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Localization of the Chloroperoxidase of the Capitellid Polychaete *Notomastus lobatus*

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Abstract. Antisera against the two constituent proteins of the chloroperoxidase enzyme of the capitellid polychaete *Notomastus lobatus* were used to identify and localize these polypeptides by immunoblotting and indirect immunofluorescence. Immunofluorescence staining showed the enzyme to be localized primarily in tissues of the tail region of the worm. Some reactivity was also observed in the epidermis of the mid-body, but none was seen in the head region. These immunofluorescence results were supported by immunoblotting experiments, which also showed that chloroperoxidase holoenzyme is localized in the tail. Immunological results were substantiated by the distribution of enzyme activity and the *in vivo* products of the chloroperoxidase, 4-bromophenol, 2,4-dibromophenol, and 2,4,6-tribromophenol. Chloroperoxidase is the principal enzyme involved in the production of bromoaromatics in *N. lobatus*. Localization of most of this enzyme in the tail region explains the detection of high levels of bromophenols in the tail, the most exposed portion of this head-down, deposit-feeding worm. This pattern of bromoaromatic distribution is consistent with the hypothesis that the worms produce these compounds as defensive chemicals against epifaunal predators.

Introduction

A variety of sediment-dwelling, soft-bodied marine invertebrates, including hemichordates and polychaetes, contain high levels of volatile brominated compounds, such as bromophenols, bromopyrroles, and bromobenzylalcohols (Woodin *et al.*, 1987; Woodin, 1991). These brominated compounds are also released by the worms into the sediments surrounding their burrows (King, 1986; Woodin *et al.*, 1987; Steward *et al.*, 1992). Many marine sediments support significant populations of infaunal worm species that produce volatile, malodorous, brominated aromatic compounds. Examples of such populations include the terebellid polychaete *Lanice conchileca* in the North Sea (Buhr, 1976; Weber and Ernst, 1978) and the hemichordate *Balanoglossus* in the southeastern United States (Peterson and Peterson, 1979).

High concentrations of bromophenols are found in the capitellid polychaete *Notomastus lobatus*, which is a head-down, sediment deposit feeder. Bromophenols can be produced through the activity of bromo- or chloroperoxidases; Ahern *et al.* (1980) had previously detected bromoperoxidase activity in the terebellid polychaete *Thelepus setosus*. Chen *et al.* (1991) purified and characterized a chloroperoxidase from *N. lobatus* and found that this enzyme has several unique properties. It is composed of two dissociable protein components, a flavoprotein and a heme protein, which associate in a 1:1 molar ratio to form holoenzyme. The flavoprotein is composed of four identical subunits (alpha) and contains flavin adenine dinucleotide (Chen *et al.*, 1991). The heme protein is a tetramer composed of two copies each of two nonidentical subunits (beta and gamma) and contains 1 mol of ferriheme per mol of protein. The *N. lobatus* chloroperoxidase thus has a subunit structure of $\alpha_4\beta_2\gamma_2$. Neither the flavoprotein nor the heme protein moiety alone has detectable chloroperoxidase activity, but they readily associate to form fully active enzyme. The chloroperoxidase of *N. lobatus* is capable of oxidizing Cl^-, Br^-, and I^-. It is the first haloperoxidase to be purified to homogeneity from a marine worm and the first haloperoxidase ever found

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Abbreviations: PBS, phosphate buffered saline; GC, gas chromatography.

to contain flavin. The enzyme can halogenate a wide variety of aromatic compounds and is the only detectable halogenating enzyme in *N. lobatus*. The enzyme is very abundant, constituting approximately 2% of soluble protein in *N. lobatus* crude extract. Using phenol and sodium bromide as substrates, it produces 4-bromophenol, 2,4-dibromophenol, and 2,4,6-tribromophenol, the compounds found in *N. lobatus* (Chen *et al.*, 1991). It is clear that this chloroperoxidase is responsible for the production of bromophenols in *N. lobatus* and that production of these toxic compounds is likely to be significant in both the ecology and physiology of this animal.

In a variety of terrestrial and marine plants as well as such sessile marine organisms as coelenterates and sponges, noxious compounds have been shown to have utility as antipredator and antifouling agents (Hay and Fenical, 1988). In at least some cases, these compounds show distinctive patterns of localization—for example, in sponge, in spherulous cells near excurrent canals (Thompson *et al.*, 1983); and in red alga, in outer cortical cells and trichoblast cells (Young *et al.*, 1980). In most, but not all cases (see Hashimoto *et al.*, 1991), the compounds have some autotoxicity properties and thus are encapsulated within vesicles apparently at the point of synthesis in the organism (Thompson *et al.*, 1983). King (1986) has hypothesized that compounds such as those produced by *Notomastus lobatus* are antimicrobial agents and act to reduce bacterial populations in the burrow lining. To be consistent with this hypothesis, the enzyme and its products should be localized in the epidermis over the entire body of the worm. In contrast, Woodin *et al.* (1987) suggested that these compounds are primarily antipredator compounds. For this hypothesis, the enzyme and its products are expected to be concentrated in the tail, which is the portion of the organism most exposed to surface predators (Powell, 1977). In this study, we used a combination of techniques, including immunohistochemistry and assays of enzyme identity and activity, to determine the distribution of the chloroperoxidase and its products in *N. lobatus*. This localization study complements a more direct test of the antipredator hypothesis; results of that test will be published elsewhere. The products of enzymes, particularly toxic and autotoxic compounds such as these, can have several simultaneous effects. Knowledge of localization and activity of the enzyme can help to discriminate among these, particularly in this case where the enzyme represents such a large percentage of the animal's soluble protein (2%).

Materials and Methods

Specimens of *Notomastus lobatus* were collected at low tide from Debidue flat, in the North Inlet estuary (Georgetown, South Carolina, USA, 33°20′ N, 79°10′ W). The worms were removed from the sediment and washed in seawater to remove external sediment. Three body regions were clearly visible from external features: the head, which lacked branchiae and was seemingly quite muscular; the mid-body, which had branchiae and was much less rigid; and the tail, which lacked branchiae and was sticky, translucent, and quite glandular in appearance. Intact worms were treated differently depending upon their planned use. Those to be used for immunochemistry were cut with a razor blade into 1-cm lengths of head, mid-body, and tail regions. Segments adjacent to the junctions between body regions were discarded. Each length was immediately placed into 10 volumes of Carnoy's fixative (95% absolute ethanol + 5% glacial acetic acid) on ice in the field and transported on ice to the University of South Carolina main campus. In the laboratory, the fixed body portions were transferred to −10°C and maintained for two days. Worms used for measurement of enzyme activity, quantification of enzyme, or extraction of enzyme were cut into head, mid-body, and tail regions, immediately frozen on dry ice, and transported on dry ice to the University of South Carolina main campus. In the laboratory, the worms were transferred to −70°C until used. Chen *et al.* (1991) found that *N. lobatus* can be stored in this manner for several months with little loss of chloroperoxidase activity.

The heme protein and flavoprotein moieties of the *Notomastus lobatus* chloroperoxidase were purified separately as described by Chen *et al.* (1991). Purity of each protein was checked by native polyacrylamide gel electrophoresis. Polyclonal antibodies to purified heme protein and flavoprotein moieties were raised separately in male New Zealand white rabbits. Purified proteins were dissolved in phosphate buffered saline (PBS, pH 7.4) at a concentration of 100 μg/ml and emulsified in Freund's complete adjuvant for the initial, subcutaneous injection. Subsequent injections of antigens were carried out using Freund's incomplete adjuvant. The antisera were tested for specificity by immunoblot analysis using purified heme protein and flavoprotein moieties, reconstituted chloroperoxidase formed by mixing the two subunits in a 1:1 molar ratio, and a crude extract of *N. lobatus*. The crude extract was prepared as described in Chen *et al.* (1991).

After fixation, the body regions to be examined by immunohistochemistry were passed through three changes of 100% ethanol and three changes of toluene for 10 min each and embedded in low-melting-point paraffin in reduced light. These methods were used because the compounds are volatile and the enzyme is sensitive to both temperature and light. The embedded tissue was sectioned to a thickness of 9 μm. Some sections were stained with hematoxylin and eosin for bright-field microscopy.

For indirect immunofluorescence microscopy, the paraffin was removed from the sections by treatment with toluene, and the sections were then rehydrated in a graded ethanol series (100, 100, 95, 85, and 70%) for 1 min each. Sections were blocked with 1% goat serum in PBS for 30 min at room temperature. Serially matched sections were incubated overnight at 4°C in a 1:100 dilution of rabbit anti-heme protein, or anti-flavoprotein serum, or a mixture of anti-heme protein and anti-flavoprotein (1:1) sera. The sections were rinsed in PBS three times for 5 min each and then incubated in diluted (1:50) second antibody (fluorescein-conjugated, goat anti-rabbit IgG (H&L) antibodies; ICN Immunobiologicals, Lisle, IL) at room temperature for 1 h. Sections were then rinsed in PBS three times for 5 min each and mounted in 10% glycerol. Sections treated with preimmune rabbit serum, blocked with goat serum, and then treated with second antibody were used as controls. Sections were examined at 16× on a Zeiss Universal microscope equipped for epifluorescence.

For determination of brominated aromatic compounds, each frozen body region (head, mid-body, or tail) was thawed to room temperature, immediately placed in HPLC-grade methanol, and refrigerated pending extraction. The body regions were then ground in ice-cold methanol and the homogenates centrifuged at 1000 × *g* for 15 min. To each supernatant a known amount of 2,6-dichlorophenol was added as an internal standard. Each supernatant was diluted initially with 10 volumes of acidified, saturated sodium chloride solution (pH 2.0) and extracted five times with 1 volume of HPLC-grade pentane. For each extraction the amount of pentane added was equal to the volume of the supernatant (typically 5 to 10 ml). The pentane fractions were pooled, concentrated, and analyzed by gas chromatography (GC) with a Varian Model 3300 equipped with a fused silica capillary column (15 m × 0.53 mm ID) coated with a 1.5-μm film of cross-linked SE-54 phase (DB-5, J & W Scientific, Folsom, CA), an electron capture detector, and an integrator. The brominated compounds were resolved with the aid of a temperature program (120° to 160°C at 10°C/min, 30 ml/min N_2 flow) and quantified with standard curves derived from known concentrations of reagent-grade bromophenols previously determined to be found in *N. lobatus* (4-bromophenol, 2,4-dibromophenol, and 2,4,6-tribromophenol; Chen *et al.*, 1991) as well as with standard curves for the internal standard, 2,6-dichlorophenol.

Frozen, dissected worm heads, mid bodies, and tails were separately thawed at room temperature, weighed (about 0.5 g dry weight), and washed several times with 50 m*M* sodium phosphate buffer, pH 4.1. Each body region was homogenized by hand grinding in an ice-cold mortar and pestle with 2 ml added cold phosphate buffer. The homogenates were centrifuged at 10,000 × *g* for 20 min, then loaded onto a Sephadex G-50 column (0.5 × 5 cm), pre-equilibrated with phosphate buffer. The proteins were eluted with 50 m*M* phosphate buffer. The products of chloroperoxidase activity in each body region were measured by GC. Known amounts of purified chloroperoxidase formed by adding purified heme protein and flavoprotein moieties in a 1:1 molar ratio were added to a reaction mixture containing 10 m*M* NaCl, 440 μ*M* H_2O_2, and 50 m*M* phenol in 100 m*M* phosphate buffer, pH 5.0 (Chen *et al.*, 1991). Assay mixtures were incubated at room temperature for 30 min. The reaction mixture was extracted five times with 1 ml of pentane per extraction. The pentane fractions were then combined, concentrated, and analyzed by GC as described above. The presence and identities of the chlorophenols produced were confirmed using both gas chromatography and mass spectroscopy (Chen *et al.*, 1991). One unit of specific enzyme activity was defined as 1 μmol of product formed by 1 mg of protein per minute. Protein concentration was assayed by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard.

For analysis by Western immunoblotting, native polyacrylamide gel electrophoresis was performed with a Bio-Rad Protean II gel electrophoresis system. The polypeptides were transferred electrophoretically to nitrocellulose membrane. The nitrocellulose membrane was immersed in a blocking solution containing 3% goat serum in PBS for 30 min prior to incubation overnight in the mixture of anti-heme protein and anti-flavoprotein sera (1:1000 dilution) at room temperature. The nitrocellulose membrane was washed in PBS and then incubated in a horseradish-peroxidase-conjugated goat anti-rabbit IgG (1:5000 dilution) (BioRad Laboratories, Richmond, CA) for 30 min at room temperature. The blot was rinsed twice with 0.05% Tween 20 for 5 min each and rinsed again in PBS for 5 min. Polypeptides from each region on the worm were visualized by reaction with hydrogen peroxide and 4-chloro-1-naphthol. Polypeptide bands were quantified using a Hewlett Packard Scanjet Plus.

To confirm its specificity, anti-heme protein serum was absorbed against purified heme protein from *N. lobatus* overnight at 4°C. Supernatant was collected after centrifugation of precipitated antibody-antigen complexes. Ten microliters of purified heme protein and 10 μl of fresh *N. lobatus* crude extract were spotted onto nitrocellulose membrane, allowed to dry at room temperature, then rinsed once with TBS (50 m*M* Tris HCl, 150 m*M* NaCl, pH 8.0). The nitrocellulose membranes were then soaked in blocking solution (5% (w/v) nonfat dry milk in TBS) for 30 min, washed twice for 5 min each with TBST (TBS + 0.05% (v/v) Tween-20), and rinsed again with TBS. The membranes were then reacted with the primary absorbed serum (diluted 1:1000 with TBS) or unabsorbed

rabbit anti-heme serum for 1 h, washed twice for 5 min each with TBST, and rinsed with TBS. They were then reacted with horseradish peroxidase conjugated with goat anti-rabbit IgG (BioRad; diluted 1:1000 with TBS) for 1 h and then washed twice for 5 min each with TBST and rinsed with TBS. Polypeptides were visualized by reaction with hydrogen peroxide and 4-chloro-1-naphthol for 15 min.

Results

To determine the specificity of the antisera raised in rabbit against the purified heme protein and flavoprotein moieties of chloroperoxidase from *Notomastus lobatus*, we used the sera to probe Western blots containing purified heme protein complex, purified flavoprotein moiety, and whole-worm crude extracts. The anti-flavoprotein antiserum reacted only with the flavoprotein. The anti-heme protein serum reacted strongly with the purified heme protein, but not with the flavoprotein moiety. Each serum thus reacted only with its respective protein in the crude extract of *N. lobatus*. The anti-flavoprotein serum produced a band at a molecular weight consistent with the chloroperoxidase flavoprotein (120,000; Chen *et al.*, 1991) (Fig. 1: lane 4). The anti-heme protein serum produced a band at a molecular weight consistent with the chloroperoxidase heme protein (54,500; Chen *et al.*, 1991) (Fig. 1: lane 5). As expected, a mixture of anti-heme protein serum and anti-flavoprotein serum reacted with crude extract prepared from *N. lobatus* tail tissue produced two bands corresponding to the molecular weights of the heme protein and flavoprotein moieties (Fig. 1: lane 3). Bands consistent with heme protein were also seen in the head and mid-region extracts (Fig. 1: lanes 1 and 2 respectively). A small band consistent with the flavoprotein complex was seen in the mid-region extract, but none was seen in the head region extract (Fig. 1: lanes 2 and 1 respectively). The constituent proteins of the chloroperoxidase were thus easily detectable with the anti-heme protein and anti-flavoprotein sera.

To determine the histological location and regional distribution of the enzyme, tissue cross sections of head, mid-body, and tail regions were treated with a 1:1 mixture of heme protein and flavoprotein antisera. Positive immune staining was observed primarily in the tail and to a lesser extent in the mid-body (Figs. 2 and 3). The head region was completely negative in all sections.

Sections of tail region were treated with anti-heme protein or anti-flavoprotein serum, or a 1:1 mixture of the sera (Fig. 2A, B, C). In all cases, intense fluorescence labeling was observed in the muscles and with less intensity in the epidermis (Fig. 2B, C). Controls consisted of sections reacted with preimmune rabbit serum in PBS and then

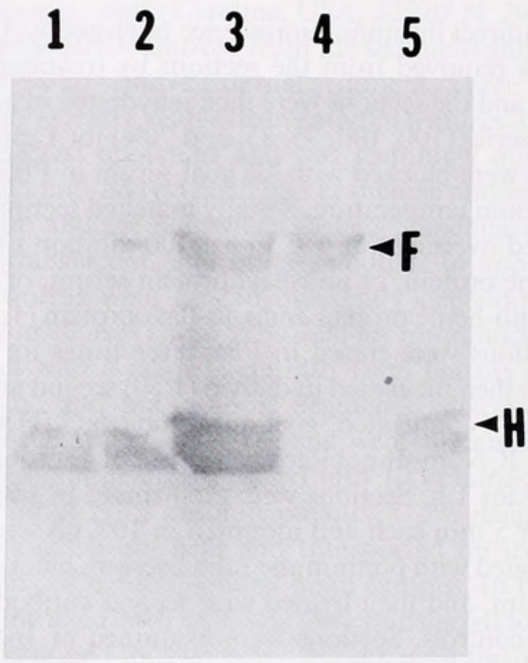

Figure 1. Western immunoblot of partially purified chloroperoxidase from *Notomastus lobatus*, reacted with a 1:1 ratio of heme protein and flavoprotein antisera. Lane 1: head region; Lane 2: mid-body; Lane 3: tail region; Lane 4: purified flavoprotein subunit of chloroperoxidase; Lane 5: purified heme protein subunit of chloroperoxidase.

rinsed and incubated with second antibody (fluorescein-tagged goat anti-rabbit IgG serum). Control sections showed no nonspecific immunoreaction (Fig. 2D). The samples treated with anti-heme protein (Fig. 2A) or anti-flavoprotein sera (Fig. 2B) were very similar to each other and to the samples treated with the 1:1 serum mixture (Fig. 2C). In addition to the staining of muscle, gut epithelium in the tail was also intensely labeled by the antibody (Fig. 3A). These results show the presence of chloroperoxidase in the muscle, epidermis, and gut lining in the tail. We also observed minor fluorescence labeling in the epidermis of the mid-body. In contrast to the Western immunoblot data, no fluorescence labeling was seen in any of the sections of the head region treated with either anti-heme protein or anti-flavoprotein sera.

As a second approach to confirmation of the immunocytochemical results on localization, we used Western immunoblotting to further examine the extracted enzyme moieties from each body region. Proteins transferred to nitrocellulose membranes were treated with the anti-heme protein and anti-flavoprotein antibodies, and the colored products of the reaction were quantified by scanning densitometry. Known amounts (Lowry *et al.*, 1951) of the purified flavoprotein and heme protein components were included as controls. As expected from the immunocytochemical results, the flavoprotein moiety was present in large quantities in the tail, but in much smaller quantities in the mid-body of the worm (Table I; Fig. 1). No

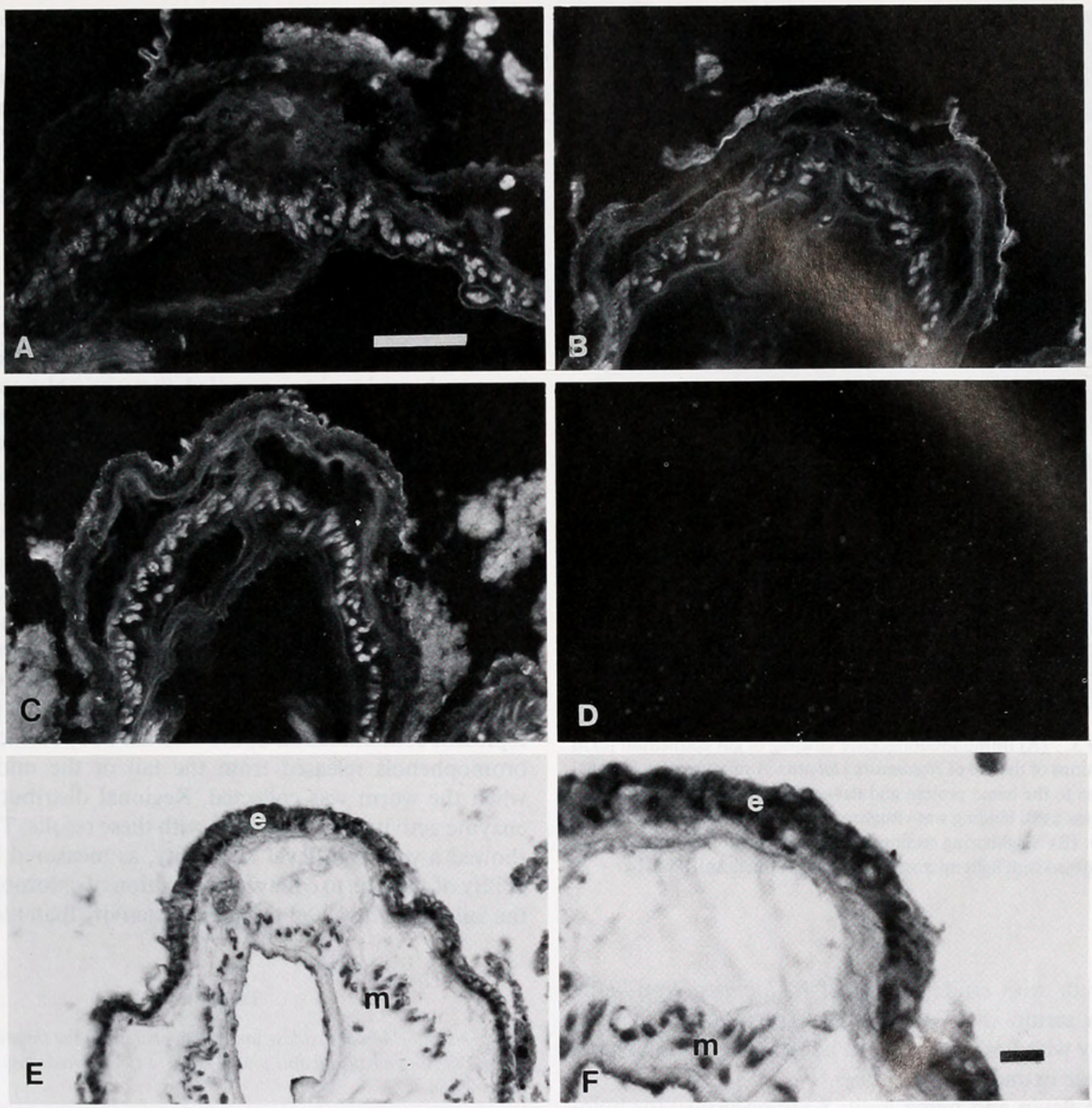

Figure 2. Immunofluorescence labeling of cross sections of the tail of *Notomastus lobatus*. Polyclonal antisera to heme protein and flavoprotein subunits of chloroperoxidase from *N. lobatus* were used; binding was visualized by fluorescein-conjugated anti-rabbit antibody. Labeling with (A) heme protein antibody; (B) flavoprotein antibody; (C) 1:1 mixture of heme protein and flavoprotein antibodies; (D) preimmune rabbit serum. (E) Neighboring section stained with hemotoxylin and eosin and examined with light microscopy (m—muscle, e—epidermis). (F) Same section as in (E), at higher magnification (m—muscle, e—epidermis). Scale bar in A = 72 μm and applies to A–E. Scale bar in F = 36 μm.

flavoprotein moiety was detected in the head region. In contrast to the immunocytochemical results, heme protein was detected in all three body regions (Table I). This could be due to nonspecific activity of the antiserum or to the actual presence of heme protein in all three body regions. If the latter, then the heme protein may have another function in the head and mid-body of the worm, but it must be complexed in some way such that we did not detect it by immunocytochemistry (Knapp *et al.*, 1991). Tests for nonspecific activity of the anti-heme protein serum were negative. When anti-heme protein serum preabsorbed with the purified heme protein moiety was reacted with crude extract and pure heme protein, no reaction was seen with either the purified heme protein

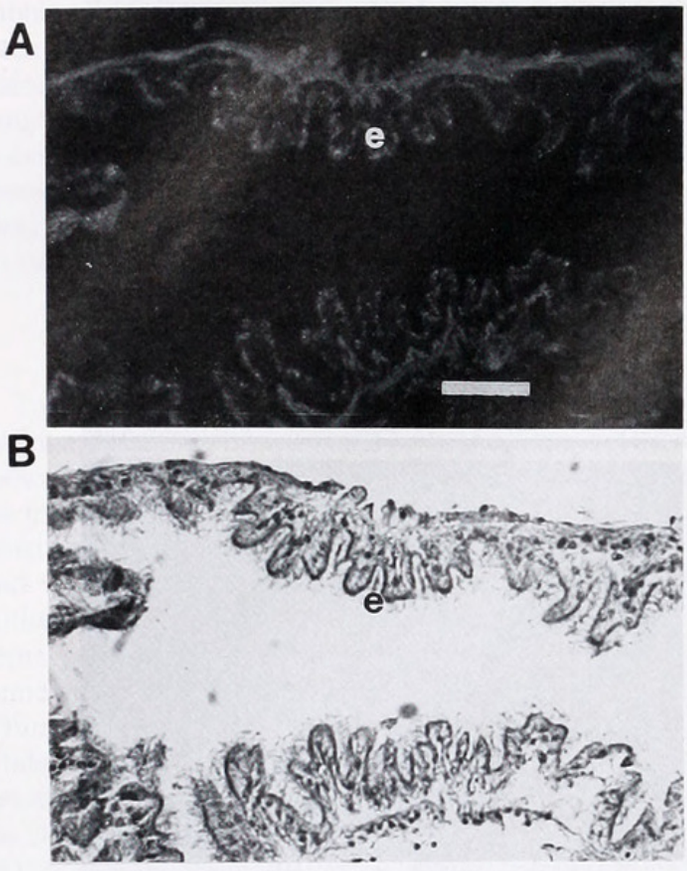

Figure 3. (A) Immunofluorescence labeling of gut epithelium (e) in cross sections of the tail of *Notomastus lobatus*. A mixture of polyclonal antibodies to the heme protein and flavoprotein subunits of chloroperoxidase was used; binding was visualized by FITC-conjugated anti-rabbit antibody. (B) Neighboring section stained with hemotoxylin and eosin and examined with light microscopy (e—gut epithelium). Scale bar = 72 μm.

moiety or with crude extract of *N. lobatus*. Anti-heme protein serum that had not been preabsorbed reacted strongly with both the purified heme protein moiety and the crude extract of *N. lobatus*.

Both flavin and heme moities are necessary for holoenzyme activity. To determine presence of holoenzyme per body region, the enzyme activity of the chloroperoxidase extracted from different regions of the body of *N. lobatus* was assayed by comparing the amount of halogenated product produced per milligram of protein. The product was assayed by GC. Since the worm naturally contains brominated aromatic products, assays for brominated products with crude extracts suffer from background contamination. The worm does not contain chlorinated aromatics, but its chloroperoxidase will catalyze the synthesis of these compounds *in vitro* (Chen *et al.*, 1991). Therefore, we used an enzyme reaction mixture including phenol and NaCl, which in the presence of enzyme produced 4-chlorophenol. This product was assayed by GC and its identity verified by mass spectroscopy. The highest enzyme activity was detected in extracts from the tail region, with much less in extracts from the mid-body and head regions (Table I). In addition, the amount of brominated aromatic compounds found in methanol extracts of each body region was measured by GC (Table I). The highest concentration of bromophenols was found in the tail, with a lower level in the mid-body (Table I) and only trace amounts in the head region.

Discussion

Chloroperoxidase and its products are localized primarily in the tail region of *Notomastus lobatus* (Table I). Immunohistochemistry revealed that the chloroperoxidase was located in muscle, epidermis, and gut lining of the tail region as well as to a lesser extent in the mid-body (Figs. 2 and 3). This corresponds closely with the data on the distribution of the natural brominated products of this chloroperoxidase (4-bromophenol, 2,4-dibromophenol, and 2,4,6-tribromophenol), which are also concentrated in the tail region (Table I). The tail region has 3.2 times more bromophenol than the mid-body and 75.2 times more than the head region (bromophenol expressed as micrograms per milligram of protein). In fact, so little bromophenol was detected in the head region that it may represent contamination of the external body surface with bromophenols released from the tail or the mid-body when the worm was collected. Regional distribution of enzyme activity was consistent with these results. The tail showed a very high level of activity, as measured by the ability of extracts to catalyze production of chlorophenol; the tail region had 29.5 times more activity than the mid-

Table I

Occurrence of chloroperoxidase and bromophenols in the three body regions of Notomastus lobatus plus activity of chloroperoxidase by body region

	Body Region		
	Head	Middle	Tail
Chloroperoxidase quantity[1] (OD · mm² band area)			
Heme-containing subunit	0.44	0.29	0.59
Flavin-containing subunit	0.0	0.07	0.23
Chloroperoxidase specific activity[2] (μmol substrate/mg crude extract protein/min)	0.20	0.06	1.86
Bromophenol compounds quantity (μg/mg protein)	5	117	376

[1] Determined by scanning densitometry of Western blot to nitrocellulose membrane.

[2] Measured as the ability of the enzyme to produce the product 4-chlorophenol.

body and 9.3 times more than the head region. The only data that appeared to be inconsistent with the conclusion that the chloroperoxidase was localized in the tail tissue were the data from Western blots. These data showed the heme protein moiety of the chloroperoxidase to be abundant in all body regions of the worm (Table I). However, the flavoprotein moiety either was not present (head) or was present in only trace amounts (mid-body). Since both proteins are required for chloroperoxidase activity, this strongly suggests that no functional chloroperoxidase enzyme existed in the head and only trace amounts in the mid-body of the worm (Table I). Thus, there is no inconsistency with respect to the absence of enzyme activity in regions other than the tail.

Localization of product (or products) and enzyme near the site of their expected use or release appears to be typical of many noxious chemicals in marine organisms (Young *et al.*, 1980; Thompson *et al.*, 1983; Hay and Fenical, 1988) as well as in terrestrial plants (Langenheim *et al.*, 1978). It also seems to be characteristic of organisms that do not synthesize their own noxious compounds but rather derive them from their foods (*e.g.*, gastropods: Jensen, 1984; Faulkner and Ghiselin, 1983; insects: Bowers and Puttick, 1986; Malcolm, 1991). Because many of the endogenously produced compounds probably have autotoxic properties, one would expect them to be synthesized at the site of sequestration and localized in a manner appropriate for their functional role (Hay and Fenical, 1988). Exceptions to this pattern are known: in some terrestrial plants, for example, alkaloids are synthesized at one location within the plant and then translocated to their site of utility (Hashimoto *et al.*, 1991). In the marine worm *N. lobatus*, it is clear that synthesis and storage of toxic bromophenols occur primarily in the tail region (Table I, Figs. 2 and 3).

Notomastus lobatus lives in sediments in a habitat that is intensively excavated by a variety of large digging predators such as rays, skates, and blue crabs (Peterson and Peterson, 1979). The predatory rays, for example, are capable of excavating to depths of more than 0.5 m in pursuit of prey (Orth, 1975). Methods of escape for sediment dwellers probably include life among partial structural refuges such as oyster reefs, rapid burrowing deep within the sediments, very rapid withdrawal into a very deep burrow, and distastefulness. *N. lobatus* is not a rapid burrower, nor does it withdraw rapidly into the deepest portions of its burrow. It lives in muds into which predators can readily dig. The localization of chloroperoxidase and its bromophenol products in the tail suggests that these compounds serve an antipredator function. If these compounds were primarily used as antimicrobial or antifouling compounds, as suggested by King (1986) and others, one would expect to see both the bromophenols and the chloroperoxidase evenly distributed across all three body regions in the epidermis; they are not (Table I; Figs. 2 and 3). The distribution of the bromophenols and the chloroperoxidase enzyme responsible for their production in *N. lobatus* is consistent with the hypothesis that they are used as antipredator compounds by this worm. *N. lobatus* lives head down, ensuring that the first portion of the body to be encountered by an epifaunal predator will be the tail, the region of the body containing the highest concentration of the brominated compounds and the highest activity of the chloroperoxidase enzyme (Table I). We surmise that encounters with the tail and its noxious brominated compounds reduce predatory pursuit. Feeding experiments that will more directly test this antipredator hypothesis are under way.

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