Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pesticide Biochemistry and Physiology 82 (2005) 52–58
PESTICIDE
Biochemistry & Physiology
www.elsevier.com/locate/ypest

Inhibitory effects of 4-dodecylresorcinol on the phenoloxidase of the diamondback moth *Plutella xylostella* (L.) (Lepidoptera Plutellidae)

Shu Dong Wang, Wan Chun Luo *, Shou Jian Xu, Qi Ding

College of Plant protection, Shandong Agricultural University, Shandong Tai'an 271018, People's Republic of China

Received 25 May 2004; accepted 16 December 2004
Available online 25 February 2005

Abstract

Phenoloxidase (PO) is a key enzyme in the developmental process of insects that is responsible for catalyzing the hydroxylation of monophenols and the oxidation of *o*-diphenols. In the present investigation, the PO of *Plutella xylostella* (L.)(Lepidoptera Plutellidae) was partially purified with 40% saturated $(NH_4)_2SO_4$ and Sephadex G-100 gel filtration, and the effects of 4-dodecylresorcinol on the monophenolase and *o*-diphenolase activity of PO were studied. The results showed that 4-dodecylresorcinol could inhibit monophenolase and *o*-diphenolase activity. In addition, following 4-dodecylresorcinol treatments, the lag time of PO for oxidation of L-tyrosine was obviously lengthened and the steady-state activity was decreased. The inhibitor was found to be competitively reversible with a K_i of 0.201 mM and an estimated IC_{50} (inhibition concentration showing 50% of the maximum inhibition) of 0.160 mM for monophenolase and 0.369 mM for diphenolase. The ability of 4-dodecylresorcinol to inhibit PO activity may be associated with its ability to directly affect copper at the active site

Keywords: 4-Dodecylresorcinol; *Plutella xylostella*; Phenoloxidase; Inhibitor; Inhibition kinetics

1. Introduction

Phenoloxidase (PO, EC.1.14.18.1), also known as tyrosinase or tyrosinohydroxylase, is a copper-containing enzyme widely distributed in plants, microorganisms and animals. This multifunctional enzyme catalyzes two distinct reactions, the hydroxylation of monophenol to *o*-diphenol (monophenolase activity) and the conversion of *o*-diphenol to the corresponding *o*-quinone (diphenolase activity), which can be spontaneously polymerized to form high-molecular-weight compounds or brown pigments (melanins) [1]. In insects, phenoloxidase is a widely distributed enzyme that plays important

* Corresponding author. Fax: +86 538 8242983.
E-mail address: wcluo@sdau.edu.cn (W.C. Luo).

0048-3575/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pestbp.2004.12.005

roles in normal developmental processes, such as cuticular tanning, scleration, wound healing, production of opsonins, and encapsulation and nodule formation for defense against foreign pathogens [2,3]. It is possible that inhibition of PO could lead to abrogation of insect defense mechanisms or abnormal body softening, both of which could lead to pest control. Thus, PO may be regarded as a potential candidate for the novel insecticide development.

PO can be inhibited by aromatic compounds such as aromatic acids, flavonols, flavones, and aromatic aldehydes. For example, 4-hexylresorcinol inhibits PO to prevent melanosis in shrimp and crabs and to control browning of fresh and hot-air-dried apple slices, avocados, potatoes, and grape juices [4]. However, there are few reports on PO inhibition in insects.

In the present study, we investigated the effect of 4-hexylresorcinol on PO mono phenolase and diphenolase activity in the diamondback moth *P. xylostella* (L.) (Lepidoptera Plutellidae). In addition, we investigated the relationship between the kinetic parameters and inhibition activity. Together, these data may provide the basis for developing novel PO inhibition-based pesticides.

2. Materials and methods

2.1. Insects

Larvae of the diamondback moth *P. xylostella* were reared on Chinese cabbage *Brassica parachinesi* (Bailey) in a greenhouse at 25 ± 1 °C with a 14:10 h light: dark photoperiod. Final (fourth) instar larvae were gathered for the experiments.

2.2. Reagents

4-Dodecylresorcinol and bovine serum albumin (BSA) were purchased from Sigma Chemical. L-Tyrosine was the product of Aldrich Chemical (USA). All other reagents were analytical grade. The water used in the experiments was twice-distilled and ion-free.

2.3. Enzyme purification

The larvae of diamondback moth *P. xylostella* were homogenized in an 8- fold weight of ice-cold 0.02 M sodium phosphate buffer (pH 6.5) containing Na_2HPO_4–NaH_2PO_4.The homogenate was filtered through gauze and centrifuged at 8000 rpm (CR22, Japan) for 30 min at 0 °C. The supernatant under the fat layer was collected as the crude enzyme extract and brought to 40% saturation with solid ammonium sulfate. The resulting precipitate was collected by centrifugation at 8000 r min^{-1} for 30 min at 0 °C, and dialyzed against the same phosphate buffer. This process increased the specific enzyme activity up to 4.08-fold that of the crude extract. The dialyzed solution was gel filtered through a Sephadex G-100 column equilibrated with the same buffer. The fractions were tested for catechol oxidase, and those having high activity were collected. After this step, the specific activity was 6.52-fold that of the crude extract. The protein content was determined using the Bradford method [5] with BSA as the standard. One unit (U) of *P. xylostella* PO activity was expressed as 0.001 change in absorbance per min per mg protein. The specific activity of the final enzyme solution was found to be 1010 U mg^{-1}.

2.4. Enzyme assays

PO catalyzes the reaction between a phenolic compound and oxygen. Here, we assayed PO activity in air-saturated solutions so that the oxygen content was not limiting. Different amounts of 4-dodecylresorcinol were dissolved in DMSO and diluted 30-fold for use in the experiments. Diphenolase activity was assayed as previously reported [6] with slight modifications. First, 1.5 ml of 14 mM catechol aqueous solution was mixed with 1.3 ml of 0.02 M phosphate buffer (pH 6.5) and 0.1 ml containing different concentrations of 4-dodecylresorcinol. The mixture was incubated at 30 °C for 10 min, then 0.1 ml of the aqueous PO was added. And the linear increase in optical density at 400 nm was monitored. The monophenolase assay was performed with L-tyrosine as a substrate. For this test,