in molar amounts of chloride ion along with millimolar amounts of bromide ion, this bromoperoxidase yielded an α,β-bromochloro-compound according to the sequence presented in Fig. 1 (Table 3).

Knowing that seawater was rich in halide salts with a Cl^-:Br^- ratio (500 mM Cl^-:1 mM Br^-) favorable for formation of the bromochloro-compounds, we tested a marine bromoperoxidase using seawater as a source of halide ions.

A crude algal bromoperoxidase isolated from a *Coralina* sp., hydrogen peroxide and allyl alcohol (an alkene substrate) were added to seawater. The results obtained (Fig. 2) clearly demonstrate bromonium ion-induced synthesis of bromochloro-compounds under conditions simulating the marine environment. Presumably, the products are formed by oxidation of Br^- to the bromonium ion which adds to the carbon–carbon double bond, this is then followed by the passive addition of Cl^- to the intermediate, as postulated from biomimetic studies.

From the data presented above, many intriguing speculations can be made. To take full advantage of the bountiful Cl^- supply in the marine sphere, why would a marine organism not have an enzyme that could oxidize Cl^-? We suggest that bromoperoxidases found in these organisms are optimized to the particular conditions of the reactor (i.e. the ocean) and to the chemical nature and biological function of the halogenated compounds desired. Do bromochloro-derivatives have novel biological properties not possessed by either chlorinated or brominated products? The organism, a good enzymologist, would recognize that a bromoperoxidase would ignore the vast excess of Cl^- present and control the minimal amount of chlorinated product needed by incorporating only as much chloride as there is bromide present. The organism, as a bright chemist, would see the enhanced versatility of a bromochloro-metabolite over a dichloro one, since halogen removal is far easier for C–Br than it is for C–Cl. Thus, the organism could synthesize a monochlorinated metabolite (BrCl addition, followed by Br loss) without needing to chlorinate directly. Direct chlori-

Table 3. Effects of concentrations and ratios of Cl^- and Br^- on product formation from allyl alcohol with lactoperoxidase [16]

$$\text{CH}_2(\text{OH})\text{–CH=CH}_2 + Br^- + Cl^- \xrightarrow[H_2O_2]{\text{Lactoperoxidase}} \text{Products}$$

Product			Product composition (%)						
	mM	NaCl	0	0	1200	2000	2000	2000	200
	mM	KBr	200	2400	1200	20	6	0	0
	Ratio	Cl^-/Br^-	—	—	1	100	333	—	—
OH OH Br CH$_2$–CH–CH$_2$*			54	6	5	6	3	0	0
OH Br Br CH$_2$–CH–CH			46	94	34	8	5	0	0
OH Cl Br CH$_2$–CH–CH$_2$*			0	0	61	86	92	0	0

*Positional isomers produced in approximately 1:1 ratio.

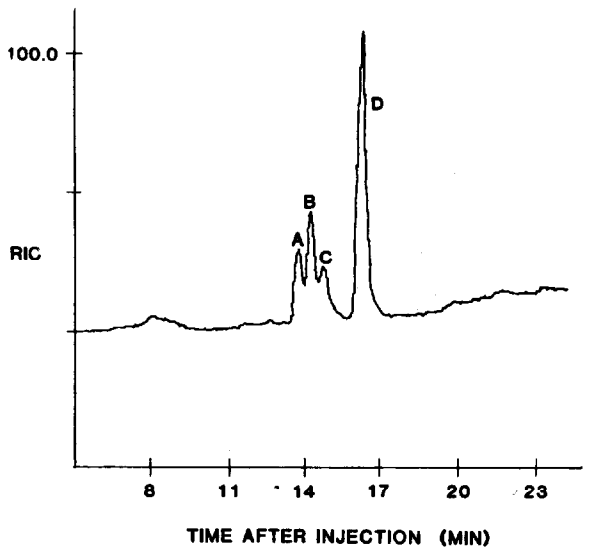

Fig. 2. Reconstructed ion chromatogram for the products formed in the enzymatic reaction of bromoperoxidase from *Coralina* sp., allyl alcohol, hydrogen peroxide and seawater. (A) 2, 3-bromochloro-1-propanol; (B) 3-bromo-1,2-propanediol; (C) 2-bromo-1,3-propanediol; and (D) 2,3-dibromo-1-propanol.

nation could also lead to more side-product formation than this indirect chlorination.

Like bromonium ion-induced biosynthesis, iodonium ion-induced biosynthesis can occur. Here, an enzyme that can only oxidize I^- (not Br^- or Cl^-), an iodoperoxidase, can passively incorporate Cl^- as well as Br^- (see Fig. 3). In addition, halogen exchange with Cl^- from seawater is far easier on organic-bound iodine than on organic-bound bromine. Such a reaction sequence is postulated to occur in the formation of chloromethanes from iodomethanes in the marine environment [6].

Therefore, we suggest that in the marine environment bromonium ion-induced (as well as iodonium ion-induced) biosynthesis of chlorinated matabolites does occur. Figure 4 illustrates some marine metabolites that might be formed from bromoperoxidases.

In conclusion, we note that there have been two reports claiming the discovery of chloroperoxidase in the marine organisms *Cystoclonium purpureum* [20] and *Iotrochota birotulata* [21]. However, the red alga *C. purpureum* could only incorporate chloride in the presence of bromide and only in the presence of substrates that lost formaldehyde during the halogenation reaction. Chlorination by the sponge *I. birotulata* was measured only by the monochlorodimedon assay; an assay well known to be non-specific for chloride ions. In addition, both of these peroxidases exhibited atypical characteristics of a chloroperoxidase: *C. purpureum* could not oxidize iodide, while *I. birotulata* could not oxidize bromide. Therefore the true identity of these peroxidases must await further confirmation.

EXPERIMENTAL

The reaction mixture consisted of 2 units bromoperoxidase (prepared according to U.S. Patent No. 4,247,641 [22] from the alga *Coralina* sp. (obtained along the coast of La Jolla, CA), 20 mM H_2O_2 and 10 mM allyl alcohol in 10 ml seawater (collected off the Monterey, CA coast). The mixture was incubated with stirring at room temp. for 15 min.

Aliquots of reaction mixtures (10 μl) were injected into a Finnigan 4021 gas chromatograph–mass spectrometer. GC conditions: 1.8 m × 4 mm coiled, glass column packed with Tenax-

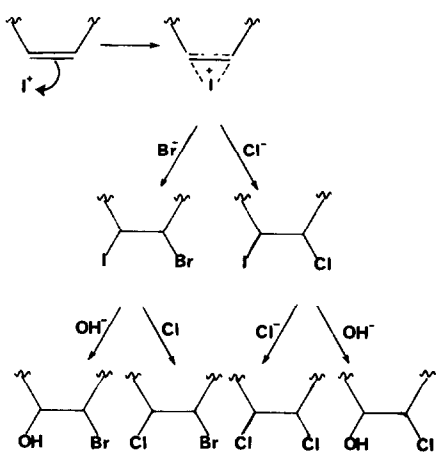

Fig. 3. Proposed iodonium ion-induced biosynthesis of bromoiodo- and chloroiodo-metabolites, and their respective mono- and dihalo-derivatives.

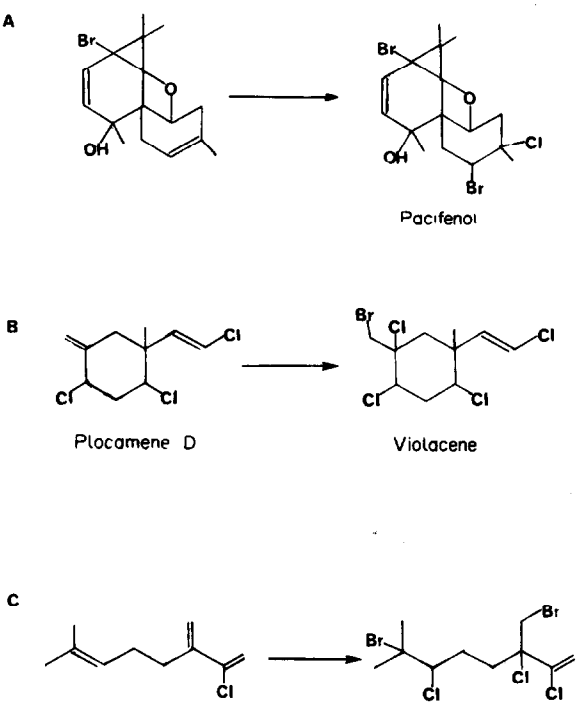

Fig. 4. Proposed involvement of bromonium ion-induced biosynthesis of specific marine algae bromochloro-metabolites [17–19]. (A) Metabolites found in *Laurencia nipponica* Yamada; (B) metabolites found in *Plocamium violaceum*; and (C) metabolites found in *Chondrococcus hornemanni*.

GC (80–100 mesh); carrier gas: He, 25 ml/min; column temp. from 100° to 250° at a rate of 10°/min, and then held at 250° for 10 min; the injector and jet separator temps. 260°. MS condition: EI mode, 70 eV; mass range from *m/z* 40 to 400 scanned every 2 sec.

Confirmations of the identities of the reaction products were made by GC retention times and mass spectral comparisons with authentic standards.

The product with R_t 13 min gave the mass spectrum diagnostic for 2,3-bromochloro-1-propanol: *m/z* 172, 174, and 176 (3:4:1, $[M]^+$ indicating one Cl and one Br on the molecule), 136 and 138 (1:1, $[M-HCl]^+$), 106 and 108 (1:1, $[CHCH_2Br]^+$) and 92 and 94 (3:1, $[M-HBr]^+$).

The two products with R_t of *ca* 14 min showed the mass spectra diagnostic for bromo-propanediols. The product with R_t 14.0 min was identified as 1-bromo-2,3-propanediol: $[M]^+$ not detected; *m/z* 123 and 125 (1:1, $[M-CH_2OH]^+$) and 61 $[M-CH_2Br]^+$. The product having R_t 14.5 min was identified as 2-bromo-1,3-propanediol: $[M]^+$ not detected, *m/z* 136 and 138 (1:1, $[M-H_2O]^+$) and 106 and 108 (1:1, $[CH_2CHBr]^+$).

A final product had an R_t of 16 min and showed the mass spectrum diagnostic for 2,3-dibromo-1-propanol: 216, 218 and 220 (1:2:1 in intensity, $[M]^+$, indicating 2 Br on the molecule), 137 and 139, and 136 and 138 (both sets 1:1 in intensity) $[M-Br]^+$ and $[M-HBr]^+$, respectively and 106 and 108 (1:1 in intensity, $[CH_2CHBr]^+$).

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