Invertebrate Reproduction and Development, 29:2 (1996) 167–172
Balaban, Philadelphia/Rehovot
0168-8170/96/$05.00 © 1996 Balaban

The conglobate gland of *Blattella germanica* (L.) (Dictyoptera, Blattellidae). Maturation, juvenile hormone dependency and changes during spermatophore formation

LLUÏSA VILAPLANA, MARIA-DOLORS PIULACHS and XAVIER BELLÉS*
Department of Agrobiology, Centro de Investigación y Desarrollo, CSIC, Jordi Girona 18, 08034 Barcelona, Spain
Tel. +34 (3) 400-6124; Fax +34 (3) 204-5904; E-mail: xbragr@cid.csic.es

Received 4 March 1996; Accepted 6 June 1996

Summary

The conglobate gland of male cockroaches is a reproductive organ anatomically close to the accessory glands. From a functional point of view it has been postulated that the conglobate gland is involved in the formation of the spermatophore, although no clear demonstration of this role has been published so far. In addition, previous endocrinological studies have suggested that juvenile hormone might influence conglobate gland maturation. In the present work, taking the conglobate gland of the cockroach *Blattella germanica* as a model, we have studied the protein pattern during maturation, the effects of allatectomy, and the depletion of gland proteins during the formation of the spermatophore. Taken together, the results suggest that juvenile hormone stimulates the accumulation of proteins in the conglobate gland and that these proteins contribute to the formation of the spermatophore.

Key words: Conglobate gland, protein pattern, corpora allata, allatectomy, juvenile hormone, cockroaches

Introduction

The conglobate gland is an internal reproductive organ typical of male cockroaches which lies beneath the accessory glands and the ejaculatory duct (Adiyodi and Adiyodi, 1975). Although the name "conglobate gland" is used currently, some authors (e.g., Happ, 1984) use "phallic gland" as a synonym. Morphological and cytological observations on the conglobate gland have been reported for *Periplaneta americana* by Beams et al. (1962) and Vijayalekshmi and Adiyodi (1973a, 1973b). In *Blattella germanica* the morphological features of the conglobate gland have been described by Feliubadaló et al. (1996). It has a plate structure, approximately triangular in shape, formed by coiled tubules which derive from a single ductule that opens directly into the genital pouch.

Generally, it is assumed that the conglobate gland is involved in the formation of the spermatophore, possibly because of its anatomical proximity and biochemical relationships with the accessory reproductive glands (see Vijayalekshmi and Adiyodi, 1973a, 1973b). However, no clear demonstration of this has been reported so far, as pointed out by Cornwell (1968), who stated that its function is unknown. This laconic statement may reflect the

*Corresponding author.

disparity of conclusions afforded by different authors. For example, whereas Gupta (1947) described that in *P. americana* the conglobate gland secretions contribute to the formation of the spermatophore, together with those from the accessory reproductive glands, Khalifa (1950) reports in *B. germanica* that only the products from the accessory glands participate in this formation. More recent studies that deal specifically with the conglobate gland (Vijayalekshmi and Adiyodi, 1973a, 1973b) do not answer the question, and the most recent reviews on the male reproductive organs of insects do not deal with the conglobate gland (Kaulenas, 1992; Happ, 1992).

Concerning the endocrine dependence of conglobate gland maturation, data available are even scarcer. Only a preliminary study carried out on *B. germanica* reports that corpora allata removal impaired the increase in size of the gland (Bellés and Piulachs, 1992), which suggested that juvenile hormone might be involved in the process of maturation.

On the basis of these antecedents, and taking the conglobate gland of *B. germanica* as a model, we have studied: (1) the total protein contents and protein pattern during maturation, comparing the results found with those of juvenile hormone production previously described by Piulachs et al. (1992); (2) the effects of allatectomy (followed or not by a treatment with juvenile hormone) on total protein contents and protein pattern; and (3) the dynamics of depletion of proteins during the formation of the spermatophore, and that of refilling after its transfer to the female.

Material and Methods

Insects

Virgin males of *B. germanica* were from a colony fed on Panlab dog chow and water, and reared in the dark at 30±1 °C and 60–70% r.h.

Dissections and protein studies

Conglobate glands were dissected out under Ringer's solution from specimens anesthetized with CO_2. Total protein contents were measured following the method of Bradford (1976). SDS-PAGE studies were carried out using the method of Laemmli (1970) with 15% polyacrylamide slab gels. Electrophoresis was run at constant voltage (150 V) for 45 min, and gels were stained with Coomassie blue. Molecular markers were from Sigma (SigmaMarker, low range: MW 6.5–66 kDa, and phosphorylase b from rabbit muscle: MW 97 kDa). At least four replicates of each electrophoretic study were performed. Protein levels in the gels were estimated by densitometry with a Molecular Dynamics computing densitometer.

Allatectomy and juvenile hormone treatments

Due to technical constraints, the whole retrocerebral complex (corpora allata+corpora cardiaca) was dissected out. The operation was performed on freshly ecdysed adults (less than 5 h of imaginal life). Sham operations were carried out by removing the cervical sclerite and adjacent main tracheae. All these manipulations were conducted on CO_2 anesthetized specimens. Juvenile hormone III (Sigma), the native juvenile hormone in *B. germanica* females (Camps et al., 1987), was applied to allatectomized specimens in two doses (10 µg each), one after allatectomy and the other 48 h later. In previous studies carried out on *B. germanica* and *Blatta orientalis* (Piulachs et al., 1992 and García-Alonso et al., 1992, respectively), this system of doses effectively restored the maturation of the accessory reproductive glands in allatectomized specimens. The hormone was applied topically in acetone. Effects induced by allatectomy alone or by allatectomy plus juvenile hormone treatment were checked on day 5 of adult life. At this age differences between intact and allatectomized males are well apparent, at least in terms of accessory gland development (Piulachs et al., 1992).

Mating studies

For mating experiments, 12-day-old virgin males and 5-day-old virgin females were used. In order to study the state of the conglobate gland during and after mating, the following physiological stages (see Vilaplana et al., 1996) were established on these males. A: virgin (control); B: some 50 min after the beginning of mating, first stages of spermatophore formation; C: some 10 min later, spermatophore formed but not transferred to the female; D: some 10 min later, spermatophore freshly transferred; E: some 10 min later, just after termination of mating; F: 6 h after termination of mating; G: 24 h after termination of mating; H: 48 h after termination of mating.

Results

Conglobate gland maturation

Conglobate gland maturation was investigated throughout the first 10 days of adult life, and an additional observation was carried out of day 15. As shown in Fig. 1, total protein contents followed a steady increase until around day 5 and then stabilized.

Fig. 1 also shows the dynamics of juvenile hormone production, according to data previously published by Piulachs et al. (1992) to illustrate the parallelism of this pattern with that of protein contents in the conglobate gland.

The electrophoretic pattern of the conglobate gland was studied on the same days. The pattern showed eight main bands which were labelled I to VIII: I corresponding to a molecular weight of ca. 60 kDa; II close to 45 kDa; III around 35 kDA; IV close to 18 kDa; V, VI and VII around 15 kDa; and VIII close to 7 kDa (Fig. 2). The gel shows that all bands increased in intensity from day 0 to day 5 (Fig. 2) and then stabilized (not shown). The densitometry of the gel (not shown) indicated that all bands increased in intensity at a similar rate, except band II, which seemed to grow more slowly.

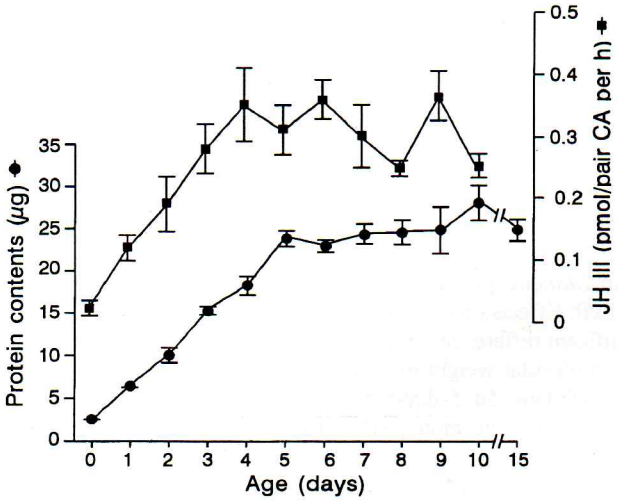

Fig. 1. Total protein contents of conglobate gland in *Blattella germanica* during the first days of adult life. Each value represents the mean ±SEM of 7–26 specimens. Also indicated are the rates of juvenile hormone III (JH III) release by corpora allata incubated *in vitro* according to Piulachs et al. (1992).

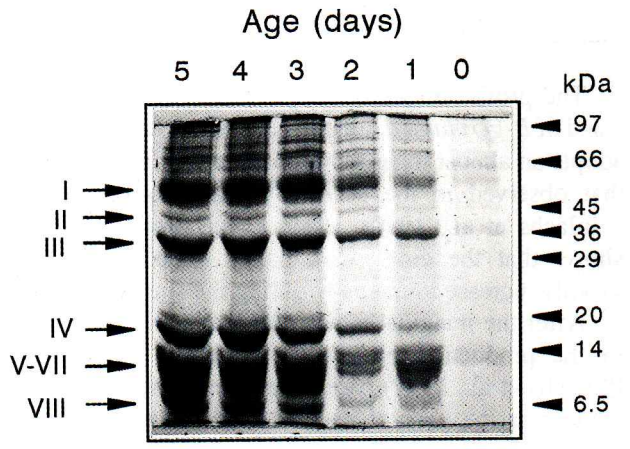

Fig. 2. Protein pattern (15% SDS-PAGE) of conglobate gland of male *Blattella germanica* during the first 6 days of adult life. The arrows to the right indicate the molecular weight markers, and the bands studied, labelled I to VIII, are indicated to the left. An individual gland was loaded in each lane.

Effects of allatectomy

The effects of allatectomy were first studied in terms of total protein contents (Fig. 3). In general, a reduction was observed, whereas juvenile hormone replacement therapy corrected these deficiencies. However, the sham-operated specimens also showed some effect. Therefore, the experiments with juvenile hormone have to be compared with sham-operated rather than with intact controls.

The electrophoretic studies (Fig. 3) showed that bands I–VIII were markedly less intense in allatectomized than in intact males, and that the patterns corresponding to sham-operated specimens and to allatectomized specimens treated with juvenile hormone were similar. Those of allatectomized specimens treated with acetone were almost identical to those subjected to allatectomy alone. The densitometry of the gels (not shown) indicated that all bands (I–VIII) were affected by allatectomy (they were between 50% and 75% less intense than in intact specimens, depending on the band), and in all cases juvenile hormone treatment fully restored the intensity measured in sham-operated specimens.

Effects of mating

To study the influence of mating on the protein contents of the conglobate gland, the eight physiological stages described in Materials and Methods and related to the spermatophore formation were selected.

Total protein contents of the conglobate gland in these stages (Fig. 4) suddenly dropped when the spermatophore began to form (stage B), maintained low values during the process of spermatophore completion and transference, and at the end of mating (stages C–F), and showed a steady recovery after mating (stages G and H). As a reference, Fig. 4 also shows the dynamics of juvenile hormone production according to data reported by Vilaplana et al. (1996).

The electrophoretic pattern of conglobate gland in stages A, C, D, E and G (Fig. 5) indicated that proteins corresponding to all main bands (I–VIII) were suddenly depleted at the beginning of spermatophore

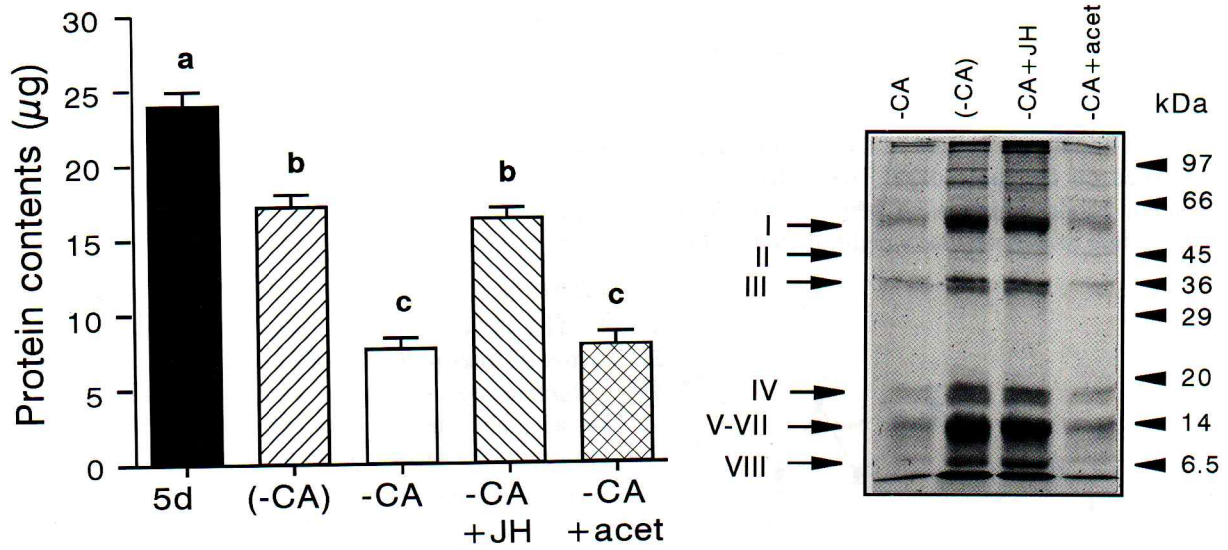

Fig. 3. Effects of allatectomy on conglobate gland proteins in *Blattella germanica*. Allatectomy and juvenile hormone treatments were carried out on day 0 and measurements on day 5. Left: Effects on total protein contents. Each value represents the mean ±SEM of 7–18 specimens. Different letters indicate significant differences (*t*-test, $p \leq 0.05$). Right: Effects on protein pattern (15% SDS-PAGE). The arrows to the right indicate the molecular weight markers, and the bands studied, labelled I–VIII, are indicated to the left. An individual gland was loaded in each lane. 5d, 5-day-old intact males; -CA, allatectomized; (-CA), sham-operated; -CA+JH, allatectomized and treated with juvenile hormone; -CA+acet, allatectomized and treated with acetone.

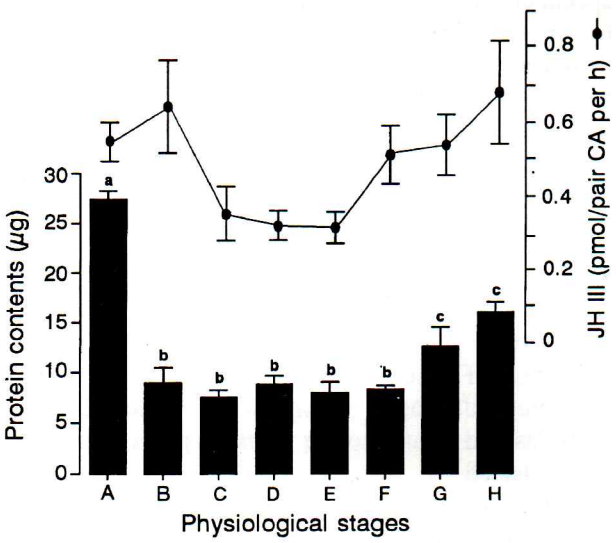

Fig. 4. Total protein contents of the conglobate gland of *Blattella germanica* during and after mating. Physiological stages: A, virgin (control); B, spermatophore in process of formation (50 min after the beginning of mating); C, spermatophore formed but not transferred to the female (10 min later); D, spermatophore freshly transferred (10 min later); E, just after termination of mating (10 min later); F, 6 h after termination of mating; G, 24 h after termination of mating; H, 48 h after termination of mating. Values are expressed as the mean ±SEM (n=6–38). Also indicated are the rates of juvenile hormone III (JH III) synthesis by corpora allata incubated *in vitro*, according to Vilaplana et al. (1996).

formation, and all simultaneously recovered intensity after mating. The densitometry of these gels (not shown) indicated that bands I–VIII lost between 70% and 80% of intensity when the formation of spermatophore began (stage B), and that they slowly recovered intensity after mating.

Discussion

The profile of protein contents of the conglobate gland of *B. germanica* during the first days of adult life adopts an almost logarithmic form, which is similar to that observed in the accessory reproductive glands (Piulachs et al., 1992). Besides, the protein pattern shows that the eight main bands (labelled I–VIII) steadily increase in intensity during the studied period.

After the imaginal moult, the progressive increase in the production of juvenile hormone (data from Piulachs et al., 1992) parallels the increase in protein content in the conglobate gland, which suggests the hypothesis of a functional link between juvenile hormone and conglobate gland maturation. The results of the experiments of allatectomy are also in agreement with this hypothesis, since after corpora allata removal, accumulation of conglobate gland secretions is significantly reduced, whereas the administration of exogenous juvenile hormone restores

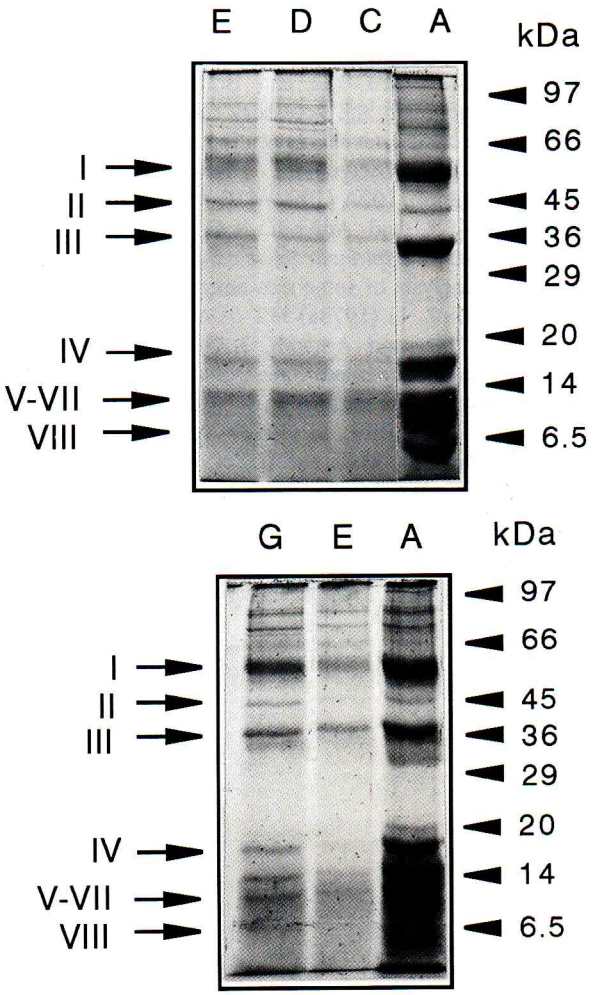

Fig. 5. Protein pattern (15% SDS-PAGE) of the conglobate gland of *Blattella germanica* during and after mating. Lanes: A, virgin (control); C, spermatophore formed but not transferred; D, spermatophore freshly transferred; E, just after termination of mating; G, 24 h after termination of mating. The main bands, labelled I–VIII, are indicated to the left, and molecular weight markers are indicated to the right. An individual gland was loaded in each lane.

the features observed in sham-operated specimens. These results agree with our preliminary data on the effects of allatectomy on conglobate gland size (Bellés and Piulachs, 1992). Therefore, juvenile hormone seems to influence protein accumulation in the conglobate gland. However, some accumulation was observed from day 0 to day 5 in allatectomized specimens, which indicates that allatectomy reduces protein accumulation rates rather than impairing that process irreversibly. Indeed, it is a situation similar to that described for the accessory reproductive glands in the same species (Piulachs et al., 1992; Bellés and Piulachs, 1992).

The observations carried out during and after mating show that a sudden depletion of conglobate gland proteins is concomitant with the first stages of the formation of the spermatophore. This suggests that proteins from this gland participate in the formation of the spermatophore from the very beginning, as occurs in the accessory reproductive glands (Feliubadaló et al., 1996). New accumulation of proteins in the conglobate gland begin after mating, and 48 h later total contents are about half those corresponding to 10-day-old virgin males or equivalent to those measured on day 3 of adult life. The increase in the synthesis of juvenile hormone after mating (data from Vilaplana et al., 1996) parallels the increase in total protein contents in the conglobate gland. This suggests that increasing rates of juvenile hormone biosynthesis are necessary to stimulate the progressive accumulation of proteins in a new cycle of gland maturation.

Acknowledgments

Financial support from the DGICYT, Spain (Project No. PB92-0026: Studies on the reproductive biology in cockroaches) is gratefully acknowledged.

References

Adiyodi, K.G. and Adiyodi, R.G., Morphology and cytology of the accessory sex glands in invertebrates. Int. J. Cytol., 43 (1975) 353–397.

Beams, H.W., Anderson, E. and Kessel, R., Electron microscope observations on the phallic (conglobate) gland of the cockroach, *Periplaneta americana*. J.R. Microsc. Soc., 81 (1962) 85–89.

Bellés, X. and Piulachs, M.D., Juvenile hormone and maturation of male accessory reproductive glands in the cockroach *Blattella germanica* (L.). In: Insect Juvenile Hormone Research. Fundamental and Applied Aspects, B. Mauchamp, F. Couillaud and J.C. Baehr, eds., INRA, Paris, 1992, pp. 101–109.

Bradford, M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of Protein-Dye Binding. Anal. Biochem., 72 (1976) 248–254.

Camps, F., Casas, J., Sánchez, F.J. and Messeguer, A., Identification of juvenile hormone III in the hemolymph of *Blattella germanica* adult females by gas chromatography-mass spectrometry. Arch. Insect Biochem. Physiol., 6 (1987) 181–189.

Cornwell, P.B., The cockroach. A laboratory insect and an industrial pest, Vol. 1. The Rentokil Library, Hutchinson, London, 1968, pp. 193–195.

Feliubadaló, E., Vilaplana, L. and Bellés, X., Morphology and protein pattern of the accessory reproductive glands of male *Blattella germanica* (L.) (Dictyoptera, Blattellidae). Eur. J. Entomol., 93 (1996) in press.

García-Alonso, M., Piulachs, M.D. and Bellés, X., Influencia de los *corpora allata* en el desarrollo de las glándulas accesorias del macho de *Blatta orientalis*. Rev. Esp. Fisiol., 48 (1992) 77–82.

Gupta, P.O., On the structure and formation of spermatophore in the cockroach *Periplaneta americana* L. Ind. J. Entomol., 8 (1947) 79–84.

Happ, G.M., Structure and development of male accessory glands in insects. In: Insect Ultrastructure, Vol. 2, R.C. King and H. Akai, eds., Plenum, New York, 1984, pp. 365–395.

Happ, G.M., Maturation of the male reproductive system and its endocrine regulation. A. Rev. Entomol., 37 (1992) 303–320.

Kaulenas, M.S., Insect Accessory Reproductive Structures. Springer Verlag, Berlin, 1992.

Khalifa, A., Spermatophore production in *Blattella germanica* L. (Orthoptera: Blattidae). Proc. Roy. Entomol. Soc. London, 25 (1950) 53–61.

Laemmli, U.K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227 (1970) 680–685

Piulachs, M.D., Maestro, J.L. and Bellés, X., Juvenile hormone production and accessory reproductive gland development during sexual maturation of male *Blattella germanica* (L.) (Dictyoptera, Blattellidae). Comp. Biochem. Physiol., 102A (1992) 477–480.

Vijayalekshmi, V.R. and Adiyodi, K.G., Accessory sex glands of male *Periplaneta americana* (L.): Part I — Qualitative analysis of some non-enzymic components. Ind. J. Exp. Biol., 11 (1973a) 512–514.

Vijayalekshmi, V.R. and Adiyodi, K.G., Accessory sex glands of male *Periplaneta americana* (L.): Part III — Histochemistry of the mushroom-shaped and conglobate glands. Ind. J. Exp. Biol., 11 (1973b) 521–524.

Vilaplana, L., Piulachs, M.D. and Bellés, X., Inhibition of juvenile hormone during the formation of the spermatophore in *Blattella germanica* (L.) (Dictyoptera, Blattellidae). Arch. Insect Biochem. Physiol., 32 (1996) in press.