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Specific binding of victorin to a 100-kDa protein from oats

(host-selective toxins/*Cochliobolus victoriae*/*Helminthosporium victoriae*/victorin receptor)

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ABSTRACT **Susceptibility of oats to victoria blight, caused by the fungus *Cochliobolus victoriae*, and sensitivity to the host-specific toxin victorin, produced by the fungus, are controlled by the dominant allele at the *Vb* locus. It has been postulated that the *Vb* locus encodes a toxin receptor, although direct evidence for such a receptor is not available. Our recent studies on structure–activity relationships of the toxin established a methodology for producing ^{125}I-labeled victorin. Electrophoretic analysis of proteins from isogenic susceptible and resistant oat genotypes following treatment of leaves with radiolabeled victorin showed that victorin binds in a covalent and a genotype-specific manner to a 100-kDa protein only in susceptible oat leaf slices. This *in vivo* binding was competitively displaced by reduced victorin, a nontoxic protective compound, and appeared to be correlated with biological activity. *In vitro* binding to the 100-kDa protein in leaf extracts showed several differences from *in vivo* binding. Binding was not genotype specific and required a reducing agent that was not required for *in vivo* binding. Differential centrifugation showed that the 100-kDa victorin binding protein was not a cytosolic protein but was enriched in a high-speed particulate fraction. The data support the hypothesis that the 100-kDa protein is the victorin receptor.**

A major objective of contemporary plant pathology is to describe the conditions that define specificity and determine the molecular mechanisms of disease resistance and susceptibility in plant host–parasite interactions. Classical genetical approaches have identified genes that determine host resistance or susceptibility and genes that determine pathogen virulence or avirulence. The genetics of some host–parasite interactions conform to the gene-for-gene relationship as classically defined by Flor (1). In these types of interactions, disease resistance (incompatibility) is determined by a dominant gene for resistance in the host and a corresponding dominant gene for avirulence in the pathogen. The absence of a dominant allele at either gene locus results in a susceptible (compatible) interaction (2).

Alternatively, many diseases involving host-selective (host-specific) toxins (HST) are best described by the genetical inverse of the gene-for-gene relationship. In these plant diseases, a dominant gene in the host confers susceptibility to the pathogen and sensitivity to its toxin (compatibility) and a corresponding gene in the pathogen confers toxin production (virulence) (3–5).

HSTs produced by certain pathogenic fungi are directly involved in pathogenesis and characteristically reproduce the visible and biochemical symptoms of the disease caused by the toxin-producing pathogen. Furthermore, the toxin is active only against genotypes of the host that are susceptible to the pathogen.

We have been investigating victoria blight of oats caused by the fungus *Cochliobolus (Helminthosporium) victoriae*, which produces the HST victorin (6–8). Only oat genotypes carrying the dominant *Vb* allele are both susceptible to the fungus and sensitive to the toxin.

Victoria blight became a major disease of oats in the 1940s as a consequence of the introduction of commercial cultivars carrying the *Pc-2* gene for resistance to crown rust caused by *Puccinia coronata* (9). It was subsequently determined that the genes determining resistance to *P. coronata* and susceptibility to *C. victoriae* are either closely linked or controlled by the same genetic locus (10). Thus, identification of the *Vb* gene conditioning susceptibility to *C. victoriae* may simultaneously identify the *Pc-2* gene, a rust resistance gene. In that event, the diseases characterized by the gene-for-gene hypothesis and those characterized by HSTs would be uniquely bridged.

It has been postulated (11) that the *Vb* gene confers sensitivity to victorin (and thus susceptibility to the fungus) by encoding a receptor to the toxin, although there is no direct evidence for such a receptor. However, the stringent structural requirements for the toxic activity of victorin (12) suggest that a specific cellular recognition site exists. Identification of the postulated receptor would permit a direct analysis of the mode of action of victorin and would lead to the elucidation of disease specificity.

Extensive structure–activity studies of victorin (12) facilitated the preparation of biologically active derivatives of the toxin for use as labeled probes while defining a number of the structural features of the molecule required for biological activity. Significantly, the presence of the masked (hydrated) aldehyde function of the glyoxylic acid residue (7) is required for toxic activity, and we speculated that this functional group may mediate a covalent attachment of the toxin to its receptor (12).

Thus, it can be predicted that a victorin receptor is present in susceptible genotypes and either absent or altered in resistant genotypes and that the toxin binds to the receptor in a covalent manner. The objective of this study was to identify likely receptor molecule(s) by electrophoretic analysis of proteins from susceptible and resistant oat genotypes following *in vivo* and *in vitro* incubation with labeled victorin.

MATERIALS AND METHODS

Plant Material. Near-isogenic lines of oats (13, 14) homozygous for and differing in the *Vb* allele (S, susceptible; R, resistant) conditioning susceptibility to the pathogen and sensitivity to the toxin were used. Plants were grown in a growth chamber under a 16-hr photoperiod (23°C light, 20°C dark, 60% relative humidity) for 7–10 days.

Dark CO_2 Fixation Assay. Dark CO_2 fixation assays were performed as described by Wolpert *et al.* (12).

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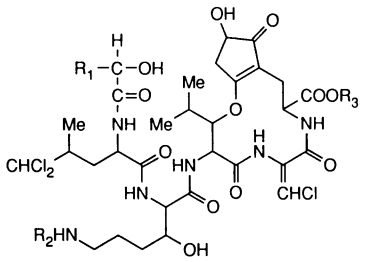
Abbreviations: HST, host-selective toxin; BHC, Bolton–Hunter derivative of victorin C; MEC, methyl ester of victorin C; RC, reduced form of victorin C; VBP, victorin binding protein; HS, high speed.

Preparation of Victorin C, Reduced Victorin C (RC), Methyl Ester Victorin C (MEC), and Radiolabeled Victorin C. Purification of victorin C and preparation of RC, MEC, and Bolton–Hunter derivative of victorin C (BHC) were performed as described (6, 7). The structures of these compounds are shown in Fig. 1.

Radiolabeled victorin C was prepared by adding 20 μl of a solution containing 80 μg of victorin C in 0.08 M potassium phosphate buffer (pH 7.5) to the dried residue of 2 mCi of ^{125}I-labeled *N*-succinimidyl-3-(4-hydroxyphenyl)propionate (specific activity, ≈2000 Ci/mmol; 1 Ci = 37 GBq; ICN), mixing thoroughly, and incubating at room temperature for 75 min. The reaction mixture was then streaked onto a silica gel thin-layer chromatography plate (Soft-Plus, Merck), developed in acetonitrile/H_2O (6:1), and the area (R_f, ≈0.5) with the labeled derivative was scraped from the plate and eluted with H_2O. Radiolabeled victorin C (^{125}I-BHC) was isolated, completely free of underivatized victorin C (R_f = 0).

Protein Labeling with ^{125}I-BHC. *In vivo*. Ten 0.5-mm leaf slices of either S or R oat genotypes containing ≈15.5 μg of chlorophyll were incubated in 100 μl of 10 mM Mops containing 1% sucrose and 20–22 μCi of ^{125}I-BHC per ml at 25°C for 4 hr with various concentrations of unlabeled toxin or toxin derivatives.

Proteins were extracted by homogenizing rinsed leaf strips with a small amount of powdered sand in 200 μl of 80% phenol in water and 200 μl of 50 mM Tris·HCl/700 mM sucrose/5 mM dithiothreitol/100 mM KCl/5 mM EDTA, pH 7.0. The homogenate was centrifuged and the upper (phenol) layer was mixed with 1 ml of 0.1 M ammonium acetate in methanol and left at 4°C overnight. The protein precipitate was collected by centrifugation, washed with cold methanol, dried under vacuum, and solubilized in $NaDodSO_4$ buffer [62.5 mM Tris·HCl, pH 6.8/2.3% (wt/vol) $NaDodSO_4$/5% (vol/vol) 2-mercaptoethanol/10% (wt/vol) sucrose]. Samples (≈3 μg of chlorophyll equivalent) were typically analyzed on 8% polyacrylamide gels in the buffer system of Laemmli (15). Molecular weight estimation of low molecular weight protein was performed using 12% polyacrylamide gels and low molecular weight markers (Bio-Rad) (data not shown). For autoradiographic analysis, dried gels were exposed to Kodak X-OMAT AR film (Eastman Kodak) with an intensifying screen at −80°C for 44 hr.



Victorin C (R_1 = OH, R_2 = H, R_3 = H)

Reduced Victorin C (R_1 = H, R_2 = H, R_3 = H)

Methyl Ester Victorin C (R_1 = OH, R_2 = H, R_3 = CH_3)

Bolton-Hunter Victorin C (R_1 = OH, R_2 = C(=O)-CH_2CH_2-⬡-OH, R_3 = H)

FIG. 1. The general structures of victorin C, RC, MEC, and BHC are shown. Reference to the substituents indicated by R_1, R_2, and R_3 presented to the right of the name of the compound will complete the structure of that compound. Victorin C is the most abundant form of the toxin produced by *C. victoriae*. The concentration of victorin required to elicit a 50% inhibition (EC_{50}) of dark CO_2 fixation in susceptible oat leaf slices in a 4-hr incubation at 25°C is 37 nM. RC is the reduced form of victorin C. Reduction of the aldehyde group of victorin C to a primary alcohol, as in RC, renders the compound nontoxic. Coincubation of susceptible leaf slices with victorin C and RC can reduce or eliminate (depending on the relative concentration of the two molecules) the activity of victorin C. BHC is the hydroxyphenyl propionate derivative of victorin C, which can be radiolabeled with ^{125}I at positions ortho to the hydroxyl group of the phenyl propionate group. BHC, although less toxic than the native toxin, retains its host-selective activity. The EC_{50} of BHC in dark CO_2 fixation assays is 3.9 μM. MEC is the methyl ester derivative of victorin C, which also retains host-selective toxic activity. The EC_{50} of MEC in dark CO_2 fixation assays is 283 nM.

In vitro. Leaf tissue (≈0.5 g fresh weight per 10 ml of buffer) from S and R genotypes was homogenized with a mortar and pestle in cold 50 mM Mops/2 mM EDTA/0.4 M sucrose, pH 7.5/6 mM 2-mercaptoethanol (except where indicated) and filtered through four layers of cheesecloth. Chlorophyll content (16) was adjusted to 155 μg/ml by addition of cold buffer. This preparation is referred to as the "crude homogenate" (CH). The CH was centrifuged at 400 × *g* for 4 min, and the pellet was resuspended in the original volume of buffer (low speed preparation). The supernatant was centrifuged at 100,000 × *g* for 30 min, and the resulting pellet was resuspended in the original volume of buffer (high-speed preparation or HS). The final supernatant was referred to as the soluble preparation.

Binding was tested by adding ≈3 μCi of ^{125}I-BHC per ml to the preparation and incubating for 1 hr at 25°C. The reaction was terminated by the addition of an equal volume of $NaDodSO_4$ buffer and the samples were incubated in a boiling water bath for 2.5 min. Samples (≈3 μg of chlorophyll equivalents) were analyzed by autoradiography (20-hr exposure) after electrophoresis on 8% polyacrylamide gel electrophoresis as described above. Molecular weight estimation of low molecular weight proteins was also performed as described above.

RESULTS

***In Vivo* Binding.** When oat leaf slices were incubated with ^{125}I-BHC, a 100-kDa protein was labeled only in the S oat genotype. ^{125}I-BHC also labeled a low molecular weight protein (≈15 kDa) in both S and R genotypes (Fig. 2). The genotype-specific toxin-induced labeling of the 100-kDa protein appeared to be covalent as it persisted after phenol extraction, precipitation, boiling in $NaDodSO_4$ buffer, and PAGE.

RC is not toxic but it behaves as a protectant, presumably by competitive displacement of victorin C (12). When S oat leaf slices were incubated with victorin C and RC, the toxic effects of victorin C, as assessed by dark CO_2 fixation assays, were decreased or eliminated, depending on the concentration of RC (Fig. 3*A*). Incubation of leaf slices with ^{125}I-BHC and RC under conditions analogous to those used for dark CO_2 fixation

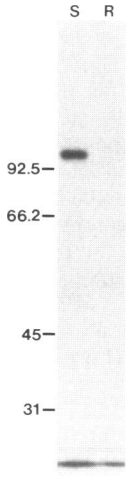


FIG. 2. Autoradiograph of PAGE analysis of proteins extracted from S and R oat leaf slices incubated in ^{125}I-BHC (22 μCi/ml) and unlabeled victorin C (100 ng/ml) at 25°C for 4 hr. Numbers on left are kDa.

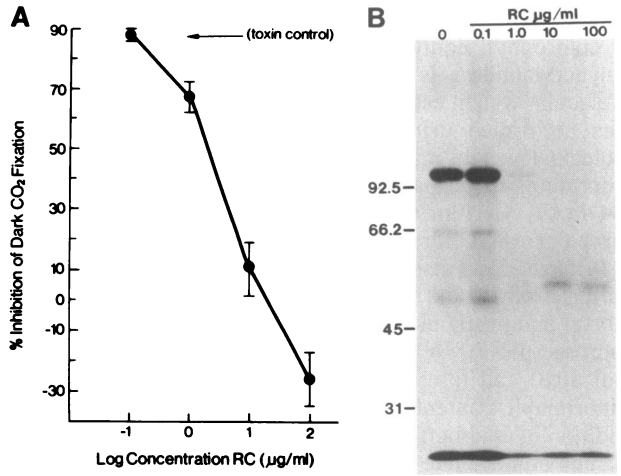


FIG. 3. (*A*) Protection of toxin-treated susceptible oat leaf slices by increasing concentrations of reduced victorin C (RC) as measured by % inhibition (compared to untreated controls) of dark $^{14}CO_2$ fixation. Oat leaf slices were incubated in the presence of victorin C (100 ng/ml) with 0 (i.e., toxin control, see arrow), 0.1, 1.0, 10, or 100 μg of RC per ml for 4 hr at 25°C. (*B*) Autoradiograph of PAGE analysis of protein extracted from susceptible oat leaf slices incubated in ^{125}I-BHC (22 μCi/ml) + unlabeled victorin C (100 ng/ml) for 4 hr at 25°C with 0, 0.1, 1.0, 10.0, or 100.0 μg of RC per ml. Numbers on left are kDa.

assays also decreased or prevented the binding of labeled BHC to the 100-kDa protein in S oat leaf slices (Fig. 3*B*).

***In Vitro* Binding.** Leaf tissue homogenates were adjusted to contain 155 μg of chlorophyll per ml, which was the same concentration used for *in vivo* binding studies with leaf slices. The homogenate was fractionated by differential centrifugation and incubated with ^{125}I-BHC to determine which fractions contained the 100-kDa victorin binding protein (VBP). Results from these analyses showed that the VBP was enriched in the 100,000 × *g* pellet (HS preparation) and that binding was greater in the HS preparation than in the crude homogenate (Fig. 4). Binding to a 21-kDa protein was also evident *in vitro*, which was not observed *in vivo*.

In vitro binding analysis revealed a requirement for an exogenous reducing agent (2-mercaptoethanol or dithiothreitol). When homogenates were prepared in the presence or absence of a reducing agent and tested for covalent binding, labeling was observed only in the presence of a reducing agent (Fig. 5). If the reducing agent was added after protein extraction, immediately before binding assays were conducted, covalent binding was restored (Fig. 5).

In vitro binding experiments demonstrated the presence of a 100-kDa VBP in both genotypes. When binding was conducted in the presence of a constant amount of labeled BHC with increasing concentrations of unlabeled victorin, competition by the unlabeled victorin was evident (Fig. 6). Incubation of HS preparations with 3.4 μCi of ^{125}I-BHC per ml with 0–100 μg of unlabeled victorin C per ml resulted in a gradual decrease in the extent of ^{125}I-BHC-mediated labeling of the 100-kDa protein in both genotypes (Fig. 6).

In addition, victorin C, MEC, BHC, and RC appeared to prevent ^{125}I-BHC-mediated labeling of the 100-kDa protein. Incubation of HS preparations from the S genotype, adjusted to contain 39 μg of chlorophyll per ml, in the presence of a constant amount of labeled BHC (3.1 μCi/ml), with increasing concentrations of unlabeled victorin C, MEC, BHC, or RC at 0–100 μg/ml decreased the extent of ^{125}I-BHC-mediated labeling of the 100-kDa protein (Fig. 7).

DISCUSSION

Several requirements should be satisfied with the binding of victorin to its site of action. The binding should (*i*) demonstrate genotype specificity—i.e., should show a marked difference in S and R genotypes; (*ii*) be correlated with biological activity; and (*iii*) be ligand specific—i.e., binding of the biologically active radiolabeled derivative of the toxin should be competitively displaced by unlabeled toxin.

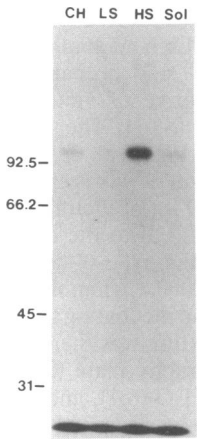


FIG. 4. Susceptible oat leaf tissue was homogenized and the homogenate was subjected to differential centrifugation and tested for binding by incubating aliquots with 3.4 μCi of ^{125}I-BHC per ml for 1 hr at 25°C. The crude homogenate volume was adjusted to contain 155 μg of chlorophyll per ml, which was the approximate chlorophyll content of leaf slices used in *in vivo* labeling experiments (Figs. 2 and 3*B*). All pellets were resuspended in the original volume of buffer before addition of ^{125}I-BHC. Shown is an autoradiograph of fractions analyzed by PAGE after binding. CH is the crude homogenate. LS denotes the resuspended pellet after centrifugation at 4000 × *g* for 4 min. HS denotes the resuspended pellet after centrifugation of the supernatant from LS at 100,000 × *g* for 30 min, and Sol denotes the supernatant of the 100,000 × *g* centrifugation. Numbers on left are kDa.

Previous studies on the structure–activity relationships of victorin revealed the importance of the aldehyde group for toxic activity (12). Removal of the glyoxylic acid residue eliminates all biological activity. The reduction of the aldehyde to a primary alcohol, as in RC, eliminates toxicity. However, RC must nevertheless associate with the active site, because treatment of oat leaf slices with RC prevents the toxic effects of victorin C. Based on those observations and the fact that many aldehydes can bind covalently to proteins (17), we speculated that the aldehyde mediates a covalent association of victorin with its site of action and that covalent binding was essential for toxicity (12). Thus, in binding analyses we anticipated a covalent association of labeled victorin with the active site.

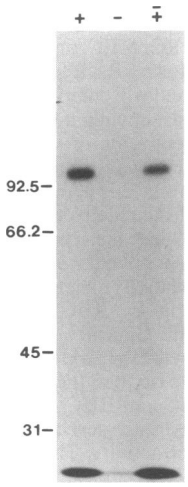


FIG. 5. HS fractions (see Fig. 4) were prepared from susceptible oat leaf tissue (crude homogenate = 155 mg of chlorophyll per ml) and tested for binding by incubation with 3.4 μCi of ^{125}I-BHC per ml for 1 hr at 25°C. Preparations tested included tissue homogenized in buffer containing 6 mM 2-mercaptoethanol (+); buffer without 2-mercaptoethanol (−); and a portion of the preparation that was adjusted to 6 mM 2-mercaptoethanol immediately prior to binding (∓). Equivalent results were obtained with dithiothreitol. Numbers on left are kDa.

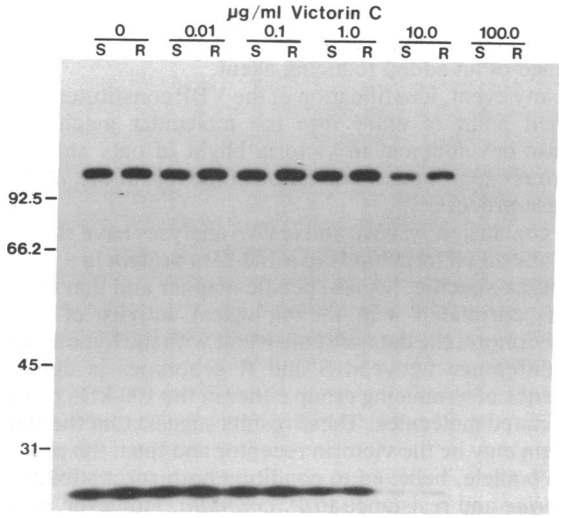


FIG. 6. HS preparations from S and R leaf tissue were tested for *in vitro* binding in the presence of a constant amount of ^{125}I-BHC (3.4 μCi/ml) and increasing concentrations of unlabeled victorin C at 0, 0.001, 0.01, 1, 10, or 100 μg/ml. Numbers on left are kDa.

In vivo binding experiments revealed that radiolabeled BHC bound in a covalent and a genotype-specific manner to a 100-kDa protein only in S oat leaf slices. Furthermore, these results were not confined to the near-isogenic lines as we obtained identical results using "unrelated" susceptible Park (homozygous dominant for the *Vb* allele) and resistant Rodney (homozygous recessive for the *vb* allele) oat lines (data not shown). Binding was prevented by the protectant RC and appeared to be correlated with biological activity, because decreased binding was associated with the decreased toxic effects of victorin C in the presence of increasing concentrations of RC.

In vitro analyses clearly demonstrated competition for binding by unlabeled victorin C, MEC, BHC, and the protectant RC. Both RC and victorin C appeared to prevent ^{125}I-BHC labeling of the VBP with comparative efficiency, indicating that they have similar affinity for the VBP. Previous structure–activity studies revealed that MEC is ≈10% as active as native victorin C (12); these results are consistent with results from the label-displacement studies. The extent of *in vitro* labeling was approximately the same for MEC at 100 μg/ml as for victorin C at 10 μg/ml. However, unlabeled

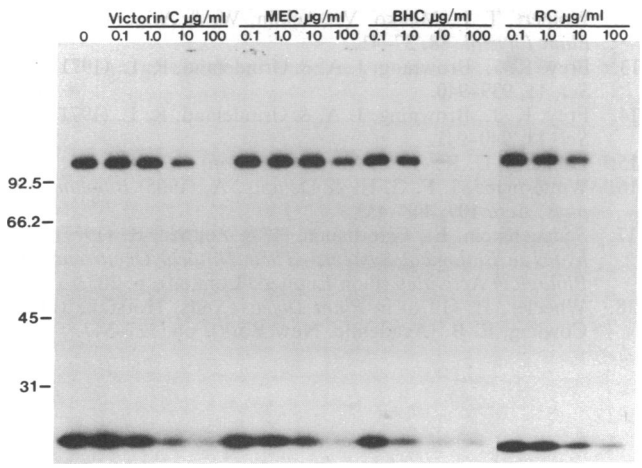


FIG. 7. HS preparations from susceptible leaf tissue adjusted to contain 39 μg of chlorophyll per ml were incubated in the presence of a constant amount of ^{125}I-BHC (3.1 μCi/ml) and increasing amounts of unlabeled victorin C, unlabeled MEC, unlabeled BHC, or unlabeled RC at 0, 0.1, 1, 10, or 100 μg/ml. Numbers on left are kDa.

BHC showed a greater displacement activity than native victorin C. Significant displacement could be observed at 1 μg of unlabeled BHC per ml. Comparison of unlabeled BHC at 10 μg/ml vs. victorin C at 100 μg/ml showed approximately equivalent results. This indicated that BHC actually has a higher affinity for the VBP than victorin C.

The discrepancy between the biological activity of BHC and binding that occurs is then attributed to factors other than binding. It is reasonable to assume that *in vivo* three events must occur for toxin binding: The toxin must (*i*) reach the site of action (transport, diffusion, etc.), (*ii*) associate with the active site, and (*iii*) covalently bind to the active site. Any modification of the molecule that decreases the rate of event *i* would decrease the biological activity but would not be apparent in *in vitro* binding experiments. Furthermore, it is possible that chemical modifications that lead to an altered rate in event *ii* or *iii* could yield an apparent discrepancy between *in vivo* activity and *in vitro* binding. For example, if BHC *in vivo* has a higher rate for event *ii* (i.e., a higher affinity for receptor associations) but a lower rate of event *iii* (covalent association) then its biological activity could be reduced. However, *in vitro*, because covalent binding required an added reducing agent, the rate of event *iii* for both compounds could be very different from *in vivo*, thus masking this effect.

In vitro binding to the 100-kDa protein exhibited a number of differences from *in vivo* binding. Binding was not genotype specific, and covalent binding *in vitro* required a reducing agent, which was not required for binding *in vivo*. After these studies, we have generated antibodies to the *in vitro* labeled 100-kDa protein and found that they react with the *in vivo* labeled protein (data to be published). Thus, the *in vitro* and *in vivo* labeled 100-kDa protein are very likely the same protein.

Differential centrifugation showed that the 100-kDa VBP is not a cytosolic protein but is enriched in a high-speed particulate fraction. These results do not necessarily indicate that the 100-kDa VBP is associated with the membrane fraction. We noted that prolonged low-speed centrifugation resulted in an enrichment of the VBP in the low-speed pellet, albeit with less efficiency than was obtained by high-speed centrifugation (data not shown) and that binding in the HS pellet, after resuspension, was much greater than in the crude homogenate. This latter observation suggests that disruption of some type of vesicle may result in the "release" of more VBP. These results indicate that the majority of the VBP could be compartmentalized within the cell. However, it is possible that different quantities of the VBP are located in two or more cellular compartments.

Attempts to quantify binding and demonstrate displaceable binding with unlabeled victorin *in vivo* failed. We attribute this to the use of leaf slices, which present a diffusional barrier to toxin. It has been well documented that victorin causes changes in permeability in sensitive oat leaves (cf. refs. 3, 11, and 18). These toxin-induced permeability changes may account for the observation that increases in concentration of unlabeled toxin did not prevent victorin binding *in vivo*. For example, progressive toxin-induced damage to peripheral cells causes permeability changes and, therefore, promotes the diffusion of toxin deeper into the tissue to more binding sites. *In vivo* binding analysis was further complicated by the fact that, as a toxin, victorin causes cell death and concomitant degradative processes.

The most probable reason that RC so effectively reduced ^{125}I-BHC binding *in vivo* is that RC not only competes for the binding sites but also prevents the toxic activity of victorin and thus the induced permeability changes and cell death. The extent of label displacement also indicates that a limited amount of binding results in a substantial biological effect. This could be interpreted to mean that there is a substantial

difference between saturable binding for a biological effect vs. saturable binding of ^{125}I-BHC. For example, if there is a relative excess of receptor in the plant cell, it is quite possible that binding of toxin to 10% of available receptor produces 100% of the biological response. Therefore, an apparent reduction of binding by 92% may very well diminish biological activity by only 20%.

Victorin-induced labeling of low molecular weight peptides was considered to be less significant, because the 15-kDa peptide was labeled *in vivo* in both genotypes, and the 21-kDa peptide was labeled only *in vitro*. Thus, these peptides did not appear to be genotype specific or their labeling was not associated with biological activity.

The finding that a 100-kDa protein binds ^{125}I-BHC in a genotype-specific manner only *in vivo* but has no genotype specificity *in vitro* complicated interpretation of the results. Questions arise not only as to whether the VBP is the site of action of the toxin (i.e., the receptor) but if so, whether it is the product of the *Vb* allele. Under the assumption that the VBP is not the site of action, toxin binding to the VBP must be a consequence and not a cause of toxic activity, and the VBP must not be the product of the *Vb* allele. This interpretation must be considered because toxin is selective and therefore damaging only toward S genotypes. Thus, toxic disruption (e.g., decompartmentalization) could lead to binding only in the sensitive genotype. However, under this interpretation the ligand-specific binding of ^{125}I-BHC must be dismissed as simply fortuitous, a condition that seems unlikely. Furthermore, if binding of S genotypes is a consequence of disruption by toxin, binding *in vivo* would presumably be analogous to *in vitro* binding. This latter point is unlikely because *in vivo* binding in S genotypes appears to be covalent without the addition of a reducing agent, a requirement for covalent binding *in vitro*.

Based on the above, we believe it is more likely that the VBP is the site of action (i.e., receptor). However, because a VBP is present in both genotypes it is unclear whether it is the product of the *Vb* allele. If the VBP is not the product of the *Vb* allele, another factor(s) conditioned by the *Vb* allele may affect the binding of victorin to the VBP. For example, the *Vb* allele may mediate the access of the toxin to the VBP or the localization of the VBP. Alternatively, if covalent binding is required for toxic activity and a reducing potential is required for covalent binding, as indicated by *in vitro* binding experiments, then the product of the *Vb* allele may mediate this reducing potential and constitute the difference between S and R genotypes.

Finally, the VBP could be the product of the *Vb* allele. If so, there must be a difference between the VBP in S and R genotypes. This difference could, for example, affect transport or a posttranslational modification of the VBP. Alternatively, the *in vivo* requirement for a reductant could be fulfilled in the S genotypes by a reducing group in the 100-kDa protein (e.g., a cysteine residue) near the victorin binding site that is absent or displaced in the resistant genotype. The absence of a nearby reducing group in R genotypes could preclude the covalent binding of victorin *in vivo* and thus its toxic activity, in R genotypes, if covalent binding is required for toxic activity. Presumably, isolation of the protein for *in vitro* binding analysis results in a displacement of this reducing group either by denaturation or oxidation and thus prevents the covalent association of victorin *in vitro* in the absence of an added reducing agent.

In any event, identification of the VBP constitutes a clearly defined point of entry into the molecular mechanism of disease development in victoria blight of oats and permits rigorous experimental evaluation of the role of the VBP in the disease process.

In conclusion, *in vitro* and *in vivo* analyses have shown that ^{125}I-labeled victorin binds to a 100-kDa protein in a covalent, genotype-specific, ligand-specific manner and that this binding is correlated with the biological activity of victorin. Furthermore, the data are consistent with the hypothesis that the difference between S and R genotypes is due to the presence of a reducing group either in the 100-kDa protein or associated molecules. These results suggest that the 100-kDa protein may be the victorin receptor and thus, the product of the *Vb* allele, believed to condition both susceptibility to *C. victoriae* and resistance to *P. coronata*. However, it should be noted that cause and effect have not been established. Until proof can be provided that victorin binding to this protein leads to the observed toxic effects, the 100-kDa protein can only be considered as a likely candidate for the victorin receptor.

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