Internal Binding of Halogenated Phenols in Dehaloperoxidase-Hemoglobin Inhibits Peroxidase Function

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ABSTRACT Dehaloperoxidase (DHP) from the annelid Amphitrite ornata is a catalytically active hemoglobin-peroxidase that possesses a unique internal binding cavity in the distal pocket above the heme. The previously published crystal structure of DHP shows 4-iodophenol bound internally. This led to the proposal that the internal binding site is the active site for phenol oxidation. However, the native substrate for DHP is 2,4,6-tribromophenol, and all attempts to bind 2,4,6-tribromophenol in the internal site under physiological conditions have failed. Herein, we show that the binding of 4-halophenols in the internal pocket inhibits enzymatic function. Furthermore, we demonstrate that DHP has a unique two-site competitive binding mechanism in which the internal and external binding sites communicate through two conformations of the distal histidine of the enzyme, resulting in nonclassical competitive inhibition. The same distal histidine conformations involved in DHP function regulate oxygen binding and release during transport and storage by hemoglobins and myoglobins. This work provides further support for the hypothesis that DHP possesses an external binding site for substrate oxidation, as is typical for the peroxidase family of enzymes.

INTRODUCTION

The two dehaloperoxidase (DHP) hemoglobins (Hbs) from Amphitrite ornata, DHP A and DHP B, are the first characterized Hbs that have natural peroxidase function (1–6). Hbs, including DHP, are readily identified by their characteristic 3/3 α-helical protein structure. Although Hbs are primarily associated with O2 storage and transport, the characteristic globin fold actually encodes a diversity of protein functions. In addition to allosteric regulation of oxygen uptake, globins minimize the autooxidation rate of the heme iron, discriminate against CO binding, and carry out other natural functions such as NO binding and oxidation to nitrate. DHP brings this functional diversity to a new level by combining the seemingly contradictory functions of reversible oxygen binding (globin) and hydrogen peroxide activation (peroxidase).

Although Hbs and heme peroxidases are structurally distinct and perform different functions, they have a common heme cofactor and iron-binding site, with a histidine residue positioned on each side (Fig. 1). The proximal histidine is coordinated to the heme iron and provides a charge relay that supports either the ferrous (Fe2+) or ferric (Fe3+) iron oxidation state (7,8) depending on the local environment of globins or peroxidases, respectively. In myoglobins (Mbs) and Hbs, the distal histidine stabilizes diatomic oxygen during uptake and transport, whereas in peroxidases, the distal histidine serves as the acid-base catalyst necessary for heterolytic O–O bond cleavage, which constitutes the activation of bound hydrogen peroxide. Allostery in Hb is vital for the proper uptake of oxygen in the lungs and release of oxygen to respiring tissues (9). Whereas allostery may modify a binding constant or facilitate communication between multimers in cooperative proteins such as Hb, allosteric inhibition in heme peroxidases is an off-switch that can cause the enzyme to become completely inactive (10–12). The significance of both allostery and inhibition is more complex in a dual-function protein like DHP because the regulation involves not only each individual function, but also the switch between functions.

The first x-ray crystal structures of DHP A (Protein Data Bank (PDB) 1EW6 and 1EWA), obtained at room temperature, showed two features that are unique in the Hb superfamily (1). First, the distal histidine was observed in two conformations at pH 6, identified as open or closed. The closed conformation is the commonly observed conformation shown in Fig. 2 a, in which the histidine is in the distal pocket and interacts with a ligand coordinated to the heme iron (13). In the open conformation (Fig. 2 b), the distal histidine (H55) has swung out to a solvent-exposed position (14). Although the open conformation is known in sperm whale Mb, it is only observed below pH 4.5 when the distal histidine is protonated (15). We previously showed by a comparison of x-ray crystal structures (3DR9 and 2QFK) at 100 K that the open and closed conformations in DHP A are correlated with the 5-coordinate (5c) and 6-coordinate (6c) forms of the heme iron, respectively (13,14). Furthermore, the unique flexibility of the distal histidine has been shown to play an important role in heme-coordinated ligand stabilization (16). The second unprecedented observation in the initial x-ray crystal structure of DHP A was the presence of a substrate analog,
Internal Binding Inhibits DHP

4-iodophenol, in a well-defined position in the distal pocket of the globin but not coordinated to the heme iron (1). This unusual mode of binding in an Hb led to the suggestion that the internal binding site is the substrate-binding site (1). We have systematically investigated this hypothesis and found that the 4-halogenated phenols (4-XP) that bind internally are inhibitors rather than substrates. The active site for oxidation of substrates such as 2,4,6-tribromophenol (2,4,6-TBP), 2,4,6-trichlorophenol (2,4,6-TCP), and 2,4,6-trifluorophenol (2,4,6-TFP) is external (17). The possibility that an external substrate-binding site is located on DHP A was recently established via backbone NMR experiments (18). Given the extensive data available on DHP A relative to the more recently characterized DHP B, the remainder of this study will focus on DHP A, which will be referred to as DHP for brevity.

In this work, we provide detailed evidence that internal binding of 4-XPs inhibits the peroxidase function of DHP. This result is contradictory to the previous hypothesis that the distal pocket binding site for 4-XP is the substrate-binding site for phenol oxidation. This work builds on a number of observations that support the existence of distinct binding sites for 4-BP and 2,4,6-TBP, and demonstrates that these distinct sites are involved in inhibition of a competitive nature. Normally, competitive inhibition implies that the substrate and inhibitor compete for the same binding site. However, the structural and kinetic evidence presented below suggests that DHP exhibits a form of competitive inhibition (formally known as allosteric or nonclassical competitive inhibition (19–22)) in which the inhibitor binds remote to the active site and creates a conformational change in the enzyme that prevents the substrate from binding. The x-ray crystallographic, resonance Raman (RR), and kinetic data presented here are consistent with competitive inhibition between the internal and external sites mediated by the distal histidine (H55). The proposed functional role for the distal histidine, as the switch that leads to peroxidase inhibition (open) and activation (closed), underlies the regulation of oxygen-binding affinity by the same histidine in the open (low affinity) and closed (high affinity) conformations traditionally observed in globins (15,23,24).

MATERIALS AND METHODS

Materials

Buffer salts were purchased from Fisher Scientific (Waltham, MA). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification.

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### TABLE 1 Data collection and refinement statistics

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*Crystal containing 4-halophenol in complex with WT DHP; the letter specifies the halogen substituent.

*Values in parentheses are the highest-resolution shell.

ᵢRmerge = [Σᵢ|Iᵢ| - |I̅ᵢ|] / Σᵢ|Iᵢ| × 100%, where Iᵢ is the ith measurement and <I(h)> is the weighted mean of all measurements of I(h).

ᵢRwork = [Σᵢ|Iᵢ| - |Fᵢ|] / Σᵢ|Fᵢ| × 100%, where Fᵢ and Fᵢ are observed and calculated structure factors, respectively; Rfree is R factor for the subset (5%) of reflections selected before, and not included in the refinement.

*Calculated using PROCHECK.

### Crystallization, data collection, and processing

Recombinant wild-type (WT) protein was expressed in *E. coli*, purified, and characterized as previously described (13). To obtain crystals of DHP complexed with substrate analogs, the protein, at a concentration of 8 mg/mL dissolved in 10 mM Na cacodylate pH 6.5, was incubated on ice for 30 min with the parahalogenated phenols (1.5 mM 4-IP, 10 mM 4-BP, or 10 mM 4-CP, respectively) and crystallized using the hanging drop vapor diffusion method, with the reservoir solution containing unbuffered 0.2 M ammonium sulfate and 32% PEG 4000 as described previously (13,14). The crystals were cryoprotected in a solution containing 0.2 M ammonium sulfate, 35% PEG 4000 (w/v), and 15% PEG 400 as the cryoprotectant. Data were collected at 100 K on the SER-CAT 22-ID beamline at the APS synchrotron facility using a wavelength of 1 Å for crystals derivatized with 4-chloro- and 4-fluorophenol, 0.91942 Å for crystals derivatized with 4-bromophenol, and 1.5 Å for crystals derivatized with 4-iodophenol. The latter two wavelengths were chosen so that two data sets could be collected in a single-wavelength anomalous dispersion mode to correctly orient the halogenated phenol in its electron density. The collected diffraction data sets were processed using the HKL2000 program suite (25). The new crystals belong to the same space group (P2₁2₁2₁) as the ferric water-ligated (metamo) form (PDB entry 2QFK), and the structures were solved by molecular replacement using 2QFK coordinates as a starting model in the Phaser molecular replacement program (26). Structure determination and refinement calculations were performed using the CCP4 suite of programs (27,28), and visualization and manual model building were conducted using Coot model building software (29).

### Electronic absorption spectroscopy and kinetic assays

Recombinant his-tagged WT protein was expressed in *Escherichia coli* and purified as previously described (17,18). Initial inhibition experiments were conducted in 100 mM potassium phosphate buffer at pH 7 using an Agilent
Internal Binding Inhibits DHP

RESULTS

Crystallography

Previously published x-ray crystal structures (13,14) and spectroscopic data (16) strongly suggest a role for distal histidine flexibility in DHP. Fig. 2, a (closed) and b (open), shows PDB structures 2QFK and 3DR9, respectively. As mentioned above, in the metaquo form, the distal His is stabilized in the closed conformation by hydrogen bonding to the heme-coordinated water molecule (Fig. 2 a), and the heme iron is 6c high spin (6cHS). However, unlike other Hb structures (34–36), in the 5c deoxy form, the His is observed in the open conformation (Fig. 2 b). Therefore, the open and closed conformations in DHP are correlated with the 5c and 6c forms of the heme iron. Fig. 2 c shows an overlay of the new heme pocket structures of DHP co-crystallized with 4-IP (3LB1), 4-BP (3LB2), and 4-CP (3LB3) following established protocols (13). The 4-XPs bind in a conformation close to that originally reported for 4-IP (1). The occupancy of the 4-IP, 4-BP, and 4-CP molecules is >90% in all three structures. The structure of DHP with 4-CP (3LB4) is not shown, due to its low occupancy (~50%) and for clarity of the figure. Upon binding of these molecules in the internal site, the heme-coordinated water molecule is displaced and the histidine is pushed into the open conformation; thus, the iron is 5cHS (see also Fig. 1 for a schematic). The secondary structure of DHP A exhibits remarkably little change when 4-XPs bind in the distal pocket. The backbone root mean-square deviations (RMSDs) from the metaquo structure are ~≤0.4 Å, and the pairwise main-chain differences between the complexed structures are on the order of 0.1–0.2 Å. On the other hand, superposition of the structures shows that as the size of the parahalogen atom increases, the position of the 4-XP molecules bound in the distal pocket shifts slightly toward the heme-7-propionate and the solvent-exposed distal histidine.

Binding of parahalogenated phenols

In this study, the x-ray crystal structures provided meaningful insight into DHP in the solid state, whereas RR spectroscopy revealed the solution-state properties of halophenol binding. Fig. 3 a compares the RR spectra of WT-DHP with those obtained upon addition of phenol, and the 4-XP molecules (X = F, Cl, Br, I). The 5cHS core size marker band frequencies (ν3 at 1494 cm⁻¹, ν2 at 1568 cm⁻¹, and ν10 at 1632 cm⁻¹) systematically become more intense at the expense of the aquo 6cHS heme state (ν3 at 1481 cm⁻¹, ν2 at 1562 cm⁻¹, and ν10 at 1611 cm⁻¹) for the series of 4-XP bound DHP relative to WT-DHP. Similar systematic changes are also observed in the corresponding electronic absorption spectra. The Soret maximum undergoes

Binding isomer analysis

A titration data set for each inhibitor binding to DHP was collected via a series of RR spectra. Each sample contained 100 μM DHP in 150 mM potassium phosphate buffer at pH 6, with concentrations of the inhibitor from 0 to 8 mM (0–1 mM for 4-IP only). Singular value decomposition (SVD) was performed on the spectral data set with the use of Igor Pro 5.0. The SVD analysis yields one-dimensional column and row eigenvectors, with columns corresponding to changes with respect to wavenumber, and rows corresponding to changes with respect to concentration. The SVD row eigenvectors representing the intensity changes and the peak shifts of the Raman data set were fit using nonlinear least-squares to the single-site binding equation

$$\theta = \frac{|I|}{K_d + |I|}$$

to determine apparent substrate dissociation constants, $K_d$, where $\theta$ is the fraction 5c high spin (5cHS) protein and $|I|$ is the concentration of inhibitor.
a systematic blue shift as the substrate halogen is changed and follows the halogen series (see Fig. S1 in the Supporting Material). Therefore, in agreement with the x-ray crystal structures, binding of 4-XP in the internal pocket is consistent with the loss of the 6cHS population and subsequent movement of the distal His to the open, solvent-exposed position. Fig. 3 shows that 4-halophenols bind in the distal pocket with a binding affinity that follows the trend I > Br > Cl > F > H, with apparent dissociation constants of 0.536, 1.15, 1.78, 3.72, and 10.0 mM, respectively. We use the term “apparent dissociation constant” because the binding isotherms represent the fraction of enzyme that is converted to 5cHS, which does not necessarily reflect total binding to the enzyme. The relative binding affinity of 4-FP reflects its low occupancy in the crystal structure. The binding isotherms were determined using the change in relative intensities and the frequency shifts of the core size heme vibrational modes measured by RR spectroscopy and obtained from the data shown in Fig. 3a by SVD (as shown in Fig. S2).

### Binding of trihalogenated phenols

In contrast to the binding of 4-XPs, binding of 2,4,6-TXP substrates produces an increase of the 6cHS species. Fig. 4 shows the change of the RR core size marker bands, indicating the formation of a predominantly 6cHS heme ($v_3$ at 1481 cm$^{-1}$, $v_2$ at 1562 cm$^{-1}$, and $v_{10}$ at 1611 cm$^{-1}$) when 2,4,6-TBP and 2,4,6-TCP bind to DHP. Although the binding of 2,4,6-TFP also produces predominantly 6cHS heme, an appreciable amount of 5cHS ($v_3$ at 1494 cm$^{-1}$, $v_2$ at 1562 cm$^{-1}$, and $v_{10}$ at 1611 cm$^{-1}$) is observed when 2,4,6-TFP binds to DHP. The final concentration of 2,4,6-TFP was 200 mM, and the final concentrations of 2,4,6-TBP and 2,4,6-TCP were 4 mM in 150 mM potassium phosphate buffer, pH 6. Excitation wavelength: 406 nm; resolution: 1.7 cm$^{-1}$; laser power at the sample: 60 mW; acquisition time: 300 s.
We therefore tested the effect of internal binding of 4-XP on the turnover of 2,4,6-TCP. Fig. 5 provides kinetic evidence that the oxidation of 2,4,6-TCP is in fact inhibited by internal binding of 4-halophenols. The substrate (2,4,6-TCP) is readily converted to product (2,6-DCQ) when DHP is activated with excess H₂O₂ (Fig. 5a). However, in the presence of even a 2:1 ratio of 4-XP/2,4,6-TCP, little turnover of 2,4,6-TCP to 2,6-DCQ is observed (Fig. 5b–e). In fact, the only appreciable turnover is observed in the presence of 4-FP, in agreement with the binding isotherms from Fig. 2b that suggest only a small fraction of 4-FP binds internally. Since the 4-XP molecules bind internally and force the distal histidine to adopt the open conformation, it is likely that they prevent the formation of compound ES (39), the H₂O₂ activated protein radical form of DHP that is analogous to compound I of cytochrome c peroxidase (40) (see Fig. 1c). Indeed, Fig. 5f shows that increasing the concentration of 4-BP systematically blocks the formation of compound ES.

Michaelis-Menten inhibition/kinetic analysis

Since the unique internal binding of 4-XPs inhibits the peroxidase function of DHP at a remote external site, it is of interest to determine the type of inhibition that occurs between the two sites. The solubility of the native substrate, 2,4,6-TBP, is relatively low (~200 μM), which limits its usefulness for kinetic studies. However, 2,4,6-TCP is an excellent substrate and has higher solubility than 2,4,6-TBP. Therefore, we used 2,4,6-TCP rather than 2,4,6-TBP for the subsequent kinetic studies to demonstrate enzymatic

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**FIGURE 5** Kinetic assays showing inhibition by internally bound 4-XPs. (a–e) Time-dependent UV-Vis spectra from 0 s (red) to 120 s (purple). In the absence of 4-XP (a), the TCP substrate (312 nm) is converted to the DCQ product (273 nm). In the presence of 4-IP, 4-BP, and 4-CP (b–d), little product is formed, with no significant decrease in the substrate band. In the presence of 4-FP (e) some turnover is observed, in agreement with the lower affinity of 4-FP to bind internally. (f) Inhibition of compound ES formation (λmax (Soret) = 420 nm) in ferric DHP at pH 7 due to increasing concentrations of 4-BP.
inhibition by 4-BP (Fig. 6). The data obtained with and without 4-BP were fit independently to a Michaelis-Menten kinetic model. The fit parameters for the kinetic data are summarized in Table 2. $K_M$ increases as the concentration of inhibitor increases, but $V_{\text{max}}$ is essentially unchanged within the limits of the fitting errors. Ideally, one would use substrate concentrations several times greater than $K_M$, i.e., $[S]_{\text{max}} >> K_M$, but even when 2,4,6-TCP is used, the substrate solubility remains the limiting factor. Thus, errors in the fit data arise as $K_M$ becomes greater than $[S]_{\text{max}}$. Although we recognize this limitation, the relative effect of the inhibitor is clearly observed. Based on these data, the substrate/inhibitor pair exhibits competitive inhibition in DHP. Although results of this type cannot be demonstrated for 2,4,6-TBP because of its limited solubility, kinetic assays of 2,4,6-TBP with 4-BP under conditions identical to those used for 2,4,6-TCP with 4-BP yield the same complete inhibition effect (Fig. S5).

**DISCUSSION**

The shifts in the relative position of 4-XPs bound in the distal pocket of DHP suggest that there are two driving forces that stabilize the molecule. First, the parahalogen atom fills a cavity in the protein. Previous studies have examined cavities in Mb by determining Xe binding sites using x-ray crystallography (41). The cavity within DHP that is filled by the parahalogen is surrounded by hydrophobic residues (Fig. S6) and resembles the Xe binding site in sperm whale Mb (41). The second factor is the inter-action of the hydroxyl group with Y38, heme-7-propionate, and, to a lesser extent, H55 (Fig. 2 c). As the atomic radius of the halogen decreases, the hydroxyl group moves into closer contact with H55, Y38, and the heme propionate. Therefore, as shown in Figs. 2 and 3, a single atom, the parahalogen X in 4-XP, determines the binding affinity of the molecule in the distal pocket. Since the bound 4-XP displaces the water coordinated to the heme iron, one can also expect that it prevents the coordination of $\text{H}_2\text{O}_2$ and thereby inhibits peroxidase activity. The finding that increased concentrations of 4-BP systematically block the formation of the radical enzymatic intermediate (compound ES (39); see Fig. 1) in ferric DHP at pH 7 supports this hypothesis. Moreover, in accord with this hypothesis, the H55V mutation effectively eliminates the enzymatic activity of DHP (42).

On the basis of these results, and in agreement with the known mechanism for the peroxidase family members, we propose that enzymatic oxidation of substrates such as 2,4,6-TXP (X = I, Br, Cl, F) occurs at an external site. Recent $^1\text{H}^{-15}\text{N}$ HSQC experiments on $^{13}\text{C}/^{15}\text{N}$-labeled DHP clearly indicate different binding interactions between 4-BP inhibitor and 2,4,6-TCP substrate (18). 4-BP binding causes deviations in the internal binding pocket residues (F24, F35, F21, H55, and V59; see Fig. S7), whereas 2,4,6-TCP binding affects the distal H55 and amino acids residing at the protein dimer interface (R122, G1, S129, and L76; see Fig. S8) (18). Of interest, the common amino acid that is affected by either binding event, as observed by NMR, is the distal H55. The combination of RR and NMR strongly supports the existence of an external substrate-binding site. Consistent with these observations, every attempt to infuse 2,4,6-TXPs into the distal pocket in crystals of DHP under conditions identical to those used for the 4-XP x-ray structures resulted in no observable binding.

It appears from the RR data in Fig. 4 that 2,4,6-TFP binds both externally and internally, which implies that it acts as substrate and inhibitor, respectively. It is not possible to directly measure whether 2,4,6-TFP inhibits itself (i.e., whether autoinhibition is occurring). However, 2,4,6-TFP is a poor substrate for DHP compared with 2,4,6-TCP or 2,4,6-TBP, which may be in part due to its propensity to bind as an inhibitor. Fig. S9 provides a kinetic summary of the inhibition of 2,4,6-TCP by 2,4,6-TFP. It is clear from the data that 2,4,6-TFP does in fact inhibit 2,4,6-TCP oxidation. The sigmoidal shape of the 2,6-DCQ product formation suggests that 2,4,6-TFP is oxidized until its concentration is reduced such that it no longer acts as an inhibitor to 2,4,6-TCP oxidation. Several previous binding studies (16,37,38) used 2,4,6-TFP as a model for the native substrate because of its high solubility and similar substitution pattern.

The proposed external active site is consistent with pH-dependent studies of enzyme activity. The greatest activity of DHP for oxidation of 2,4,6-TCP was observed at pH 7.5.

**TABLE 2** Michaelis-Menten fit parameters

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</table>

**FIGURE 6** Michaelis-Menten analysis of inhibition, showing the initial reaction velocity versus substrate (2,4,6-TCP) concentration. Assays were conducted on WT-DHP without the presence of inhibitor, WT-DHP with 125 $\mu$M 4-BP, WT-DHP with 250 $\mu$M 4-BP, and WT-DHP with 500 $\mu$M 4-BP. The Michaelis-Menten fit parameters are given in Table 2.
Since at this pH, 2,4,6-TCP (pKa 6.4 (44)) is in the phenolate form, it is unlikely that it would be able to enter the distal pocket. On the other hand, the pKa of 4-BP is 9.3 (45), so the inhibitor would be protonated and hence neutral at pH 7.5. It is well known that buried charges are not stable in proteins. Attempts to place a buried charge in Mb by the mutation V68D resulted in ligation of the negatively charged carboxylate to the ferric heme iron, thus neutralizing the charge (46). Hence, neither the structural nor the functional observations of enzymatic activity are consistent with substrate binding in the distal pocket, but the same considerations are consistent with the internal binding of 4-bromo-phenol as an inhibitor. In line with this reasoning, the pKa of 2,4,6-TFP is ~7.2 (47), the highest of all of the TXPs studied. Thus 2,4,6-TFP would have the highest percentage of the phenol form at physiological pH, and therefore the greatest propensity to enter the distal cavity of the enzyme.

The correlation of the x-ray structural and RR data elucidates the key role played by the flexibility of the distal histidine. The x-ray crystal structure of Lebioda et al. (1) (PDB 1EWA) was the first observation of internal binding, it was reasonable to consider that the internal site may serve as the active site (1). However, structural, functional, and spectroscopic studies have repeatedly contradicted that assumption (17,18,37,38,43,51). The finding that 4-BP and 2,4,6-TBP act as an inhibitor and a substrate, respectively, for DHP is noteworthy because both molecules are present in benthic ecosystems (52). However, 4-BP is not an oxidation product of 2,4,6-TBP, this inhibitor-substrate pair is not part of a feedback system for this enzyme. Organisms such as Notomastus lobatus (among many others) synthesize 4-BP and 2,4,6-TBP, but this does not appear to be the case for A. ornata (3,53). Instead, DHP, which is the most abundant protein in A. ornata, oxidizes 2,4,6-TBP to 2,6-dibromo-1,4-benzoquinone (Fig. 1). The substrate 2,4,6-TBP acts as both a repellent, protecting marine organisms from predators, and a potentially lethal toxin. Therefore, the degradation of 2,4,6-TBP must be a protective function that minimizes the concentration of the highly toxic molecule in A. ornata (52). Although the reason for the inhibition of 2,4,6-TBP oxidation by 4-BP is not known, it is clear that DHP is a finely tuned enzyme that has an unusual mechanism for inhibitor specificity.

CONCLUSIONS

Since the x-ray crystal structure of DHP showing the substrate analog 4-IP bound in the distal pocket (PDB 1EWA) was the first observation of internal binding, it was reasonable to consider that the internal site may serve as the active site (1). However, structural, functional, and spectroscopic studies have repeatedly contradicted that assumption (17,18,37,38,43,51). The finding that 4-BP and 2,4,6-TBP act as an inhibitor and a substrate, respectively, for DHP is noteworthy because both molecules are present in benthic ecosystems (52). However, 4-BP is not an oxidation product of 2,4,6-TBP, this inhibitor-substrate pair is not part of a feedback system for this enzyme. Organisms such as Notomastus lobatus (among many others) synthesize 4-BP and 2,4,6-TBP, but this does not appear to be the case for A. ornata (3,53). Instead, DHP, which is the most abundant protein in A. ornata, oxidizes 2,4,6-TBP to 2,6-dibromo-1,4-benzoquinone (Fig. 1). The substrate 2,4,6-TBP acts as both a repellent, protecting marine organisms from predators, and a potentially lethal toxin. Therefore, the degradation of 2,4,6-TBP must be a protective function that minimizes the concentration of the highly toxic molecule in A. ornata (52). Although the reason for the inhibition of 2,4,6-TBP oxidation by 4-BP is not known, it is clear that DHP is a finely tuned enzyme that has an unusual mechanism for inhibitor specificity.

SUPPORTING MATERIAL

Nine figures and two references are available at http://www.biophysj.org/biophys/supplemental/S0006-3495(10)00716-2.

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The atomic coordinates and structure factors for the reported crystal structures have been deposited with the Protein Data Bank under accession codes 3LB1, 3LB2, 3LB3, and 3LB4.
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4. Reference deleted in proof.


