NOTE

Intraspecific Variation in the Effects of Parasitism by Asobara tabida on Phenoloxidase Activity of Drosophila melanogaster Larvae

Phenoloxidases (monophenol, l-DOPA: oxygen oxidoreductase, hereafter abbreviated as POs) are key enzymes in the immune system of invertebrates. In insects this enzymatic participates in melanization of humoral or cellular capsules formed around foreign bodies (Ashida and Yamazaki, 1990; Rizki and Rizki, 1990). Several Hymenopteran endoparasitoids, whose development is achieved within the hemocoel of an insect host, are known to severely disrupt the PO activity of their host (Stoltz and Cook, 1983; Beckage et al., 1990; Strand and Pech, 1995). The braconid Asobara tabida develops as a solitary endoparasitoid in larvae of several Drosophila species. Until now, successful parasitism on Drosophila melanogaster hosts was considered to rely on parasite eggs’ ability to adhere to host tissues, thus providing protection against encapsulation (Kraaijeveld and van Alphen, 1994; Esslin et al., 1996). Different eggs’ adhesiveness properties were observed and associated with geographical variations in parasitoid virulence (Kraaijeveld and van Alphen, 1994). However, the impact of parasitism by A. tabida on its hosts’ immune system and notably on the PO activity remains largely unknown.

The objectives of our study were to investigate (i) the effect of parasitism by A. tabida on the hemolymph PO activity of D. melanogaster host larvae and (ii) whether parasitization by a virulent (A1) or a nonvirulent (WOPV) strain could alter this enzymatic system.

PO activity was estimated by spectrophotometrically recording at 490 nm the dopachromes formed from the conversion of l-dihydroxyphenylalanine (l-DOPA). First instar larvae (2 days old) of D. melanogaster were parasitized by A1 or WOPV females and then kept at 20°C until experimentation (i.e., 48, 72 or 96 h post-parasitization). Twenty-four hours after parasitization, the small size of larvae (and hence their available hemolymph volume) precluded their use for the tests. Therefore, 3-day-old larvae were parasitized and their PO activity was measured at 24 h after parasitization. Whole hemolymph (i.e., plasma and hemocytes) was collected in 11 μL of 5 mM morpholinopropanesulfonic acid (Mops), pH 5. As the body size of larvae varies during larval development, the number of larvae bled into each well had to be adjusted, as noted in figure legends. The hemolymph samples were incubated for 45 min at 35°C, then added to 50 μL of 10 mM l-DOPA in 5 mM Mops, and incubated for 1 h at 20°C to reach a plateau in the formation of dopachrome. PO activities were expressed as the mean percentage of PO activity relative to that of nonparasitized larvae. Negative controls were performed by adding 0.01% 1-phenyl-2-thiourea (PTU), a known inhibitor of POs, to the hemolymph of nonparasitized larvae.

The addition of PTU induced a 90% reduction of PO activity for each time tested (Fig. 1). The production of dopachrome recorded was thus mostly due to the only PO activity that was present in our hemolymph samples.

Parasitism by A1 occurred with a depletion of host PO activity at 24, 48, and 72 h post-parasitization (65.8 ± 6.1, 64.6 ± 10.0, and 61.8 ± 10.9%, respectively) compared to nonparasitized larvae PO activity, whereas WOPV did not seem to significantly alter the PO activity of host larvae (Fig. 1). The limited number of hosts exhibiting a melanization process toward A1 eggs (usually <5%) restricted our evaluation of their PO activity for individuals at 96 h post-parasitization. At this time, no significant difference was observed between PO activities of larvae hosting either a melanized or a nonmelanized A1 egg (120 ± 21 and 113 ± 26%, respectively) and PO activity of nonparasitized larvae. On the contrary, PO activity measured in larvae parasitized by WOPV was significantly higher (244.9 ± 44.5%) (Fig. 1). Parasitism by the virulent A1 strain of A. tabida thus seems to have a stable depressive effect on hemolymph PO activity of its hosts during the first 72 h after parasitization which was not induced by the nonvirulent WOPV strain. This is the first demonstration of intraspecific variation in the ability of a parasitoid to induce a deleterious effect on the PO activity of its host.

To test whether this effect was specifically induced by factors originating from the parasitoid, 3-day-old D. melanogaster larvae were wounded using a glass microneedle (25 μm diameter) and assayed for their PO activity at various times (Fig. 2). These larvae exhibited a significantly reduced PO activity at 24 and 72 h after wounding (45.6 ± 5.7 and 65.1 ± 7.0%, respectively) as compared to nonwounded larvae. Conversely, no significant effect on PO activity was observed at 48 and 96 h after wounding. These unexpected transient
depletions of the PO system at 24 and 72 h postwounding seem to indicate that, under our conditions, a nonparasitic aggression could generate an endogenous down-regulation of hemolymph PO activity of D. melanogaster larvae. Several authors have reported the existence of endogenous mechanisms controlling PO activity, therefore avoiding the generation of cytotoxic quinoid components (Sugumaran and Kanost, 1993). However, it appears unlikely that such endogenous mechanisms are involved solely in the depletion of PO activity in larvae parasitized by A1 since their PO activity is not restored at 48 h after parasitization. Thus, factors from A1 would also participate in the depletion of host PO activity, definitely at 48 h, and possibly at 24 and 72 h postparasitization.

Our study shows that a virulent strain of A. tabida induces a deleterious effect on host PO activity contrary to what is observed upon parasitization by a nonvirulent strain. This suggests that, in addition to egg adhesiveness properties, the ability of this parasi-
toid to counteract its host immune defenses could also involve depressive factors altering the host PO system. In the absence in A. tabida of any virus-like particles (Eslin et al., 1996), which are often found to be associated with host immune suppression by parasitoids (Strand and Pech, 1995), the nature of the parasitic factors and their contribution to the virulence of A. tabida remain to be determined.

Key Words: Asobara tabida; Drosophila melanogaster; endoparasitoid; phenoloxidase; hemolymph; immune depression.

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