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Phytogenesis of halomethanes: A product of selection or a metabolic accident?

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Abstract. Phytoplankton (microalgae), seaweeds (macroalgae), higher plants and fungi produce halomethanes. Algae and fungi produce both methyl halides and polyhalomethanes, whereas plants are known to produce only methyl halides. Why these organisms produce halomethanes is a question frequently asked by chemists and biologists. This question implies that halomethanes have a function and have a selective value to the producing organism. Except for some fungi, the evolutionary advantage of producing halomethanes may not presently exist. Polyhalomethanes are by-products of halogenation of certain organic compounds by haloperoxidases in marine algae and perhaps some fungi, and they may be indirectly produced in aquatic environments by algal release of oxidized halogen species. A main function of this enzyme is to rid the cell of harmful oxidants such as hydrogen peroxide. Monohalomethanes (methyl halides) are products of methyltransferase activity. It has been proposed that methyl halide production may provide a mechanism to regulate chloride levels in halotolerant plants. The examination of halide cellular concentrations, halomethane production rates, and enzyme characteristics raises questions about this possible function. In algae, plants and some fungi, methyl halides may be a result of the insertion of ubiquitous halides into the active site of numerous methyltransferases. Therefore, halomethanes may be by-products or 'accidents' of metabolism.

Abbreviations: AP = ascorbate peroxidase; BrPO = bromoperoxidase; ClPO = chloroperoxidase; CH_3X = methyl halide; $CHBr_3$ = bromoform; CH_2Br_2 = dibromomethane; CH_2I_2 = diiodomethane; $CHIBr_2$ = iododibromomethane; $CHCl_2I$ = dichloroiodomethane; CH_2ClI = chloroiodomethane; $CHCl_3$ = chloroform; DCMU = dichlorophenyl-dimethylurea; DNP = 2-dinitrolphenol; DOM = dissolved organic matter; H_2O_2 = hydrogen peroxide; IPO = iodoperoxidase; K_m = Michaelis-Menten constant; OTM = *O*-methyltransferase; ppm = part per million (dry); PSI = photosystem 1; PSII = photosystem 2; SAH = S-Adenosylhomocysteine; SAM = *S*-adenosyl-L-methionine; SOD = superoxide dismutase

Introduction

Cyanobacteria, marine algae (phytoplankton and seaweeds), higher plants and fungi produce halomethanes. Algae and fungi produce both methyl

halides (monohalomethanes, CH_3X) and polyhalomethanes, while plants and cyanobacteria produce only methyl halides. Polyhalomethanes are a by-product of the halogenation of certain organic molecules by haloperoxidases, whereas, methyl halides are products of methyltransferase activity (Theiler et al. 1978; Wuosmaa & Hager 1990). Naturally produced halomethanes are important vectors of halogen transport among the hydrosphere, atmosphere, pedosphere and biosphere. Halomethanes are a source of halogen atoms that destroy stratospheric ozone. As a major source of bromine and iodine atoms in the troposphere, iodo- and bromo-methanes strongly influence tropospheric chemistry, especially of the marine boundary layer (Carpenter et al. 2000).

Halomethanes could be considered 'secondary compounds.' Primary compounds are those metabolites associated with the basic metabolism: photosynthesis, respiration, and intermediates of common biosynthetic pathways (e.g. proteins, lipids, etc.). These compounds are the starting materials for many other types of unusual, mainly complex organic compounds termed secondary compounds that are produced by 'secondary metabolism.' (see Luckner 1984 for the evolution and the meaning of the term 'secondary metabolism'). The function of many of these compounds in plants, algae or fungi is not known (i.e. verified scientifically). Many secondary compounds may not yet have a function, that is, the evolutionary advantage of producing such a compound may not yet exist. Photoautotrophs are never carbon-limited and, if successful, are not critically nutrient limited (i.e. do not experience minimal or no growth); they may have carbon available for 'metabolic experimentation.' It may be advantageous for photoautotrophs and fungi to produce new compounds because of the potential that a compound may eventually confer a selective advantage. For example, terrestrial fungi are important decomposers and as such share many habitats with bacteria. A strong selective pressure to produce antibiotics that protect mycelia from bacterial attack exists in this environment. Such fungal antibiotics are halogenated (mainly chlorinated) 'secondary' compounds. Marine organisms produce the greatest diversity of brominated, iodinated and chlorinated secondary compounds, perhaps reflecting the non-depleting source of seawater halogens (Neidleman & Geigert 1986; Gribble 1992).

Phytogenesis of polyhalomethanes

Polyhalomethanes are produced indirectly from haloperoxidase activity. There are two distinct types of haloperoxidases seen in eukaryotes, those that contain Fe-heme and those that contain vanadium as a cofactor. Although a complete phylogenetic distribution of these two haloperoxidases is lacking,

all plants and most fungi appear to contain the former while red and brown algae contain V-haloperoxidase (Butler 1998; Neidleman & Geigert 1986; Wever & Krenn 1990). Both types have been reported in green algae and diatoms (Wever & Krenn 1990; Moore et al. 1996; Verdel et al. 2000). Nearly all haloperoxidases from terrestrial organisms contain the Fe-heme prosthetic group, whereas those haloperoxidases from marine organisms tend to contain vanadium (Butler 1998).

Haloperoxidases have been named according their ability to oxidize halides (Neidleman & Geigert 1986). Chloroperoxidases (ClPO) can oxidize all halides except F^- and are the primary haloperoxidases of fungi. Bromoperoxidases (BrPO) can oxidize both I^- and Br^- but not Cl^- and are primarily found in marine algae. Iodoperoxidase (IPO) can oxidize only I^- and exist in some marine algae and higher plants.

Haloperoxidase K_m values for halides are high compared to the K_m for H_2O_2:

- V-BrPO (seaweeds, Wever & Krenn 1990; Soedjak & Butler 1991):
  K_m = 1–24 mM Br, K_m = 17–92 μM H_2O_2
- V-BrPO (diatom, Moore et al. 1996):
  K_m = 35 mM Br
- V-IPO (seaweeds, Almeida et al. 1998, 2001):
  K_m = 0.82–5.7 mM Br, K_m = 84–490 μM H_2O_2
- V-ClPO (fungi, van Schijndel et al. 1993):
  K_m = 25 mM Cl (pH=7), 2.5 mM Cl (pH= 5.5)
- Fe-ClPO (fungi, Hager et al. 1970):
  K_m = 18 mM Cl (pH 3.5), K_m = 1.4 mM H_2O_2

These high values may reflect a low affinity of the active site for halides or loss of an activator during purification. The low affinity of haloperoxidases towards halides in marine algae may have evolved in response to an elevated tissue halide concentration (see methyl halide section for details), whereas H_2O_2 is maintained at a relatively low concentration.

The substrates for haloperoxidases are halides, H_2O_2 and a wide variety of organic compounds (Neidleman & Geigert 1986). Two such organic substrates, β-diketones and β-ketoacids are produced from the polyketide pathway (Figure 1). BrPO has been shown to brominate these compounds to yield a polybrominated ketone that is susceptible to non-enzymatic hydrolysis, resulting in formation of $CHBr_3$ and CH_2Br_2 (Figure 2; Theiler et al. 1978; Beissner et al. 1981).

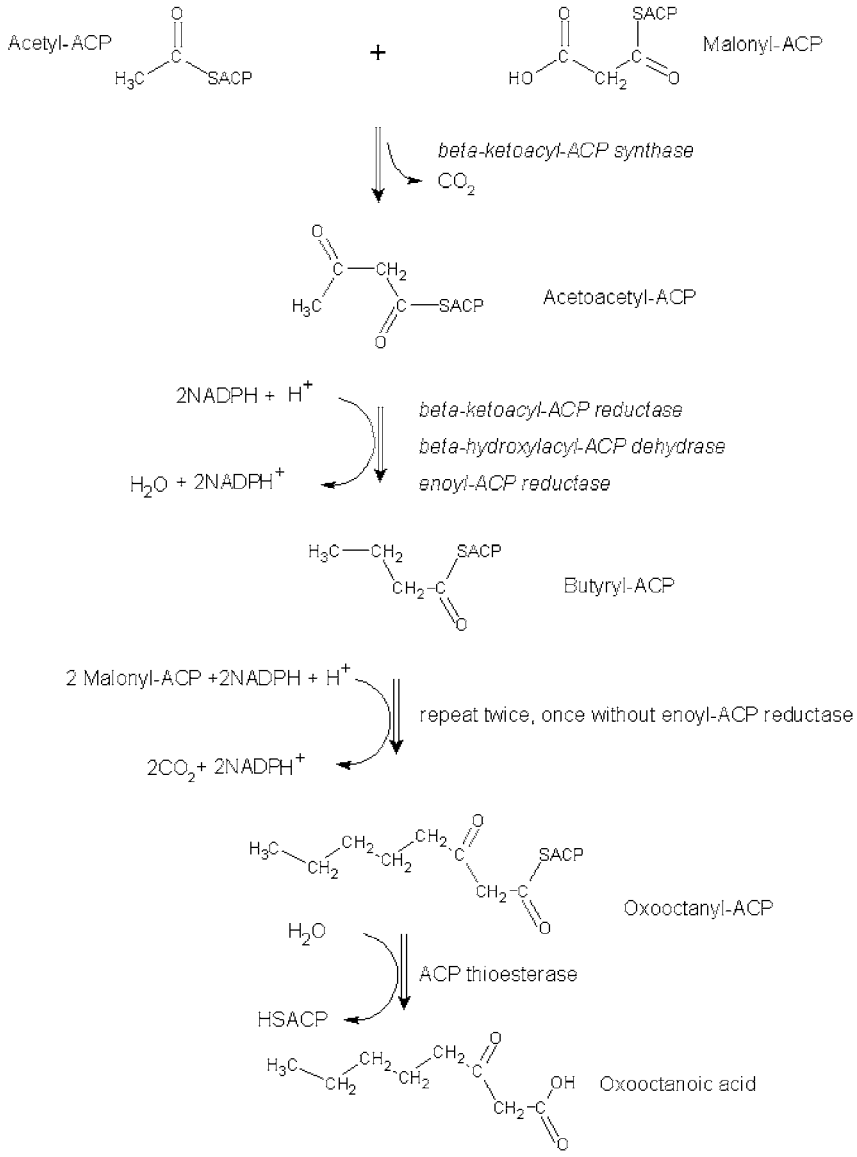

Figure 1. Biosynthesis of β-keto acids (i.e. 3-oxooctanoic acid).

Algae

Most biochemical and physiological studies have focused on polyhalomethane production by marine algae. These studies have resulted in several possible explanations as to their role in algae.

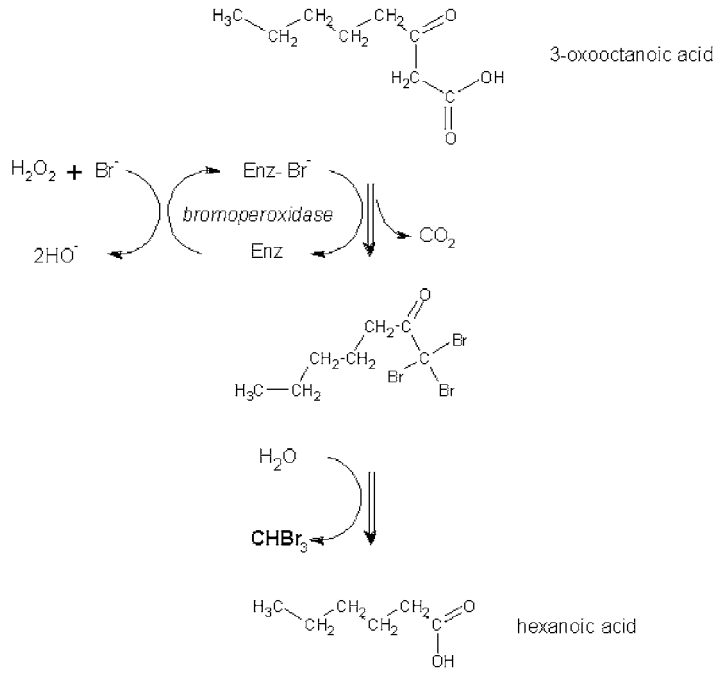

Figure 2. Biosynthesis of bromoform (after Theiler et al. 1978).

Scavenging of harmful H_2O_2

H_2O_2 is a product of photosynthesis, photorespiration, respiration, and other metabolic processes. H_2O_2 is produced from the enzymatic activity of glycollate oxidase, urate oxidase and amino acid oxidases. The major source of H_2O_2 in photoautotrophs is conversion from superoxide produced from the transfer of an electron from ferredoxin of PSI to O_2 (Mehler reaction; Falkowski & Raven 1997) by the action of superoxide dismutase (SOD). SOD also produces H_2O_2 from other superoxide sources including mitochondrial electron transport, cytochrome P450 system, and the enzymatic activity of xanthine oxidase and galactose oxidase (Cadenas 1989; Elstner 1987).

Marine algae contain BrPO and IPO and are major global sources of polyhalomethanes (Gribble 1992; Manley et al. 1992; Nightingale et al. 1995; Moore et al. 1996). A function of these haloperoxidases in algae is to scavenge harmful H_2O_2 (Pedersén et al. 1996; Manley & Barbero 2001), which impairs both photosynthesis and respiration (Manley & Barbero 2001). Experiments with marine algae have consistently demonstrated increased polybromomethane production in light as compared to darkness (Figure 3) (Goodwin et al. 1997; Manley & Barbero 2001). Field observations confirm this finding (Ekdahl et al. 1998; Carpenter et al. 2000). Experiments with

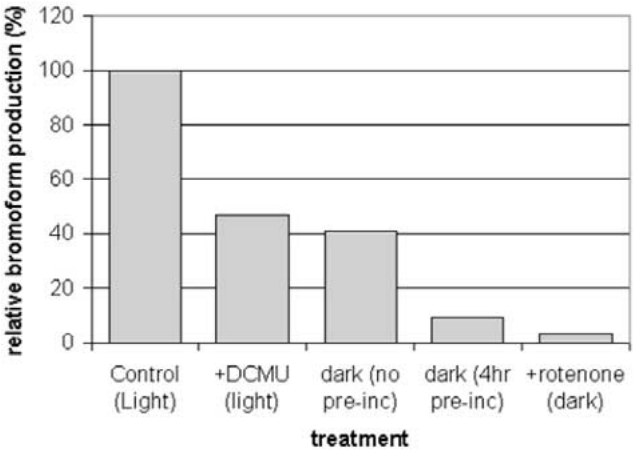

Figure 3. Bromoform production in the light and dark. + rotenone tissue in dark with 4 hour preincubation in dark prior to rotenone addition (Manley & Barbero 2001).

seaweeds have shown an inhibition of polybromomethane production in light when exposed to photosynthetically inhibiting concentrations of DCMU (Goodwin et al. 1997; Manley & Barbero 2001), which blocks electron flow on the acceptor side of PSII and thus production of H_2O_2. Manley and Barbero (2001) also have demonstrated a reduction in bromoform production in the dark in the presence of physiologically active concentrations of rotenone, an inhibitor of mitochondrial electron transport (Figure 3).

In addition to BrPO, other enzymes that destroy H_2O_2 exist in marine algae. These include catalase, which is associated with the peroxisome, and ascorbate peroxidase (AP). Ascorbate peroxidase, which catalyzes the oxidation of ascorbate, is found in the chloroplasts of higher plants (Halliwell 1984) and some green algae (Takeda et al. 1997; Pedersén et al. 1996). Brown, red and those green algae that contain glycollate oxidase (also in the peroxisome) contain high activities of catalase (Gross 1993). Some green algae may have little or no catalase, which is not surprising because they lack glycollate oxidase, a producer of H_2O_2 (Pedersén et al.1996).

BrPO may replace or coexist with AP in algal chloroplasts. BrPO activity has been localized in chloroplast fractions of the red alga *Odonthalia floccosa* (Manley & Chapman 1979) and the green alga *Ulva lactuca* (Manley unpub). Other results suggest the presence of a chloroplastic BrPO: (1) Br has been located in relatively high concentrations in the red algal chloroplasts of *Odonthalia dentata* presumably in bromophenols, the main brominated compounds identified in this seaweed (Hofsten & Pedersén 1980) and (2) cyanobacteria produce brominated phenols (Pedersén & DaSilva 1973).

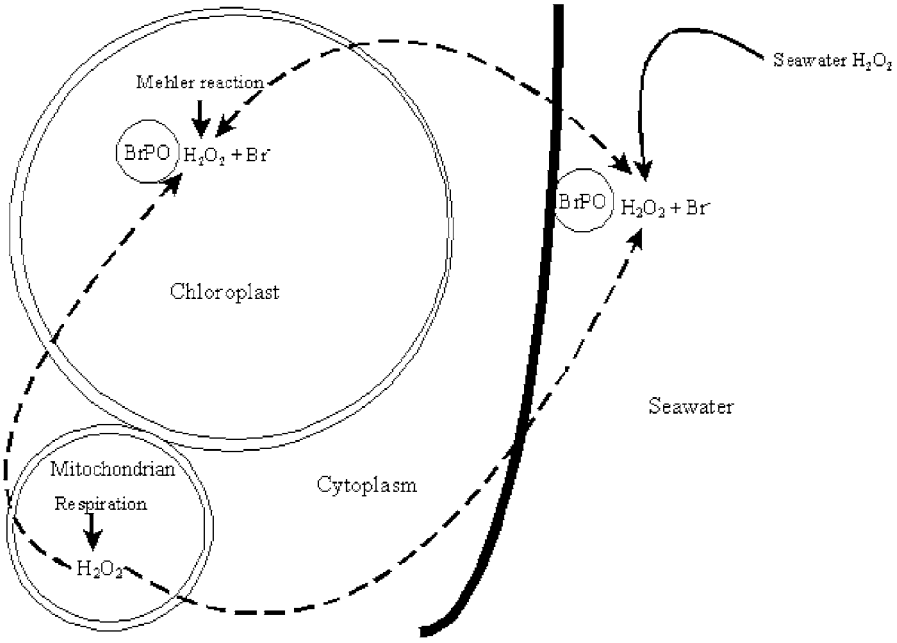

Figure 4. Cellular localization of BrPO in *Ulva lactuca* (from Brockette 1998).

Haloperoxidase has also been located on the outer cellular surface of marine seaweeds (Vitler 1983; Krenn et al. 1989) and may serve to protect tissue from extracellular H_2O_2 that is present in seawater due to abiotic photochemistry and leakage from algae (Manley & Barbero 2001). The *in situ* bromination of phenol red by seaweeds has also been interpreted as the result of an external BrPO (Wever et al. 1991; Pedersén et al. 1996; Sundström et al. 1996; Manley & Barbero 2001). The location of haloperoxidase in the algal cells strongly suggests that it functions to protect cells from harmful internally and externally produced H_2O_2 (Figure 4; Manley & Barbero 2001). Vreeland and Ng (1999) identified an extracellular haloperoxidase in brown algae that catalyzes the assembly of polyphenolic adhesives involved in cell adhesion. Also, iodine uptake may involve an extracellular haloperoxidase in kelps (Küpper et al. 1998).

Indirect production through oxidized inorganic bromine species

Independent of the polyketide pathway (Figures 1 & 2), there is evidence for an alternative pathway for formation of polyhalomethanes, which utilizes the vanadium-BrPO of brown and red algae. Oxidized bromine species (HOBr, Br_2, Br_3^-) produced by an extracellular BrPO may be released from the enzyme to react with seawater DOM to form unstable brominated intermedi-

ates (Wever et al. 1993). The unstable intermediates subsequently breakdown and indirectly form polybrominated methanes. The reaction of oxidized bromine species with DOM is extremely rapid (Song et al. 1996) and has been extensively studied by water treatment chemists (Minear & Amy 1996).

Butler (1999) has demonstrated that the oxidized bromine intermediate remains enzyme-bound for many BrPO mediated brominations. It is untenable to suggest that any of the oxidized bromine (or halogen) species would be released from the active site within the cell because of their extreme reactivity. The resulting indiscriminate halogenation would be detrimental to cellular processes. Mechanistic studies of BrPO activity, however, have not differentiated between the various isozymes present. It is, therefore, possible that the internal BrPO does not release HOBr while the extracellular form does.

Chemical defense

Bromoperoxidases catalyze the production of a variety of secondary halocarbons of unknown function. Many of these compounds may have antiherbivory activity (McConnell & Fenical 1980) although seaweeds containing even high levels of halocarbons are grazed, albeit selectively. These compounds also have antimicrobial properties (Fenical 1982; Neidleman & Geigert 1986), yet many seaweeds containing these compounds do support epiphytic microbial populations. Ohsawa et al. (2001) have demonstrated, however, that bromoform emissions across a membrane in seawater at rates equivalent to those seen for coralline red algae prevented growth of microscopic marine algae (i.e. diatoms). Furthermore, the surfaces of such coralline algae emitting $CHBr_3$ and CH_2Br_2 lack epiphytic diatoms, whereas the surfaces of dead corallines (no emissions) were heavily encrusted (Ohsawa et al. 2001). However, kelps, which are known to produce large amounts of polyhalomethanes, are usually epiphytized with diatoms, bacteria and a few fungi (Manley unpub). There is a recent report (Malin et al. 2001) that products (oligoguluronates) from the breakdown (presumably by bacteria) of brown algal (*Laminaria digitata*) alginate-rich cell walls stimulate a short-lived burst in H_2O_2 production. This burst is followed by the sudden release of iodide and the elevated production of volatile iodocarbons including iodomethanes. Although this response may be specific for brown algae, and kelps in particular, it is intriguing because it strongly suggests that the increased polyhalomethanes production is a response to pathogenic attack.

Global significance of polyhalomethane production

As major global producers of polyhalomethanes, algae supply reactive halogen atoms to the atmosphere where they can promote the destruction of both tropospheric and stratospheric ozone. Polyhalomethanes containing

iodine and bromine have very short photolytic lifetimes (e.g. CH_2I_2, $CHIBr_2$, $CHBr_3$), producing iodine and bromine atoms in the marine boundary layer that results in dramatic reduction of tropospheric ozone. While monohalomethanes have much greater atmospheric lifetimes and are more inclined to reach the stratosphere, deep convective events can transport polyhalomethanes into the stratosphere where they contribute to ozone destruction (Sturges et al. 2000). It is curious that these compounds, formed in the presence of photosynthetically derived oxygen via superoxide/H_2O_2, find their way into the stratosphere to damage the ozone layer that is also a product of photosynthetic oxygen (Falkowski & Raven 1997).

Fungi

Fungi are known to produce a variety of chlorinated compounds many of which are antibiotics (Neidleman & Geigert 1986). Cultures of the white rot fungus *Bjerkandera adusta* produced the polyhalomethanes CH_2I_2, CH_2ClI and $CHCl_2I$ (Spinnler et al. 1994), and $CHCl_3$ production by *B. adusta* and several other fungi also has been reported (Hoekstra et al. 1998). Whether these fungi produce halomethanes in their natural habitat is unknown and would be dependent on the *in situ* level of available halides. Fungal biosynthesis of polyhalomethanes is not unexpected because fungi use the polyketide pathway to produce a variety of compounds, are known to produce H_2O_2, and because some terrestrial fungi contain Fe-heme containing ClPO (Griffin 1994; Neidleman & Geigert 1986). In addition to producing H_2O_2 via SOD, fungal microbodies contain numerous H_2O_2 generating oxidases (Carson & Cooney 1990). Wood rotting fungi contain Fe-heme peroxidases that degrade lignin (Griffin 1994). These exoenzymes are provided relatively high amounts of H_2O_2 from intra- and exo- cellular generating systems. A fungal Fe-heme ClPO was the first haloperoxidase to be extensively characterized and is responsible for producing halogenated organics (Hager et al. 1970). A V-ClPO has been isolated and characterized from certain phytoparasitic terrestrial fungi (hyphomycetes). Apparently this V-ClPO does not catalyze *in vivo* halogenation of organic products but produces HOCl for the degradation of host plant cell walls (Simmons et al. 1995).

Plants and cyanobacteria

Polyhalomethane emissions from plants and cyanobacteria have not been shown. This is not surprising, as it pertains to higher plants, since only iodoperoxidase activity has been demonstrated in plants (Neidleman & Geigert 1986). This activity is associated with Fe-heme containing peroxidases (e.g. horseradish and turnip root, wormseed fruit) that primarily function to cata-

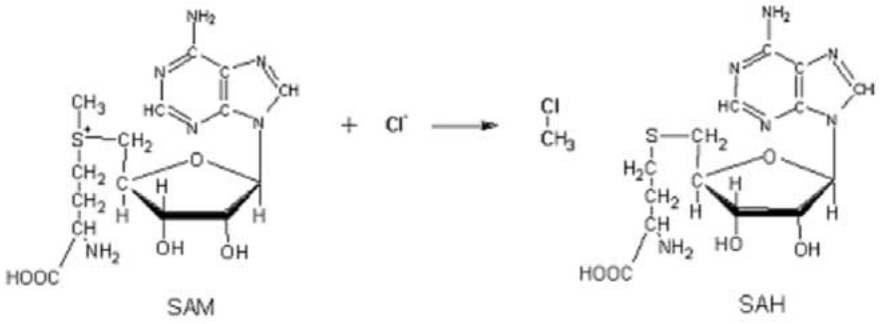

Figure 5. Reaction of SAM with chloride to form CH_3Cl.

lyze the oxidation of a variety of compounds as part of biosynthesis or catabolism. The rare instance of the identification of non-artifactual chlorinated or brominated products (Neidleman & Geigert 1986) suggest ClPO or BrPO activity in a few plants. Brominated and chlorinated compounds have been isolated from cyanobacteria (Gribble 1992); their presence suggests haloperoxidase activity.

Phytogenesis of methyl halides

Although the oceans (i.e. marine algae) were at first thought to be the most important global source of CH_3X, recent focus has shifted to terrestrial biomes and the microbes and plants found there. Cyanobacteria, algae, plants and fungi produce methyl halides (Scarratt & Moore 1998; Manley & Dastoor 1987; Wuosmaa & Hager 1990; Rhew et al. 2000; Redeker et al. 2000; Harper 2000) apparently all by the same mechanism. Methyl halides are produced when Cl^-, Br^- or I^- are enzymatically methylated by *S*-adenosyl-L-methionine (SAM) utilizing methyl transferases (Figure 5; White 1982; Wuosmaa & Hager 1990; Ni & Hager 1999). There appear to be at least two distinct 'methyl halide transferases.' Methyl transferase from the plant family Brassicaceae has the ability to methylate HS^- to form methanethiol (Attieh et al.1995) in addition to methylating halides whereas others do not have that ability (Ni & Hager 1999).

A review of known Michaelis-Menten constants (K_m) for methyltransferases suggests that halides are not the intended substrate (i.e. intended methyl acceptor). The K_m of methyl transferases for halides is high compared as compared to SAM:

- 'CH_3Cl transferase' (*Endocladia muricata*, a marine red alga, Wuosmaa & Hager 1990):
  $K_m(Cl) = 5$ mM, $K_m(Br) = 40$ mM, $K_m(SAM) = 16\ \mu$M

- 'CH_3Cl transferase' (*Batis maritima* or saltwort, Ni & Hager 1999):
  K_m(Cl) = 155 mM, K_m(Br) = 18.5 mM, K_m(I) = 8.5 mM
  K_m(SAM) = 29 μM
- thiol methyl transferase (*Brassica oleracea*, cabbage, Attieh et al. 1995):
  K_m(Cl) = 5 mM, K_m(Br) = 40 mM, K_m(SAM) = 16 μM

or when compared to organic methyl acceptor substrates of known methyl transferases:

- thiol methyl transferase, 3 isoforms (*Brassica oleracea*, cabbage, Attieh et al. 2000a):
  K_m(I) = 0.37, 2.3, 34 mM, K_m(SAM) = 21, 52, 70 μM,
  K_m(thiocyanate) = 0.03, 0.08, 3.5 μM
- lignin-specific *O*- methyl transferase (*Populus tremuloides*, aspen, Meng and Campbell 1996):
  K_m(SAM) = 6.2 μM, K_m(caffeic acid) = 40 μM,
  K_m(5-OH- ferulic acid) = 19 μM
- γ- tocopherol methyl transferase (*Capsicum annum*, pepper, d'Harlingue & Camara 1985):
  K_m(SAM) = 2.5 μM, K_m(γ-tocopherol) = 14 μM

These relatively high K_m values may reflect a low affinity of the active site for halides or loss of activator during purification. The apparent low affinity of 'methyl halide transferase' towards halides in marine algae and halophytes may have evolved in response to elevated tissue halide concentrations (Marine algal tissues: Cl: 2–21% dwt, Br: 0.03–2% dwt, I: 0.005–1% dwt, Shaw 1962; Whyte & Englar 1976; Manley & Dastoor 1988. Halophytic plant tissues: Cl: 10–25% dwt, Br: 0.01–0.4% dwt, I: 6–8 ppm, Markert & Jayasekera 1987; Manley unpub), while SAM is maintained at a relatively low concentration. The tissue chloride concentration in the halophyte *Batis maritima* (600 mM, Manley unpub) is much higher than the K_m (see above), supporting the idea that high K_m values may reflect an ample supply of halide in tissues. In contrast, the bromine and iodine concentrations in *Batis maritima* (6.4 mM Br and 7.7 mM I, at 11% gdwt) are much lower than the reported K_m values.

The halide concentration in non-halophytic plants is relatively low (*B. oleracea* tissue-Br = 22 ppm or 0.18 mM; Gan et al. 1998), while the K_m remains high. The halide concentrations of non-halophytic plant tissues range from of 2–4 ppm Br (or 16–33 μM) and 0.3–20 ppm I (or 1.6 μM–0.1 mM) (Dobrovolsky 1994; Martin 1966). Chloride content of plant tissue can vary widely. A mean value of 0.2% dwt (or 0.12 mM) has been presented (Dobrovolsky 1994) although values over 1% (0.6 mM) are common for non-halophytes (Eaton 1966). The chloride content of xylem fluid in non-halophytes is in the range of 1.3–3.3 mM (Robson & Pitman 1983). A

comparison of these tissue values with the above K_m values suggests that the low affinity of the methyl transferases is because halides are not the normal substrate for these enzymes. Minimal catalytic efficiency is achieved when the substrate concentration is far less than K_m ($v \ll V_{max}$).

Methyl halide production may be a result of non-specific methylation of halides by SAM utilizing methyl transferases

SAM-utilizing methyltransferases have been identified in algae and fungi, and both produce a variety of methylated secondary compounds including polyketides, phenolics, isoprenoids, steroids and alkaloids (fungi only). Higher plants contain a number of SAM-utilizing methyl transferases such as lignin-specific *O*-methyltransferases, flavonoid-specific *O*-methyltransferases, γ- tocopherol methyltransferase, alkaloid methyltransferases and sterol methyltransferases (Meng & Campbell 1996; d'Harlingue & Camara 1985; Schröder et al. 2002; Joshi & Chiang 1998). Perhaps it is these enzymes that catalyze the methylation of halides because the ubiquitous halide ions may easily intercalate into the active site of methyltransferases.

Thiol methyltransferase has been shown to methylate halides and has been implicated in detoxification of glucosinolate breakdown products (Attieh et al. 2000b). *In vivo* incubations with plant leaf disks (*B. oleracea)* show that the addition of caffeic acid (CA), a substrate of lignin-specific *O*-methyl transferase (OTM), inhibits methyl bromide production (Manley & Redeker unpub). The reaction catalyzed by this OTM is one of several methylations of hydroxycinnamic acid intermediates in the production of lignin. These known methylation reactions utilizing SAM are not tightly coupled to primary metabolism, especially photosynthesis. The production of CH_3X by algae is not enhanced in the light (Manley & Dastoor 1987). The production of CH_3Br from incubations of leaf disks (*B. oleracea*), however, does show inhibition ($\sim$50%) in the dark that is attributed to stomatal closure (Manley & Redeker unpub).

Methyl halide production as a means of halide excretion?

It has been proposed by Ni and Hager (1999) that CH_3X production is a means by which marsh plants (specifically *B. maritima*) regulate their tissue halide levels; a way to rid their tissues of excess halides. Production of CH_3Cl by *B. maritima* can be as high as 3.5 ng gfwt^{-1} hr^{-1} (Manley unpub). A comparison of this production rate with the tissue halide levels shows that the daily loss of halides as CH_3Cl is but a small fraction of *Batis* tissue chloride concentration ($\sim$3 μg CH_3Cl/g tissue-Cl). This loss is much less than the variability in chloride content (17–25%) in *Batis* (Manley unpub).

A comparison of known rates of CH_3X production with tissue halide levels from other plants and algae show similar results: *Brassica*: 120–690 μg Br

lost as CH_3Br/g tissue-Br (Gan et al. 1998); *Macrocystis*: 11 ng Cl lost as CH_3Cl/g tissue-Cl (Whyte & Englar 1976; Manley & Dastoor 1987); 112–176 ng I lost as CH_3I/g tissue-I (kelps, Manley & Dastoor 1988). Such analysis suggests that it is unlikely that *B. maritima*, other plants and algae rely on CH_3X production to maintain a functional halide level in tissue. It is possible, however, that a methyl transferase, if compartmentalized in a small membrane bound vesicle, could maintain a proper chloride level for the functioning of adjacent enzymes. Much more work is needed to answer this question.

CH_3Cl as a primary methyl donor in fungi

Although CH_3X production in plants and algae may be an 'accidental' by-product of metabolism, it has a function in certain fungi. A metabolic role of CH_3Cl in such fungi has been presented in a series of studies by Harper (2000). Methyl chloride is a methyl donor in the biosynthesis of the secondary compound veratryl alcohol (3,4-dimethoxybenzyl alcohol) a 'key component of the lignin degrading system of many white rot fungi' (Harper 2000). Harper has proposed that the CH_3Cl biosynthetic and utilizing enzymes are membrane bound in close proximity and that CH_3Cl emissions occur when there is an uncoupling of the two processes. Such CH_3Cl emissions in themselves may be advantageous as a mechanism to rid the cell of excess methyl fragments acquired from methoxy groups of lignin during the decomposition of wood (Harper 2000).

Conclusion

Polyhalomethanes

The formation of polyhalomethanes results from the halogenation of larger organic compounds by haloperoxidases. The main function of haloperoxidases in marine algae and perhaps many fungi is to rid the cell of harmful hydrogen peroxide (the role in iodine uptake in brown algae requires more elucidation). More investigations of the physiological and spatial coupling of H_2O_2 generating systems and haloperoxidase in algae and fungi are needed. It would be useful to quantify the amount of haloperoxidase and the other H_2O_2 utilizing enzymes (e.g. catalase, ascorbate peroxidase) present in algal cells and to follow any changes that result from changes in oxidative stress. It is also extremely important to determine the component(s) of seawater DOM that yields polyhalomethanes after halogenation and whether this component is halogenated within or outside (via HOBr) of the active site of the extracellular BrPO.

There is no evidence that halomethane production serves a function in photoautotrophs, and except for CH_3Cl production by a few fungi, halomethanes appear to be by-products or 'accidents' of normal metabolism. For algae, plants, cyanobacteria and many fungi, methyl halide production probably results from insertion of ubiquitous halides into the active sites of the numerous methyltransferases involved in specific biosynthetic pathways. Further work is required to determine the halide methylating capacities of purified and well-characterized methyltransferases of known function (e.g. lignin-specific *O*-methyl transferase) and to determine if 'methyl halide transferases' are specific for the methylation of halides only.

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