Trehalase Incorporated into the Spermatophore from the Bean-Shaped Accessory Gland of the Male Mealworm Beetle, *Tenebrio molitor*

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1. Is trehalase a structural protein in the spermatophore ?

In many insects, male adult transfers sperm to the female via spermatophore or sperm sac (Mann, 1984). In the mealworm beetle, *Tenebrio molitor*, the spermatophore is formed through the secretions from two pairs of accessory glands, the bean-shaped accessory glands (BAGs) and the tubular accessory glands (TAGs) (Happ, 1984, 1990). The eight secretory cell types of BAGs synthesize each protein and secrete it into the lumen of BAGs, in which a semisolid plug, a precursor of the wall of the spermatophore, is formed (Dailey *et al.*, 1980; Grimnes and Happ, 1986; Grimnes *et al.*, 1986; Shinbo *et al.*, 1987). BAGs arise from a larval mesoderm pouch (Huet, 1966) which grow in size by cell division during pupal development (Happ *et al.*, 1982, 1985; Yaginuma *et al.*, 1988). After adult ecdysis, the secretory proteins are actively synthesized in BAGs (Happ *et al.*, 1982; Shinbo *et al.*, 1987). High activities of energy metabolism may be required to support the high rates of production of the secretory proteins. Since one source of energy is thought to be trehalose in the blood (Wyatt, 1967), BAGs might be expected to contain trehalase (α, α-glucoside-1-glucohydrolase, EC 3.2.1.28). In fact, high activity of trehalase was found in mature BAGs of male adult (Yaginuma and Happ, 1988).

During pupal development, trehalase activity in BAGs was very low. After adult ecdysis, the total activity of trehalase increased drastically from 2 days to 6 days and then reached maximum levels at 9 days. The specific activity increased 20-fold from the time of adult ecdysis to 6 days. In 10-day adult, the specific activity of trehalase in testes, seminal vesicles, vas deferens, TAGs, or ejaculatory ducts, was lower by two orders of magnitude than in the BAGs, but the specific activity in the spermatophore was similar to that in the BAGs. Thus, trehalase may be related in part to glucose production from blood trehalose for energy production, but rather appears to be secreted from the BAGs and incorporated into the spermatophore (Yaginuma and Happ, 1988).

Trehalase from BAGs was purified as a homogeneous protein using DEAE-cellulofine and Sephacryl S-300 column chromatographies. Molecular mass of native form or subunit form was estimated to be 43 kDa using gel filtration method or 62 kDa by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), respectively. An optimum pH was at 5.7-5.8. Km value for trehalose was 4.4 mM. Using anti-trehalase serum, the immunohistochemical experiment showed that trehalase protein was localized in the specific secretory cell type within BAGs and in the semisolid plug within the lumen of the BAGs. Further, by SDS-PAGE and immunoblotting analyses, an immunoreactive polypeptide with a molecular mass of about 62 kDa was detected in the spermatophore. The immunohistochemical observation also showed the localization of trehalase in the surface of the wall of spermatophore. Thus, the results confirmed the suggestion that trehalase protein was synthesized by specific secretory cell type within BAGs, secreted into the lumen of the BAGs, and incorporated into the spermatophore.

Significant levels of trehalose were also found in the spermatophore (Yaginuma and Happ, 1988). What then is the physiological role of this enzyme and its substrate? Is the trehalase-trehalose system important for spermatophore evacuation (Gadzama and Happ, 1974) ? When a fresh spermatophore is immersed in phosphate-buffered saline (PBS), it can evacuate the sperm in the same manner as in bursa copulatrix of the female. Addition of Validoxylamine A, an inhibitor specific for trehalase (Kameda *et al.*, 1987), did not affect

the evacuation of spermatophore in PBS. Thus, this result suggested that the trehalase was a structural protein of the spermatophore. However, a possibility is not yet ruled out that the trehalase is absorbed *in vivo* and then utilized within the female, because the trehalase of the spermatophore is soluble and is released into the surrounding PBS before the rupture of the spermatophore in PBS.

2. Hormonal control on the synthesis of trehalase in mature BAGs

During the period of cell division within pupal stage, the many cells which will give rise to the secretory epithelium of the BAG are morphologically similar to one another. At the end of the pupal stage and in the first 2 days after adult ecdysis, the eight morphological cell types characteristic of the adult glands become clearly defined (Dailey *et al.*, 1980; Dailey and Happ, 1983). When the definitive adult morphology of the BAGs is established, there are rapid synthesis and accumulation of the adult-specific proteins involved in the formation of spermatophore (Happ *et al.*, 1982; Grimnes and Happ, 1986; Grimnes *et al.*, 1986; Shinbo *et al.*, 1987; Happ, 1990).

Since adult-specific proteins in BAGs increased markedly even in isolated adult male abdomens, factors from the cephalic and thoracic centers are not thought to be required for production of adult-specific proteins in the adult BAGs (Yaginuma and Happ, 1989). However, pupal ecdysteroid hormone is suggested to affect the production of adult-specific proteins in BAGs (Happ, 1987; Happ, 1992). For example, leucine incorporation into spots on fluorographs and dot blots with monoclonal antibodies showed that BAGs became competent to produce adult-specific proteins during the ecdysteroid peak between pupal days 3 and 6 (Happ, 1987; Grimnes and Happ, 1987).

To detect expression of that commitment, trehalase was used as a quantitative index of adult differentiation of the BAGs. When pupal BAGs were transplanted into 0-day female adults, 8 days later, trehalase activity did not increase in implanted BAGs from 1- and 2-day pupae (before the ecdysteroid peak), but did in those from 4- and 5-day pupae (at the time on the pupal ecdysteroid peak). Next, BAGs from 0-day pupae were exposed to 20-hydroxyecdysone for 24 hr *in vitro* and then transplanted into 0-day female adults. 8 days later, an increase in trehalase activity was observed in implanted BAGs and dose dependent (Yaginuma and Happ, 1989). Thus, this result clearly showed that 20-hydroxyecdysone acted in the male pupa to commit BAGs toward trehalase production.

Now, our effort is concentrated on understanding the molecular mechanism how ecdysteroid hormone commits the pupal BAGs to produce the trehalase protein. Northern blots using cDNA for trehalase from BAGs showed that mRNA with about 2 kb was abundant in mature BAGs among male reproductive organs, and that it appeared in BAGs 1 day after adult ecdysis and increased to 4 days (Takiguchi *et al.*, 1992). For the purpose described above, gene structure of the trehalase has further to be understood.

Acknowledgments: We thank Drs. O. Yamashita and M. Kobayashi for their encouragements and fruitful discussions in our laboratory.

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