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Purification and Characterization of Polyphenol Oxidase from Glandular Trichomes of *Solanum berthaultii*

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**ABSTRACT**

Type A glandular trichomes of the wild potato (*Solanum berthaultii* Hawkes) entrap insects by rapidly polymerizing the trichome contents after breakage by insect contact. Polymerization of trichome exudate appears to be driven by a soluble polyphenol oxidase (PPO). PPO constitutes up to 70% of the protein in individually collected trichomes and reaches a concentration approaching 200 μM in these organs. Trichome PPO has been purified and shown to be a monomeric copper metalloprotein with an isoelectric point of 5.5, possessing only o-diphenol oxygen oxidoreductase activity, and is larger than most other reported PPOs, with relative molecular weight of 59,000. Chlorogenic and caffeic acid were the most readily oxidized of 14 phenolic substrates tested. Polyclonal antibodies raised against the relative molecular weight 59,000 *S. berthaultii* trichome PPO were used to show that *S. tuberosum* L. trichomes express low levels of a cross-reactive protein that lacks detectable PPO activity.

The selection process that has given rise to the modern cultivated potato (*Solanum tuberosum* L.) has drastically narrowed its genetic base. A few genetically similar varieties now account for nearly all of the acreage grown in the United States. Selection for horticultural quality may have been accompanied by the inadvertent removal or reduction in levels of important pest-resistance factors (e.g. steroidal glycoalkaloids) (34), and until recently, relatively little success has been achieved toward breeding for resistance to insects that have adapted to cultivated potato (e.g. the Colorado potato beetle, *Leptinotarsa decemlineata*). Hence, it is not surprising that the cultivated potato is susceptible to attack from a wide array of arthropod herbivores; little inherent insect resistance remains in *S. tuberosum*. There is, however, a broad diversity of genetic traits available in wild germplasm. Wild potato species exhibit substantial resistance to insect pests. The wild Bolivian potato species *Solanum berthaultii* Hawkes displays a high degree of resistance to aphids, leafhoppers, flea beetles, spider mites, and the Colorado potato beetle (11). This resistance is conferred by high densities of foliar glandular trichomes (8, 9, 11).

Glandular trichomes are modified epidermal cells that frequently confer resistance to arthropod herbivores by the action of secreted toxic and behavior-modifying allelochemicals, or by physical entrapment. *S. berthaultii* possesses two types of glandular trichomes, A and B. Type B trichomes are 600 to 950 μm in length and secrete a viscous mixture of short- and medium-chain fatty acids esterified to succrose (16). *S. berthaultii* sucrose esters function in entrapment of small arthropods and also act as potent feeding deterrents for the green peach aphid (22).

The type A trichome of *S. berthaultii* is 120 to 210 μm in length, each with a 50- to 70-μm diameter tetralobulate head (composed of four to eight cells) at its apex. Type A trichomes confer resistance to insects by entrapment in polymerized trichome exudate. The fragile type A trichome head ruptures upon contact by insects, discharging an exudate that rapidly undergoes oxidative polymerization. Polymerization and hardening of this exudate on the insect impedes movement, occludes the mouthparts, and ultimately entraps the small arthropod on the foliage, with ensuing mortality (8, 9, 11).

Previous work (4, 8, 25) has attributed the oxidative polymerization of *S. berthaultii* type A trichome exudate to two types of oxidative enzymes, PPOs (EC 1.10.3.) and PO (EC 1.11.1.7). PPOs and POs are Cu(II)- and heme-containing enzymes, respectively, which are capable of oxidizing and cross-linking a wide range of phenolic substrates. PPOs are ubiquitous, membrane-associated, plastid enzymes of Mr, 40,000 to 45,000 that oxidize phenols to quinones at the expense of O₂ (19, 20, 31, 32). The POs are a heterogenous group of enzymes, localized both intra- and extracellularly, that utilize H₂O₂ to oxidize a wide array of substrates (7). Gibson (8) observed that in the absence of O₂, the contents of ruptured type A trichomes would not polymerize, indicating that oxygen is the primary oxidant. In contrast, previous studies (25) also suggested that PO accounts for a substantial portion of the oxidative capacity of the type A trichome. However, these workers did not sample individual trichomes, and it is likely that the majority of PO they observed was nontrichomal. Bouthyette et al. (4) tentatively identified both PPO and PO in the type A trichome, but because individual trichomes were not sampled it was not clearly established whether these enzymes were derived from the trichomes or nontrichomal cells of the leaf.

The cultivated potato, *S. tuberosum*, lacks trichome-mediated insect entrapment resistance. Although the outward

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2 Abbreviations: PPO, polyphenol oxidase; PO, peroxidase; DOPA, dihydroxyphenylalanine; IEF, isoelectric focusing; pI, isoelectric point.
morphology of *S. berthaultii* and *S. tuberosum* type A trichomes is substantially conserved, the trichomes on the foliage of the cultivated potato are present at low density and do not readily rupture. Furthermore, when ruptured, the contents of *S. tuberosum* type A trichomes fail to oxidize and are incapable of entrapping insects (9). Although the trichome-based resistance mechanism of the wild potato has been successfully transferred to tetraploid hybrids of *S. berthaultii* × *S. tuberosum* possessing high densities of trichomes capable of insect entrapment (29), the reasons for the failure of *S. tuberosum* trichome exudate to undergo oxidative polymerization remain unknown.

The purpose of this study was to analyze individual trichomes to identify, purify, and characterize the primary oxidative enzymes involved in insect entrapment by *S. berthaultii* type A trichomes and, further, to determine whether failure of *S. tuberosum* trichomes to provide insect resistance is related to an absence of oxidative enzymes in the type A trichome.

**MATERIALS AND METHODS**

**Plant Materials**

Seeds of *Solanum berthaultii* Hawkes, plant introduction number 473334, were treated with 10% (v/v) Clorox for 10 s, washed twice with water, and then soaked in 9 mM GA₃ for 24 h prior to sowing. The soil mix used was Promix BX (Premier Brands). Plants were on a weekly fertilization schedule with 20-10-20 NPK (Premier Brands). Plants were grown at 25°C under 1000-W metal halide lamps, with a PPFD of 3.6 × 10³ μmol photons m⁻² s⁻¹, on a light schedule of 14 to 16 h light/8 to 10 h dark.

**Individual Trichome Collections**

For each electrophoretic analysis, 250 to 7000 trichomes were manually collected by touching each trichome head with the end of a 300-μm diameter glass capillary that had been drawn out to a diameter of approximately 50 μm. Upon contact, the trichomes rupture and their contents are drawn into the capillary, which is filled with 200 mM DTT or 10 mM cysteine, 50 mM sucrose, 100 mM sodium phosphate, pH 7.0. The samples were centrifuged at 12,000g for 5 min to remove debris, and prior to electrophoresis the supernatant was either mixed with 2% carrier ampholytes or boiled in Laemmli sample buffer. SDS-PAGE electrophoresis was carried out on 10% polyacrylamide minigels. Gels were run at 200 V (constant) for 45 min at 25°C. IEF was performed using pH 3.0 to 9.5 precast 5% polyacrylamide gels (Pharmacia-LKB Biotechnology) according to the manufacturer’s instructions.

To determine protein content of type A trichomes and subcellular distribution of trichome PPO, 7000 type A trichomes were collected individually into 10 mM cysteine, 50 mM sucrose, 100 mM sodium phosphate, pH 7.0. The final volume of the trichome collection was 27 μL; this was brought to 65 μL with collection buffer, and the sample was centrifuged at 7000g for 5 min at 4°C. The supernatant was centrifuged at 75,000g for 30 min at 25°C in an airfuge (Beckman Instruments, Inc.). The supernatants and washed pellets were diluted to 65 μL with collection buffer, and assayed for PPO activity and protein (Bradford assay, Bio-Rad, using BSA as standard).

**Protein and Activity Gel Stains**

For PPO activity detection, the gels were placed in a solution of 0.1 mM sodium phosphate, pH 7.0, 0.3 mM catechol, and 90 mM p-phenylene diamine for 5 to 10 min, thoroughly washed with water, soaked in 10% citric acid for 2 min, and dried. PO activity stain was composed of 6 mL of 45 mM 3-amino-9-ethylcarbazole in dimethylformamide, 92 mL of 0.1 mM sodium acetate, pH 5.0, and 1.5 mL of 30% H₂O₂. The gel was incubated for 5 min, washed thoroughly with water, and dried. For Coomassie staining, SDS-PAGE and IEF gels were fixed in a solution of 10% (w/v) TCA acid, 25% (w/v) methanol, 3% (w/v) sulfosalicylic acid. IEF gels were then extensively washed in 5% (v/v) methanol, 7.5% (v/v) acetic acid to remove carrier ampholytes, prior to staining with Coomassie brilliant blue R. Silver-stained gels were fixed and washed identically prior to development (National Diagnostics Silver Stain Kit EC-710).

**Purification of PPO**

Fully expanded leaves of *S. berthaultii* were wiped with cotton swabs moistened with a solution of 200 mM DTT. Approximately 30 leaflets were wiped abaxially and adaxially per swab, with a total of 4000 leaflets being wiped. The crude trichome exudate was squeezed from the swab using a syringe and centrifuged at 15,000g for 15 min at 4°C. The supernatant (approximately 20 mL) was decanted, mixed with 2 mL of 40% carrier ampholytes (pH 4–6), and brought to a final volume of 40 mL with water. This solution was then loaded into a preparative IEF cell (Rotofor, Bio-Rad) and electrophoresed at 40 W constant power. Fractions were collected simultaneously under vacuum and the presence of PPO was determined using a spectrophotometric PPO activity assay (6). Active fractions were pooled and dialyzed at 4°C for 12 h against two 4-L changes of 0.5 mM NaCl, followed by two 4-L changes of H₂O. Purified PPO was concentrated by lyophilization and stored at −70°C.

Copper-depleted PPO was reconstituted by incubating the enzyme in 33 mM sodium malate, pH 5.0, 1 mM CuSO₄, for 13 h at 4°C. As a control, an aliquot was incubated under the same conditions but without CuSO₄. Removal of malate and excess copper ions was accomplished with a 1-mL Sephadex G-25 spin column equilibrated in H₂O. The reconstituted enzyme was assayed spectrophotometrically for PPO activity and by activity staining of IEF gels.

**Substrate Specificity**

Initial rates of reaction of PPO with various substrates were determined by spectrophotometric assay of 2-nitro-5-thiobenzoic acid oxidation by quinones generated from various phenols by PPO activity (6). Reaction mixes were composed of 10 mM substrate, 100 mM sodium phosphate, pH 7.0, and 57 μM 2-nitro-5-thiobenzoic acid in 1 μL, to which was added 2 μg of PPO in 100 mM of H₂O.
Figure 1. Electrophoresis of proteins from type A trichomes. Lane 1, 1850 S. berthaultii type A trichomes, developed for PPO activity; lane 2, 1850 S. berthaultii type A trichomes, Coomassie stained for protein; lane 3, 500 S. tuberosum type A trichomes, developed for protein using silver stain; lane 4, 500 S. berthaultii type A trichomes, developed for protein using silver stain; lane 5, 1850 S. tuberosum type A trichomes, developed for PPO activity; lane 6, 1850 S. berthaultii type A trichomes, developed for peroxidase activity.

ELISA and Immunoblotting

Polyclonal antibodies against trichome PPO were prepared by injecting two New Zealand White rabbits subcutaneously with 300 μg of purified PPO emulsified in 1 mL of complete Freund’s adjuvant. Subsequent injections of 100 μg in incomplete Freund’s adjuvant were carried out at 15 and 21 d. Titering of antisera was carried out by ELISA using 100 ng of purified PPO per well in polystyrene 96-well plates developed with affinity purified goat anti-rabbit immunoglobulin G alkaline phosphatase conjugate (1). Under these conditions, the titer of the pooled antisera collected at 10 and 30 d after the second boost was 250,000. A 1:4000 dilution of antiseraum was routinely used for immunoblotting. Electrophoresis or SDS-PAGE minigels were equilibrated for 20 min in a transfer buffer of 25 mM Tris, 192 mM glycine, 20% (v/v) methanol, pH 8.3, and then transferred to nitrocellulose using a Mini Transblot Unit (Bio-Rad) operated at 0°C, 100 V, 0.25 A for 1 h (1). Secondary antibody was goat anti-rabbit immunoglobulin G horseradish peroxidase or alkaline phosphatase conjugate.

RESULTS

PPO and PO in Type A Trichomes of S. berthaultii

Contents of 500 to 1850 type A trichomes were collected and analyzed electrophoretically to determine the contribution of PPO and PO to the complement of oxidative enzymes in the type A trichome. Manual isolation of type A trichomes permitted electrophoretic analysis of trichome proteins without contamination from other leaf or epidermal cells. On wide pH range (pH 3.5–9.5) electrophoresing gels loaded with the contents of 500 S. berthaultii type A trichomes, a single protein species (pl 5.5) predominated as 50 to 70% of the total trichome protein, and possessed strong PPO activity when 1850 trichomes were analyzed (Fig. 1). The predominance of the pl 5.5 PPO was evident in both silver- and Coomassie blue-stained focusing gels (Fig. 1). Two-dimensional electrophoresis corroborated these findings (not shown). SDS-PAGE (Fig. 2) of 500 type A trichomes also shows that this M, 59,000 PPO is the predominant protein in S. berthaultii type A trichomes.

Although activity of two anionic POs was also present in trichomes (Fig. 1), discrete bands of protein corresponding to the PO activities were not apparent from Coomassie or silver staining. Although activity-stained gels do not provide an accurate comparison of the contribution PO or PPO activity makes to oxidation of trichome exudate, tropolone, a specific inhibitor of PPO, yet a PO substrate (13), selectively inhibits the bulk of oxidative activity in individual collections of type A trichomes, suggesting that PPO is the predominant oxidative activity of S. berthaultii type A trichomes (Table I).

Table 1. PPO Activity of 7000 S. berthaultii Type A Trichomes

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Activity</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>7,000g pellet</td>
<td>21.2</td>
<td>≤0.06</td>
</tr>
<tr>
<td>+22 μM tropolone</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>75,000g supernatant</td>
<td>137.1</td>
<td>6.72</td>
</tr>
<tr>
<td>+22 μM tropolone</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td>75,000g pellet</td>
<td>12.5</td>
<td>≤0.06</td>
</tr>
<tr>
<td>+22 μM tropolone</td>
<td>NDa</td>
<td></td>
</tr>
</tbody>
</table>

* Not detectable.
PPO Is a Soluble Protein in S. berthaultii Type A Trichomes

Seven thousand type A trichomes were collected individually to assess whether PPO is associated with membrane or plastid components of the trichome and to estimate the concentration of PPO in trichomes. S. berthaultii type A trichomes contained approximately 1 ng of soluble protein per trichome. Most of the PPO activity (>80%) and the bulk of trichome protein remained in the supernatant after high-speed centrifugation (75,000g) (Table I), suggesting that trichome PPO is a soluble protein.

Purification of Trichome PPO

Although PPO is a major constituent of S. berthaultii type A trichomes, the rapidity with which quinones generated by PPO oxidatively modify proteins, and the small size and numbers of trichomes, are significant obstacles to purification of PPO from these organs. Therefore, a one-step procedure for the purification of trichome PPO was devised. Trichomes were harvested by wiping the leaf surface with a cotton swab saturated with buffer. A high level of reducing agent (200 mM DTT) was incorporated into the buffer to prevent the oxidative modification of the PPO. These preparations yielded a pale yellow-green crude trichome extract with none of the browning characteristic of oxidation. In a representative purification, about 4000 leaflets were wiped, yielding 1 mg of protein in 20 mL of 200 mM DTT. After centrifugation to remove debris, the crude trichome preparation was submitted to preparative electrofocusing, a representative run yielding 100 µg of homogeneous PPO (Figs. 3 and 4).

Prolonged exposure of PPO to high DTT concentrations during isolation partially inactivated the enzyme, apparently by removal of copper. Partially inactivated PPO was reconstituted by treating it with Cu²⁺, yielding a 27-fold increase in enzyme activity. That the catalytic activity recovered was trichome PPO was verified by electrofocusing the apoenzyme and reconstituted holoenzyme, and staining for PPO activity (Fig. 5).

Figure 4. Silver stained SDS-PAGE of 4 µg of purified PPO from pooled fractions 12 to 15 from preparative electrofocusing (Fig. 3).

Purified PPO from the preparative electrofocusing cell exhibited a single band, M₁, 59,000, on silver-stained SDS-PAGE (Fig. 4). In some preparations, a band of M₀, 17,000 became visible after boiling in Laemmli sample buffer (data not shown). This band is not observed when purification is done rapidly, and is absent in western blots of PPO from freshly collected trichomes stained with antibody against the purified preparation, suggesting that it arises by proteolysis.

Figure 5. Copper reconstitution of purified trichome PPO. A, PPO activity, assayed as described in “Materials and Methods.” Solid bar, PPO activity, reconstituted in the presence of 1 mM Cu²⁺. Hatched bar, PPO activity prior to Cu²⁺ reconstitution. B, PPO activity-stained IEF gel, pH 3.0–9.5, loaded with 2 µg Cu²⁺ reconstituted PPO (lane 1) and copper-depleted PPO (lane 2).
of the M, 59,000 polypeptide. This hypothesis was confirmed by incubating a crude trichome preparation overnight at 0°C. Under these conditions, the M, 17,000 band detected by silver stain accumulated with a corresponding decline in the higher M, band (not shown). The purified, lyophilized protein was stable at −70°C for several weeks, or in water at 4°C overnight.

The amino acid composition of the purified PPO was consistent across four preparations (Table II). The acidic pI of the protein is consistent with the high levels of Glx and Asx, some of which must represent glutamate and aspartate, respectively.

**Characterization of PPO Antibodies**

Polyclonal anti-trichome PPO immunoprecipitated nearly 80% of the PPO activity from a crude trichome preparation, although it did not inhibit PPO activity directly (17). The antiserum neither precipitated nor inhibited mushroom tyrosinase (17). Immunoblotting of SDS-PAGE and IEF confirmed that the antibodies were specific for PPO in type A trichome preparations. On immunoblots of SDS-PAGE and native IEF gels, the anti-trichome PPO antibodies cross-reacted strongly against a M, 45,000 band from freshly prepared extracts of *S. berthaultii* and *S. tuberosum* whole leaves and not at all with commercially available (Sigma) mushroom tyrosinase (17).

**PPO in Type A Trichomes of *S. tuberosum***

The predominant pI 5.5 protein extracted from the trichomes of *S. berthaultii* is absent in *S. tuberosum* trichomes. *S. tuberosum* type A trichomes had only a trace of protein in the acidic range of the gel occupied by the pI 5.5 PPO of *S. berthaultii* trichomes (Fig. 1). In addition, PPO activity was not detectable at the pI 5.5 band characteristic of the *S. berthaultii* trichome PPO (Fig. 1). SDS-PAGE of 500 *S. tuberosum* type A trichomes identified a weakly silver-staining band of M, 59,000 (Fig. 2). Immunoblotting of SDS-PAGE (Fig. 2) revealed that this band cross-reacted with polyclonal antibodies against the *S. berthaultii* M, 59,000 PPO. However, the intensity of immunostaining relative to the same number of *S. berthaultii* trichomes indicated that PPO is present in trichomes of *S. tuberosum* at a much lower concentration than in *S. berthaultii*. In blots of IEF gels of *S. tuberosum* type A trichomes, the antibody stained a band of pI 5.1, slightly more acidic than *S. berthaultii* trichome PPO. Again, the immunostained band from *S. tuberosum* was much fainter than that from *S. berthaultii* (17) and had no detectable PPO activity (Fig. 1, lane 5).

**Substrate Specificity**

PPOs catalyze two distinct reactions, the hydroxylation of monophenols to o-diphenols (cresolase, tyrosinase, or monophenol monoxygenase activity [EC 1.14.18.1]), and the dehydrogenation of o-dihydroxyphenols to o-quinones (catechol oxidase or diphenol oxygen oxidoreductase activity [EC 1.10.3.2]) (20). PPO from various sources may possess both catechol oxidase and cresolase activity (15, 23), only cresolase activity (27, 28), or only catechol oxidase activity (18, 24). In most cases where only catechol oxidase activity is apparent, cresolase activity can be initiated by the addition of reducing agents or catalytic amounts of an o-diphenol to the reaction (5, 14, 26).

*S. berthaultii* trichome PPO catalyzed the oxidation of some o-diphenolic substrates (Table III). In agreement with previous studies (4), phenylpropenoids were the preferred substrates. No monohydroxylase (cresolase; tyrosinase) activity was detectable, even when the assays were carried out in the presence of 1 mM DTT or when primed with an o-diphenol (200 μM catechol). Laccase (*p*-diphenol oxidase [EC 1.10.3.1]) activity was not exhibited by this enzyme. Tropolone, an inhibitor of PPO (20), inhibited PPO activity up to 75% at a concentration of 0.2 mM.

**DISCUSSION**

PPO is the predominant protein and oxidative enzyme in type A trichomes of *S. berthaultii*. IEF of individually collected trichomes (Fig. 1) clearly shows that PO and PO activity are minor components of type A trichomes. PPO constitutes up to 70% of the total trichome protein in the type A trichome of *S. berthaultii*, corresponding to a concentration of 14 mg of PPO protein/mL. To prevent phenolic oxidation prior to trichome rupture, PPO must be compartmentalized within the trichome, and the concentration of PPO in this compartment may be significantly higher. Even if we assume that PPO is not compartmentalized, its concentration in the trichome approaches 200 μM. This calculation demonstrates, at the level of protein expression, the remarkable degree of specialization of these organs in *S. berthaultii*. Although the specialization of glandular trichome metabolism for a high level of biosynthesis or accumulation of a few organ-specific secondary products is well established (33), this is the first case for which this specialization has been identified with a single protein species.

Rupture of type A trichomes in the absence of sufficient

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**Table II. Amino Acid Composition of *S. berthaultii* Trichome PPO**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Number of Residues*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asx</td>
<td>49.8 ± 4.8</td>
</tr>
<tr>
<td>Glx</td>
<td>42.6 ± 1.4</td>
</tr>
<tr>
<td>Ser</td>
<td>24.4 ± 1.1</td>
</tr>
<tr>
<td>Gly</td>
<td>39.9 ± 4.4</td>
</tr>
<tr>
<td>His</td>
<td>11.8 ± 2.9</td>
</tr>
<tr>
<td>Arg</td>
<td>19.3 ± 0.9</td>
</tr>
<tr>
<td>Thr</td>
<td>22.8 ± 2.7</td>
</tr>
<tr>
<td>Ala</td>
<td>24.9 ± 1.4</td>
</tr>
<tr>
<td>Pro</td>
<td>33.0 ± 2.5</td>
</tr>
<tr>
<td>Tyr</td>
<td>31.9 ± 5.0</td>
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<tr>
<td>Val</td>
<td>30.5 ± 1.4</td>
</tr>
<tr>
<td>Met</td>
<td>7.0 ± 1.4</td>
</tr>
<tr>
<td>Cys</td>
<td>11.2 ± 4.1</td>
</tr>
<tr>
<td>Ile</td>
<td>23.0 ± 1.4</td>
</tr>
<tr>
<td>Leu</td>
<td>31.0 ± 0.9</td>
</tr>
<tr>
<td>Phe</td>
<td>23.3 ± 1.8</td>
</tr>
<tr>
<td>Lys</td>
<td>25.7 ± 0.9</td>
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</tbody>
</table>

* Mean ± SD of four separate preparations.
reducing agents leads to the oxidative formation of enormous cross-linked PPO aggregates with Mr values in excess of 1,200,000, previously referred to as "β PPO" (4). When adequate reducing agent (DTT) was used in our preparations, the appearance of these cross-linked molecules was minimized. Titration of PPO extraction buffer with increasing concentrations of reducing agent (DTT) progressively removed both multiple, high Mr, forms from the crude extract, along with β PPO and IEF "isozymes," clearly indicating that these molecules are oxidative artifacts (17). In preparative IEF of PPO, the use of high concentrations of reducing agents is critical, because oxidatively modified proteins possess altered pI values. In this study, PPO was purified from the type A glandular trichomes of *S. berthaultii* in a single step by a preparative IEF procedure, to apparent homogeneity with minimal covalent modification and limited proteolysis.

In chloroplasts, PPO of *M. 45,000* is present on the thylakoid membrane in a latent form (10, 12, 21, 30). In vivo, *M. 45,000* PPOs exist as active oxidases only in senescent or damaged tissues, and PPO can be activated in isolated thylakoid preparations by the addition of detergents, alkali, and proteases, i.e. conditions mimicking senescence or damage to plastid membranes (19-21, 31, 32). Membrane association is linked to latency. Although the cross-reactivity of *M. 45,000* PPO with the anti-trichome PPO (Mr 59,000) antibodies suggests that a number of antigenic sites are conserved between these enzymes, trichome PPO is not membrane-associated and displays no latency, being fully active upon release from the trichome (17).

Although the *M. 45,000* potato leaf and tuber PPOs cross-react with anti-trichome PPO antibodies, glandular trichome PPO differs substantially from *S. tuberosum* tuber PPO in its substrate specificity. Whereas trichome PPO displays a strict specificity for oxidizing o-dihydroxyphenylpropenoids, the *M. 45,000* tuber PPO shows substantial cresolase activity without priming, utilizing p-coumaric acid, p-cresol, tyrosine, and p-coumaric acid (3, 23). In terms of its substrate specificity for different o-diphenolic compounds, trichome PPO also differs from tuber PPO. Whereas tuber PPO oxidizes chlorogenic acid, caffeic acid, and catechol at nearly equal rates (3), trichome PPO displays significantly different activity toward these substrates, with preference for phenylpropenenes

### Table III. Substrate Specificity: Trichome PPO

<table>
<thead>
<tr>
<th>Substrate</th>
<th>R</th>
<th>R'</th>
<th>Percent Activity</th>
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<tbody>
<tr>
<td>Chlorogenic acid</td>
<td>-OH</td>
<td>-HC = CHCOOH</td>
<td>100</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>-OH</td>
<td>-HC = CHCOOH</td>
<td>46.7</td>
</tr>
<tr>
<td>Hydrocaffeic acid</td>
<td>-OH</td>
<td>-H2CCH2COOH</td>
<td>19.1</td>
</tr>
<tr>
<td>Catechol</td>
<td>-OH</td>
<td>-H</td>
<td>7.9</td>
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<tr>
<td>Protocatechuic acid</td>
<td>-OH</td>
<td>-COOH</td>
<td>1.5</td>
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<tr>
<td>Ferulic acid</td>
<td>-OH3</td>
<td>-HC = CHCOOH</td>
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<tr>
<td>Protocatechuic aldehyde</td>
<td>-OH</td>
<td>-COH</td>
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<td>-H2CCHCOOH</td>
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<td>p-Hydroxybenzoic acid</td>
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<td>Tyrosine</td>
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<td>-H2CCHCOOH</td>
<td>0</td>
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<tr>
<td>Coumaric acid</td>
<td>-H</td>
<td>-HC = CHCOOH</td>
<td>0</td>
</tr>
<tr>
<td>3-(4-hydroxyphenyl) propionic acid</td>
<td>-H</td>
<td>-H2CCH2COOH</td>
<td>0</td>
</tr>
<tr>
<td>3,4-dihydroxymandelic acid</td>
<td>-OH</td>
<td>-HCOHCOOH</td>
<td>0</td>
</tr>
<tr>
<td>Catechin</td>
<td>-OH</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

* Percent Activity of various substrates for trichome PPO.

...
(Table III). Furthermore, tuber PPO oxidizes DOPA, whereas the trichome PPO reacts relatively poorly with DOPA.

From the substrate specificity of trichome PPO, one would expect certain structural requirements for the phenolic constituent(s) in trichomes whose oxidative polymerization results in insect entrapment. Avé et al. (2) tentatively identified a phenolic constituent of *S. berthaultii* type A trichomes as a glucose ester of *p*-hydroxyphenylpropionic acid and proposed that this represents the PPO substrate responsible for insect entrapment. However, the activity of trichome PPO toward this compound was not examined. Based on the strict substrate preferences described here, it is unlikely that this molecule would be utilized as a substrate for the trichome PPO.

Although the biochemistry of the *M*. 45,000 class of thylakoid PPOs has been extensively investigated, the physiological function(s) of these enzymes remains unclear. Many theories have been put forth as to their role, e.g. synthesis of *o*-diphenols, promotion of wound healing, defense against pathogens, or participation in an electron transport chain or some aspect of oxygen chemistry, possibly mediation of pseudocyclic photophosphorylation (19, 20, 31, 32). It remains possible that the oxidative activity of these enzymes is an artifact of wounding, senescence, or preparation, and is not directly related to their in vivo function (31).

In contrast, the *M*. 59,000 trichome PPO appears to possess a defined in vivo function: catalysis of the rapid oxidative polymerization of phenolics with the subsequent formation of hardened trichome exudate on the mouthparts and tarsi of insect herbivores. The epidermal localization, absence of latency, and soluble nature of this PPO may be important for its function in trichome-mediated insect defense. The high concentration of PPO in the trichome may be critical for driving oxidative polymerization at rates high enough to facilitate entrapment of mobile insects.

We have determined that the type A trichomes of *S. tuberosum* contain very low levels of protein and PPO compared with those of *S. berthaultii*. The reason(s) for such low expression of PPO in *S. tuberosum* trichomes is yet to be ascertained. However, the differences between wild and cultivated potato with respect to trichomal PPO levels and ability of ruptured trichomes to undergo oxidative polymerization strongly suggest a key role for the *M*. 59,000 trichome PPO in this insect-resistance mechanism.

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