

COMPARATIVE BIOCHEMISTRY OF MYCOPHAGOUS AND NON-MYCOPHAGOUS GRAIN BEETLES. CHITINOLYTIC ACTIVITIES OF FOREIGN AND SAWTOOTHED GRAIN BEETLES*

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Abstract—1. The chitinolytic enzymes in a mycophagous insect, the foreign grain beetle, *Ahasverus advena*, and a non-mycophagous insect, the sawtoothed grain beetle, *Oryzaephilus surinamensis*, were measured.

2. Larvae and adults of *A. advena* exhibit three-fold higher chitinase levels than do the same stages of *O. surinamensis*.

3. The foreign grain beetle adult contains more than two-fold higher β -*N*-acetylglucosaminidase than the sawtoothed grain beetle adult.

4. The results suggest that feeding stages of the mycophagous beetle have elevated levels of chitinolytic enzymes so that fungal chitin can be utilized as a food source.

INTRODUCTION

Many species of insects are associated with fungi. Some are mycophagous and utilize fungi as a source of nutrients (Wheeler and Blackwell, 1984). Insects that live in stored grain, especially those that feed on fungi, contribute to the spread of fungal spores into and throughout the grain mass. The developing mold and insect populations cause heating (hot spots) in the grain and result in damage and downgrading of the grain when it is sold. This can cause a significant economic loss to the farmer, elevator operator, shipper and ultimately the consumer.

The sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), is one of the most common cosmopolitan grain pests. The larval and adult stages attack foods of vegetable origin, including grain and grain products. The foreign grain beetle, *Ahasverus advena* (Waltl), is a smaller reddish-brown beetle that is similar in appearance to the closely related sawtoothed grain beetle. Although the foreign grain beetle is also distributed throughout the world, it is rarely found in clean grain. Instead, it is attracted to damp and moldy grain where it feeds on the molds (David *et al.*, 1974).

The cell wall of fungi contains several polysaccharides, including chitin, the β (1 4)-linked polymer of *N*-acetylglucosamine, mannans and glucose (Bartnicki-Garcia and Reyes, 1968; Davis and Bartnicki-

Garcia, 1984). To digest fungi in moldy grain, the foreign grain beetle might be expected to exhibit levels of chitin-hydrolyzing enzymes higher than those in a non-fungal-feeding beetle. In insect molting fluid chitin is depolymerized to *N*-acetylglucosamine by the tandem action of two enzymes, an endo-splitting chitinase and an exo-splitting β -*N*-acetylglucosaminidase (Fukamizo and Kramer, in press, a, b). In the present study we describe the results of a search for both types of chitin-degrading enzymes in various developmental stages of the foreign grain beetle and the sawtoothed grain beetle. The feeding stages of the mycophagous foreign grain beetle were found to exhibit higher chitinase levels than the corresponding stages of the lesser grain borer.

MATERIALS AND METHODS

Insects were reared in the laboratory at $27 \pm 2^\circ\text{C}$ and 60% RH on a diet of rolled oats (95%) and brewer's yeast (5%). To encourage mold development, the medium for the foreign grain beetle was always admixed with spent medium from a previous culture. In addition, a small vessel containing wet gauze was placed on the diet surface. Active larvae of mixed ages (larvae in premolt quiescent state and molting larvae were rejected), pupae and adults 1–21 days old were weighed and placed in a small volume of 50 mM sodium phosphate, pH 7.0, containing 0.1 mM diisopropylphosphorofluoridate to inhibit proteases and 0.1 mM 1-phenyl-2-thiourea to inhibit tyrosinase, and either frozen at -20°C or homogenized immediately. The homogenate was centrifuged at 20,000 g for 15 min at 4°C and the supernatant recovered for enzyme analysis. Chitinase activity was measured using ethylene glycol chitin as a substrate according to Imoto and Yagishita (1971), where the reducing end group produced by the enzymatic reaction at 32°C is quantitated colorimetrically at 420 nm after incubation with potassium ferriferrocyanide reagent at 90°C . β -*N*-Acetylglucosaminidase activity was determined colorimetrically at 337 nm using *p*-nitrophenyl-2-acetamido-2-deoxy- β -D-glucopyranoside (pNp β GlcNAc) as a substrate at 25°C (Koga *et al.*, 1982). Protein concentration was determined by the method of Bradford (1976).

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RESULTS AND DISCUSSION

We measured chitinase and β -*N*-acetylglucosaminidase levels in supernatants from homogenates of various developmental stages of the foreign grain beetle and the sawtoothed grain beetle. Insect chitinolytic enzymes are soluble and stable under the conditions of extraction used (Kramer *et al.*, in press). There were significant amounts of the two types of chitinolytic enzymes in both the mycophagous and non-mycophagous species. In addition, there were differences in the overall levels of individual enzymes depending on stage of development. The highest overall chitinase activity was present in *Ahasverus* larvae (Fig. 1A). Foreign grain beetle larvae and adults exhibited chitinase levels that are about three times greater than those detected in the corresponding stages of the sawtoothed grain beetle. *Ahasverus* pupae had about 70% of the chitinase activity of *Oryzaephilus* pupae. Thus the feeding stages of the mycophagous species contained higher levels of the endo-splitting chitinolytic activity.

The pupal stages of the grain beetles contained the highest exo- β -*N*-acetylglucosaminidase activity (Fig. 1B). Exo-splitting chitinolytic activity was lower but comparable in the larval stage of the two species. In the adult stage the foreign grain beetle had a significantly higher β -*N*-acetylglucosaminidase level than the sawtoothed grain beetle. The observation that the pupal stage of both species of grain beetles contained the highest level of β -*N*-acetylglucosaminidase is perhaps not unexpected, since this hydrolase is commonly associated with lysosomal fractions and extensive histolysis occurs during the pupal instar of these species.

Probably the most interesting result of this study is the appreciably higher chitinase in the feeding stages of the mycophagous species, indicating that the foreign grain beetle is utilizing chitin in the fungi upon which it feeds nutritionally. Both species of beetles probably also feed on live and dead insects encountered in their environment. Fungi are often a component of decomposing materials and contain enzymes required for digestion of polysaccharides such as chitin. They may supply chitinolytic enzymes to mycophagous insects (Martin, 1979). Thus there may be multiple sources of chitinolytic enzymes, including the insect itself, detritus, fungi and other microflora present in the insect's tissues.

Chitinolytic enzyme levels vary from tissue to tissue and species to species (Kramer *et al.*, in press). One of the richest sources of chitinases in insects is molting fluid. For example, pharate pupal molting fluid from the tobacco hornworm, *Manduca sexta* (L.), contains $6.95 \pm 0.37 \Delta A_{420}/hr/mg$ and $958 \pm 54 \text{ nmol pNp}\beta\text{GlcNAc hydrolyzed}/\text{min}/\text{mg}$ of chitinase and β -*N*-acetylglucosaminidase, respectively (Fukamizo and Kramer, in press, a). These amounts are more than twice those detected in the grain beetle homogenates. However, the labial gland of *M. sexta* larvae contains chitinolytic enzyme levels that are comparable to those present in grain beetles, about $1 \Delta A_{420}/hr/mg$ and $125 \text{ nmol pNp}\beta\text{GlcNAc hydrolyzed}/\text{min}/\text{mg}$ of chitinase and β -*N*-acetylglucosaminidase, respectively. Labial glands supply enzymes for digestive purposes in some insect species, such as attine

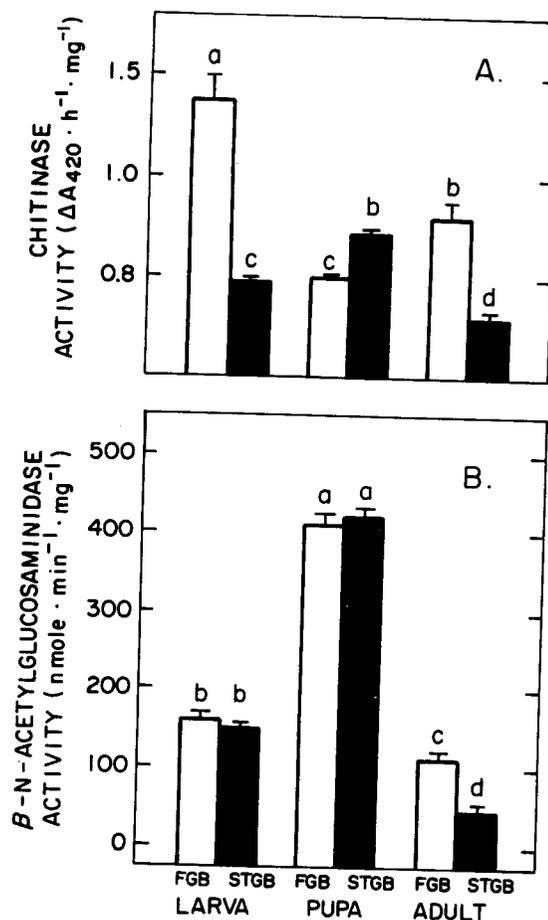


Fig. 1. Chitinolytic enzyme activities in developmental stages of grain beetles. FGB, foreign grain beetle; STGB, sawtoothed grain beetle. (A) Chitinase, $S_0 = 0.5 \text{ mg ethylene glycol chitin per ml}$. (B) β -*N*-Acetylglucosaminidase, $S_0 = 1 \times 10^{-4} \text{ M pNp}\beta\text{GlcNAc}$. The lower case letters above histogram show groupings whose members are not significantly different as determined by Duncan's multiple range test ($P = 0.0001$).

ants, which utilize a nest fungus as a food source (Febvay *et al.*, 1984). Whether the hornworm utilizes the labial gland chitinolytic enzymes for digestion or for preapoptosis body wetting is unknown (Kramer *et al.*, 1981).

In molting fluid, chitinase catalyzes the rate-limiting step in the overall process of cuticular chitin degradation (Fukamizo and Kramer, in press, a, b). The major difference in chitinolytic activity between mycophagous and non-mycophagous grain beetles is the more elevated chitinase in the former species. This result suggests that carbohydrate digestion in mycophagous insects requires biochemical specialization, with the digestion of fungal chitin being dependent primarily on chitinase. This enzyme initiates chitin depolymerization and eventually generates small oligomeric fragments from which *N*-acetylglucosamine is released by the action of β -*N*-acetylglucosaminidase.

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