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Xenobiotic metabolism of plant secondary compounds in the English grain aphid, *Sitobion avenae* (F.) (Hemiptera: Aphididae)

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ABSTRACT

Plant secondary compounds have been documented to be deleterious to insects and other herbivores in diverse ways. In this study, the effect of catechol (phenolics), gramine (alkaloid) and L-ornithine-HCl (non-protein amino acid) on the activities of xenobiotic metabolizing enzymes in English grain aphid, *Sitobion avenae,* was evaluated. Phase I enzymes investigated in this study included carboxylesterase (CarE), and oxidoreductase, whereas Phase II enzymes were represented by glutathione S-transferase (GST). In general, CarE and GST activities in *S. avenae* were positively correlated with the concentration of plant secondary compounds in artificial diets. Oxidoreductase activity, however, displayed a different profile. Specifically, peroxidase (POD) and polyphenol oxidase (PPO) activities in *S. avenae* were positively correlated with concentrations of dietary catechol and gramine, respectively, whereas catalase (CAT) activity was significantly suppressed by the higher concentration of catechol, gramine and L-ornithine-HCl. These combined results suggest that CarE and GST in *S. avenae* are key enzymes to breakdown a broad spectrum of plant secondary compounds, whereas oxidoreductase, including PPO and POD, degrades specific groups of plant secondary compounds.

1. Introduction

Plant secondary compounds have been documented to be important biochemical basis for the plant defense against herbivore insects [1–3]. Those compounds such as phenolics, alkaloids and non-protein amino acids are deleterious to insects and other herbivores in diverse ways. Thus, they play a key role in plant defensive response to pests through acute toxicity and enzyme inhibition.

Phenolics have been shown to be important factors in resistance to insect and pathogens. Gossypol is a well-known phenolic that has been demonstrated to be deterrent against *Aphis gossypii* and *Heliothis zea* [4]. Silencing of a gossypol-degrading P450 monooxygenase through *in planta* RNAi leads to substantial mortality in the cotton bollworm, *Helicoverpa armigera* [5]. Pyrocatechol and ferulic acid have been known to be the most effective feeding deterrents to English grain aphids [6]. Alkaloids have also been considered as one of the important resistance factors against herbivorous insects. Higher alkaloid concentrations in plant tissues generally reduce phytophagous insect attack of plants [7]. Gramine, a simple indole alkaloid widely found in Gramineae plants, displays a wide range of biological activities against insects, mammals and bacteria [1,2,6,8], and is responsible for aphid resistance in barley [9,10]. Non-protein amino acids are another groups of plant secondary compounds which are toxic to insects and other herbivores [11]. For example, Bennett and Wallsgrove [12] reported that β-isoxazolinonyl-alanine from pea roots is effective against pests and diseases. Highly significant negative correlations were found between the r_m values of *S. avenae* and concentrations of L-2,3-dihydroxyphenylalanine and ornithine in winter wheat cultivars [13].

Biotransformation involves an array of xenobiotic detoxification enzymes, each with broad specificity. Those enzymes, generally, can be divided into two functional groups including Phase I enzymes such as P450, carboxylesterase (CarE), and oxidases, and Phase II enzymes such as glutathione S-transferases (GSTs) [14–17]. GSTs play a pivotal role in detoxification and antioxidant defense of insects against natural and synthetic exogenous xenobiotics, including insecticides, allelochemicals, and endogenously activated compounds [18]. In aphids, increased GST activity has been linked to their detoxification capability and host adaptability [19–21]. An elevated GST activity was found in *Sitobion avenae* and *Rhopalosiphum padi*, respectively, fed on a low aphid-resistant wheat cultivar [22]. Moreover, GST activity in *S. avenae* was positively correlated with the content of xenobiotics in their host plants [23]. On the other hand, CarE (EC 3.1.1.1) and oxidases

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including peroxidase (EC 1.10.3.1, POD), polyphenol oxidase (EC 1.11.1.7, PPO) and catalase (EC 3.1.1.1, CAT) are the major Phase I detoxification enzymes against insect-active allelochemicals from plants. CarE in sap-sucking herbivores has diverse functions in dietary detoxification and insecticide resistance [7,24]. PPO and POD, derived from aphid's saliva or gut, can oxidize a wide range of phenolic compounds from plants [25–29]. When exposed to DIMBOA in plant tissue, CAT activity was significantly increased in *S. avenae* [30], whereas exposure to plant o-dihydroxyphenols caused a decrease in CAT activity [31]. These evidences indicate that insects employ both Phase I (carboxylesterase and oxidoreductase) and Phase II (glutathione S-transferases) enzymes to develop their resistance to exogenous insecticides and their adaptability to endogenous secondary compounds from host plants [32–36].

Previous studies on the relationships between insect detoxification enzymes and the metabolism of plant secondary compounds in aphids have focused on the response of aphids to different host plants. Few studies investigated how detoxification enzymes were directly affected when aphids were exposed to allelochemicals in plants. The objectives of this study are to (1) document the impacts of various secondary compounds on different groups of detoxification enzymes in aphids, and (2) investigate the role of oxidoreductases in aphids.

2. Materials and methods

2.1. Aphid culture

S. avenae nymphs and adults collected from field-grown wheat in early summer of 2007 were reared on the seedling of 'Beijing 411', a susceptible wheat cultivar (QNC, unpub data), which was planted in plastic bowl (Φ = 22 cm) with soil substrate mixed by vermiculite and turf, in climate incubator at 24 ± 1 °C, 60–70% relative humidity and 14 h illumination, aphids were transferred to new seedlings weekly to maintain optimal aphid growth and development. To ensure sufficient aphids and individual homogeneity, the aphids were reared for two generations before use in the experiment.

2.2. Artificial diet

Artificial diet contained 300 mg agrinine, 50 mg cysteine, 200 mg histidine, 200 mg lysine, 100 mg methionine, 100 mg phenylalanine, 100 mg serine, 100 mg tryptophan, 20 mg tyrosine, 10 mg ascorbic acid, 0.1 mg biotin, 50 mg choline chloride, 1.0 mg folic acid, 10 mg *p*-aminobenzoic acid, 10 mg nicotinic acid, 2.5 mg pyridoxine HCl, 2.5 mg thianine HCl, 500 mg K_3PO_4, 200 mg $MgCl_2 \cdot 6H_2O$, 100 ml distilled water and 25 g sucrose. The pH of the diet was adjusted to 6.0 with K_3PO_4 [37,38]. All reagents were from Beijing Chemical Reagent Company, China.

2.3. Aphid culture for enzyme assays

To assess the respective impacts of gramine (alkaloids, Kingsley and Keith LTD, UK), catechol (phenolic) and L-ornithine-HCl (non-protein amino acid, Beijing Chemical Reagent Company, China) on the detoxification enzymes and oxidoreductases of *S. avenae*, serial dilutions of each technical grade compound were incorporated into the artificial diet to reach final concentrations of 0.025, 0.05, 0.1 and 0.2 mM. These concentrations were set below the LC_{50} to ensure enough surviving aphids for biochemical analysis.

The second and third stage nymphs of *S. avenae* were starved for 12 h to standardize their hunger status before engaged in the feeding bioassays with plant secondary compounds [39]. Two layers of the stretched Parafilm were placed in a transparent cylindrical Plexiglas tube (60 mm in height and 22 mm in diameter) and were covered with gauze on top to allow air and humidity exchange. All treatments were replicated three times. The aphids feeding on artificial diets were cultured in an incubator at 24 ± 1 °C, 60–70% relative humidity and 14 h illumination. 72 h after feeding [39], the aphids were collected for enzyme activity assay.

2.4. Enzyme activity assay

Carboxylsterase (CarE) activity was measured following Ni and Quisenberry [35] with minor modifications. Fifty milligrams of frozen *S. avenae* were homogenized in 2 ml potassium phosphate buffer (0.02 M, pH 7.0) at 0 °C. The homogenate was centrifuged at 5000*g* for 15 min at 4 °C. An aliquot of 0.1 ml of resulting supernatant was mixed with 0.9 ml solution with α-NA and Fast blue RR salt (10 mg α-NA and 20 mg Fast blue RR salt were dissolved in 2 ml acetone, and then were diluted to 25 ml by adding the above phosphate buffer). The reaction mixture was incubated for 2 min at room temperature and the absorbance was measured at 405 nm against blank containing 0.1 ml of potassium phosphate buffer (0.02 M, pH 7.0) instead of the aphid homogenate with a spectrophotometer (SP-756P, Shanghai Spectrum Ltd., China), The change of A_{405} was expressed as the enzyme activity.

Glutathione S-transferase (GST) activity was measured following Ni and Quisenberry [35] with minor modifications. Fifty milligrams of frozen *S. avenae* were homogenized in 2 ml Tris–HCl buffer (0.1 M, pH 8.0) at 0 °C. The homogenate was centrifuged at 10,000*g* for 15 min at 4 °C. The reaction mixture contained 0.1 ml of the resulting supernatant, 1.4 ml of the Tris–HCl buffer mentioned and 50 μl reduced glutathione (40 mM). After a pre-incubation period of 5 min at 25 °C, 60 μl of CDNB (30 mM) was added to the mixture against blank containing distilled water instead of CDNB. The change of A_{340} nm was recorded using a spectrophotometer (SP-756P, Shanghai Spectrum Ltd., China).

Oxidoreductase activity, including peroxidase (POD), polyphenol oxidase (PPO) and catalase (CAT), was measured following Hildebrand et al. [40] and Hori et al. [41] with the following modifications. For enzyme preparation, 50 mg of frozen aphids from each sample were ground in liquid nitrogen before being homogenized in 1 ml of ice-cold potassium phosphate buffer (1.0 M) with Polyvinylpyrrolidone (PVP, 1%, w/w) and Triton X-100 (1%, w/w, pH 7.0). The homogenate was centrifuged at 10,000*g* for 15 min at 4 °C, and the supernatant was used for the subsequent analyses.

For the POD activity assay, 20 μl of enzyme extract were mixed with the substrate containing 10 μl hydrogen peroxide (30% w/w), 300 μl guaiacol (18 Mm) and 100 μl 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 200 mM, pH 7.0) in deionized water. The optical density of the mixture was measured at 470 nm against the control containing potassium phosphate buffer instead of the enzyme extract. POD activity was indicated by the increase of A_{470}. For the PPO activity assay, 20 μl of enzyme extract was mixed with a solution containing 500 μl catechol (1.6%, w/w) in HEPES buffer, 100 μl HEPES (200 mM, pH 6.0) and 380 μl deionized water, blank contain potassium phosphate buffer instead of enzyme extract. PPO activity was estimated at A_{470}. For the CAT activity assay, 20 μl of enzyme extract were mixed with a solution containing 100 μl hydrogen peroxide (75 mM), 100 μl HEPES (200 mM, pH8.0) and 780 μl deionized water, the blank contain potassium phosphate buffer instead of enzyme extract CAT activity was measured at A_{240}. All the enzymatic activity assays were independently replicated three times.

The total protein concentration was determined using the Bradford method [42]. Absorbance of the reaction mixture was read at 595 nm using a spectrophotometer (SP-756P, Shanghai Spectrum Ltd., China) and the protein concentration was determined from