An Unusual Dehalogenating Peroxidase from the Marine Terebellid Polychaete Amphitrite ornata*

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The terebellid polychaete Amphitrite ornata produces no detectable volatile halogenated secondary metabolites, but frequently inhabits coastal marine sediments heavily contaminated with anthropogenic or biogenic haloaromatic compounds. This animal contains high levels of two very unusual enzymes, dehalogenating peroxidases. We have purified and partially characterized one of these dehaloperoxidases, DHP I. DHP I is a heme enzyme (M_r = 30,790) composed of two identical subunits (M_r = 15,529) and is very rich in the amino acids aspartic acid (+ asparagine) and glutamic acid (+ glutamine). The enzyme converts trihalogenated phenols, such as 2,4,6-tribromophenol, into dihalogenated quinones. The optimum pH for this reaction is 5.0. DHP I is also active against di- and monohalogenated phenols and will oxidize bromo-, chloro-, and fluorophenols. We have identified similar dehaloperoxidase activities in other infaunal polychaetes, including halometabolite-producing species.

Contamination of coastal marine sediments by anthropogenic haloaromatic compounds found in agricultural, industrial, and urban runoff is well known (1, 2). Less recognized, but common and very widespread sources of haloaromatics are biotic (3-5). Among these are species of sediment-dwelling marine polychaetes and hemichordates which produce high levels of volatile brominated secondary metabolites, such as bromophenols, bromopyrroles, bromoindoles, bromohydroquinones, and bromobenzylalcohols (5-8), through the action of haloperoxidases (9, 10). Such worms occupy immense areas of coastal sediments (11-15), contaminating them with volatile, malodorous, and toxic bromometabolites. Bromophenols and related compounds are respiratory inhibitors and presumably express their toxicity through inhibition of mitochondrial function. The toxicity of these compounds to vertebrates is well established (16-19), but has not been as thoroughly examined in invertebrates. It is clear from field and laboratory studies that sediment contamination with bromoaromatics inhibits recruitment of non-bromometabolite-producing invertebrate species (15) and appears to select for specific organisms which may be resistant to the toxic effects of haloaromatic compounds. Both these resistant species and the bromometabolite producers themselves, which face bromometabolite autotoxicity, must have some means of detoxifying these compounds. Dehalogenating enzymes provide one such detoxification mechanism.

Dehalogenating enzymes are uncommon in higher organisms. The cytochrome P-450 enzymes are exceptions, being broadly distributed among animals, plants, fungi, and bacteria and capable of reductive dehalogenation of alkyl halocarbon to the corresponding alkenes under anaerobic conditions (20). Other oxygenases may also participate in similar reactions, but haloaromatic compounds present a unique problem due to their relative stability and toxicity. The terebellid polychaete Amphitrite ornata produces no detectable volatile halometabolites of its own and lacks detectable halogenase activity. However, this animal is often found in beds also inhabited by Notomastus lobatus (Polychaeta) and Saccoglossus kowalevskyi (Hemichordata), which produce and contaminate sediments with bromophenols and bromopyrroles (8, 9, 13, 21). We examined A. ornata to determine the basis for its tolerance of haloaromatic compounds and found that, instead of a typical oxygenase, it produces high levels of two dehalogenating peroxidases. We have purified and partially characterized dehaloperoxidase I (DHP I), which has a number of unusual properties.

EXPERIMENTAL PROCEDURES

Materials—A. ornata was collected at low tide from mixed oyster rubble and sandy mud at Debidue Flats, in the North Inlet estuary (Georgetown, SC, 33°20'N, 79°10'W) and immediately frozen on dry ice. Worms could be stored frozen at -70°C for at least 6 months without substantial loss of dehaloperoxidase activity, but recently collected material was used in most purifications.

Dehaloperoxidase Activity, Products, and Protein Assays—A. ornata dehaloperoxidase I activity was assayed on the basis of disappearance of substrate, typically 2,4,6-tribromophenol. Activity was assayed using a reaction mixture containing 50 mM KH_2PO_4, pH 5.0, 5 mM H_2O_2, 1 mM NaCl, 0.15 M NaOH, from 1 to 20 μM halophenol substrate, and 0.15 μg of pure DHP I in a 1-ml reaction volume, for 5 min at room temperature. An internal standard (2,6-dichlorophenol) was then added, and the reaction was stopped immediately by extraction with high performance liquid chromatography grade pentane. Substrate remaining after incubation was resolved via gas chromatography and quantified using standard curves derived from known concentrations of reagent grade substrates halophenols and of the internal standard (9, 10). Product identities were determined via mass spectroscopy (8, 9). Halophenol disappearance was linear with time and with enzyme quantity. The DHP I reaction achieved maximum velocity at 1.5 mM H_2O_2 and 20 μM 2,4,6-tribromophenol. Saturating concentrations of chlorophenols and fluorophenols were lower. Optimum pH for enzyme activity was determined using reaction mixtures buffered to different pH values with 100 mM sodium acetate buffer (pH 3.8-6.0) or 50 mM potassium phosphate buffer (pH 5.0-7.0). One unit of dehaloperoxidase activity was defined as 1 nmol of dehaloperoxidase I activity in 1 min at 25°C.

Preparation of A. ornata Extract and Purification of Dehaloperoxidase—Frozen A. ornata tissue was thawed at room temperature and homogenized by hand grinding with washed sand using an ice-cold mortar and pestle. The homogenate was diluted with 50 mM sodium phosphate buffer, pH 5.0, and filtered through cheesecloth. All subse-

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1 D. E. Lincoln, K. T. Fieldman, R. A. Marinelli, and S. A. Woodin, manuscript in preparation.

2 The abbreviation used is: DHP, dehaloperoxidase.
approximately 0.1 M NaCl), while Peak 2 eluted at about 76 ml chromatography fractions. Peak 1 eluted at about 25 ml (ap-

ity. This major activity loss is accounted for by separation of the
section of dehaloperoxidase I with loss of 38% of total DHP activ-

roughly 3% of soluble protein recovered in crude extracts (Table

lated using a Sephacryl S-300 column with PB as the mobile phase. Fractions containing peak DHP I activity were again pooled and concentrated, loaded onto a Sephacryl S-300 (Sigma) column, and separated using PB as the mobile phase. The DHP I recovered was homo-
geous by native and SDS-polyacrylamide gel electrophoresis (Ref. 23 and Fig. 1).

Gel Electrophoresis and Molecular Weight Determination—The molecular weight of the native DHP I was determined from gel permeation chromatography using a Sephacryl S-300 column (1.2 X 57 cm) cali-

brane of one heme group per dimeric holoenzyme molecule. No other metals were

determined by atomic absorption

sorption Spectra—

Metal content of DHP I was determined by atomic

absorption spectroscopy (Model 965 Plasma Atomcomp; Jarrell-Ash Co., Waltham, MA). Amino acid composition was determined from pure fractions by hydrolysis in 6 N HCl at 110 °C for 24 h under N2, followed by analysis with a Beckman System 6300 amino acid analyzer (Beck-

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amid gel is shown in Fig. 1.

Determination of Metal Content, Amino Acid Composition, and Ab-
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molecular weight of the native DHP I was determined from gel permeation chromatography using a Sephacryl S-300 column (1.2 X 57 cm) cali-

brated with proteins of known molecular weight. Electrospray mass spectroscopy was used to determine subunit molecular weight. SDS-
polyacrylamide gel electrophoresis with proteins of known molecular weight (Bio-Rad Low Range Molecular Weight Marker Set) was also used in estimating subunit molecular weight. A typical SDS-polyacry-

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autotoxicity. Since A. ornata produces no detectable volatile halometabolites and lacks detectable halogenase activity, autotoxicity seems unlikely. It lives, however, in close proximity to N. lobatus and S. kowalewskyi which contaminate the sediments with bromophenols and bromopyrroles (8, 13, 21).

A. ornata feeds on surface deposits with its tentacles and lives in a mudlined tube; its lifestyle therefore involves intimate contact with the sediments and associated contaminants (24). The range of toxic bromoaromatic compounds encountered by A. ornata requires a detoxification mechanism which can neutralize high levels of several haloaromatic compounds having dif-

![Fig. 2. Mass spectra of major reaction products of the Amphitrite ornata dehaloperoxidase I using 2,4,6-tribromophenol (A) and 2,4,6-trichlorophenol (B) as substrates.](image)

### Table II
Amino acid composition of the A. ornata dehaloperoxidase

| Amino acid | Molar % Amino acid composition | Flavin | Heme | Native |
|------------|---------------------------------|--------|------|--------|
| Asx        | 15.7 (1.1)                      | 21.9 (3.0) | 12.7 | 14.5  |
| Threonine  | 3.8 (0.4)                       | 4.9 (0.0)  | 3.0  | 5.0   |
| Serine     | 5.5 (0.1)                       | 7.8 (0.4)  | 6.7  | 6.8   |
| Glx        | 11.4 (0.3)                      | 13.7 (0.6) | 10.8 | 18.7  |
| Proline    | 2.2 (0.0)                       | 3.2 (0.1)  | 3.7  | 2.1   |
| Glycine    | 6.4 (0.3)                       | 8.5 (0.5)  | 11.6 | 14.1  |
| Alanine    | 9.8 (0.3)                       | 14.1 (0.6) | 13.4 | 7.7   |
| Valine     | 6.8 (0.2)                       | 10.1 (0.3) | 4.8  | 4.4   |
| Methionine | 2.6 (0.1)                       | 3.7 (0.3)  | 3.0  | 1.4   |
| Isoleucine | 1.8 (0.0)                       | 2.6 (0.1)  | 3.4  | 3.7   |
| Leucine    | 9.9 (0.1)                       | 13.5 (0.4) | 8.2  | 7.9   |
| Tyrosine   | 3.2 (0.1)                       | 4.4 (0.2)  | 0.8  | 1.0   |
| Phenylalanine | 6.8 (0.1)                  | 9.8 (0.3)  | 5.2  | 2.1   |
| Lysine     | 8.4 (0.2)                       | 11.7 (0.5) | 5.2  | 5.4   |
| Histidine  | 0.4 (0.3)                       | 0.7 (0.6)  | 1.9  | 0.8   |
| Arginine   | 5.6 (0.1)                       | 7.7 (0.2)  | 5.6  | 4.4   |
| Cysteine   | ND                              | ND       | ND   | ND |
| Tryptophan | ND                              | ND       | ND   | ND |
| Polarity   | 50.8                            | 45.9     | 55.6 | 49.0 |
| Hydrophobicity | 31.0                         | 25.4     | 20.5 | 23.8 |

### Table III
Dehalogenation of halogenated phenols by A. ornata dehaloperoxidase I

| Substrate            | µmol substrate oxidized | Turnover number |
|----------------------|-------------------------|-----------------|
| 2-Bromophenol        | 32.7                    | 2.9 x 10² µmol enzyme⁻¹ min⁻¹ |
| 3-Bromophenol        | 24.5                    | 2.0 x 10² |
| 4-Bromophenol        | 48.0                    | 4.0 x 10² |
| 2,4-Dibromophenol    | 29.3                    | 3.0 x 10² |
| 2,6-Dibromophenol    | 30.2                    | 3.1 x 10² |
| 2,4,6-Trichlorophenol| 33.4                    | 3.5 x 10² |
| 2-Chlorophenol       | 1.9                     | 0.2 x 10² |
| 3-Chlorophenol       | 2.4                     | 0.2 x 10² |
| 4-Chlorophenol       | 8.3                     | 0.6 x 10² |
| 2,4-Dichlorophenol   | 4.2                     | 0.5 x 10² |
| 2,6-Dichlorophenol   | 4.0                     | 0.5 x 10² |
| 2,4,6-Trichlorophenol| 6.3                     | 0.7 x 10² |
| 3-Fluorophenol       | 6.7                     | 0.7 x 10² |
| 3,4-Difluorophenol   | 8.2                     | 0.8 x 10² |
| 3,5-Difluorophenol   | 7.0                     | 0.7 x 10² |
| 2,3,4-Trifluorophenol| 3.1                     | 0.4 x 10² |
different structures and degrees and positions of bromine substitution. Sediments contaminated by anthropogenic sources, such as sediments downstream from sulfate process pulp mills, can also contain high levels of a diversity of haloaromatic compounds including chlorophenols, chloroguaiacols, and chlorocatechols, all potentially toxic to sediment-dwelling invertebrates (2,25). The high rates of A. ornata DHPI activity and its broad substrate specificity are consistent with its proposed function in neutralization of environmental haloaromatic toxins. We hypothesize that production of dehaloperoxidases allows A. ornata to survive in locations contaminated with these toxic compounds and that similar animals inhabiting sediments contaminated with haloaromatic compounds from both anthropogenic and biogenic sources will also produce these unusual dehaloperoxidases. Further structural and catalytic investigations of the A. ornata DHPI are ongoing.

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TABLE IV
Distribution of dehaloperoxidase activity in several infaunal marine worms

| Family and organ | Specific activitya |
|-----------------|-------------------|
| Capitellidae | Capitella lobatus 260 |
| Terebellidae | A. ornata 300 |
| | T. crispus 180 |

a Specific activity is expressed as units of dehaloperoxidase activity per mg of protein. One unit of dehaloperoxidase activity is defined as 1 nmol of 2,4,6-tribromophenol debrominated · min⁻¹.

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