Virus-like particles in the poison gland of the parasitic wasp *Opius concolor*

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(Accepted 19 February 1997)

Summary

Virus-like particles (VLP's) have been found in the poison glands of adult females of the parasitic wasp *Opius concolor* Szèpl. (Hymenoptera, Braconidae). These VLP's are found in the secretory cells either free in the cytoplasm or within cytoplasmic vesicles, sometimes associated to a secretory apparatus.

Negative staining of these VLP's has revealed the occurrence of two different particles. The first type exhibits icosahedral symmetry (diameter around 70 nm) and hollow surface spikes, this morphology being typical of the genus *Cypovirus* (Reoviridae). The other type is pleomorphic and presents an envelope with club-shaped projections (diameter ranging from 30 to 60 nm), as classical textbook examples of Coronaviruses, but smaller. Function and full characterisation of these particles are not yet known.

Key words: Braconidae, *Opius concolor*, poison gland, parasitoid virus, Reo-like virus, Corona-like virus

Introduction

Endoparasitoids complete their development within other arthropods. In order to achieve this goal, immatures have to cope with the host immune system. Insects usually react against introduced foreign bodies, such as the eggs of parasitoids, by hemocyte-mediated encapsulation. Consequently, endoparasitic insects have evolved different strategies for overcoming these reactions (Vinson, 1990). In some cases avoidance of encapsulation is simply passive, whereas more often, parasitoids actively disorganise the host immune system by different substances either injected by the adult parent at the time of oviposition or directly produced by the parasitoid larva (Lavine & Beckage, 1995).

Among wasp factors implicated in host immunosuppression, are some viruses. The calyx of the ovaries of different taxa within the superfamily Ichneumonoidea shelters replication of specific Polydnnaviruses (Fleming, 1992). Several members of this family have been extensively studied since their discovery in the 70's. Polydnnaviruses help the parasitoid avoid encapsulation by either altering or eliminating host haemocytes (Strand & Pech, 1995). In 1982, Edson, Barlin & Vinson described virus-like particles (VLP), not from the ovaries, but

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from the venom apparatus of two out of the nine braconids they studied. More recently, Lawrence & Akin (1988) confirmed that the venom glands of one of these wasps, *Diachasmimorpha longicaudata* (Ash.), contained two VLP's that morphologically resembled Entomopoxviruses and Rhabdoviruses. These viruses are also suspected to help the parasitoid suppress host defence mechanisms (Rolle & Lawrence, 1994a,b). Although a venom apparatus is present in all female braconids (Edson & Vinson, 1979), its function in species which are not known to paralyse their hosts remains largely unknown.

In this paper VLP's from the venom apparatus of *Opius concolor* Szèpl. (Hymenoptera, Braconidae) are described. This wasp is a palaearctic species, native of the southern Mediterranean Basin, non-paralysing parasitoid of different tephritids of economic importance, including the medfly, *Ceratitis capitata* Wied., the olive fly, *Bactrocera oleae* (Gmel.), the capper fly, *Capparimyia savastani* Martell., the jujube fly, *Carpomya incompleta* Beck. and *Dacus frontalis* Beck.

Materials and Methods

Insects

Adults of *O. concolor* were reared at $25 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ r.h. and a 16 h light photoperiod in *C. capitata* last instar larvae, according to a standardised methods (Jacas & Viñuela, 1994). Females used in this study were collected from the rearing cages where they had been housed with males and offered hosts for parasitisation for one week.

Prior to dissection, females were kept at -9°C for about 10 min. They were then placed on ice for further manipulation. Their internal genitalia were extracted by simply pulling the sting with forceps and placing in a drop of Ringer's solution under binocular. The venom glands (= acid glands) from six adult females were carefully separated and prepared for transmission electron microscopy. A homogenate of poison glands obtained from adult females was prepared for negative staining electron microscopy.

Ultrastructure

Transmission electron microscopy

Immediately after dissection, the poison glands were transferred for fixation to a 2.5% glutaraldehyde solution for 4 h at 4°C . They were then rinsed in Crison® buffer (potassium phosphate and disodium phosphate, pH 7.02) and kept in this medium overnight. The day after, specimens were postfixed in 4% osmium tetroxide at room temperature for 1 h. After three rinses in distilled water, the tissues were dehydrated through a graded ethanol-acetone series at room temperature. They were then infiltrated with Durcupan® through a graded acetone-durcupan series (2:1; 1:1; 1:2) at room temperature for 2 h each, left overnight in the last one and finally embedded in pure Durcupan® in a polymerisation mould and kept at 70°C for 3 days. Ultramicrotome sections (50 nm) were stained with acidic uranyl acetate and Reynold's lead citrate and examined under an electron microscope at 120 kV accelerating voltage.

Negative staining

Poison glands from 200 females were transferred to 0.5 M sodium citrate (pH = 6.5) and kept at 4°C from that moment onward. These were homogenised in a 2 ml Eppendorf by passage through a syringe fitted to a 25G (Burkard) needle. The homogenate was centrifuged

at 12 600 rpm (rotor 6373) for 15 min. The supernatant was retained and the pellet resuspended in sodium citrate and further homogenised. This homogenate was centrifuged as previously and the supernatant retained. Both supernatants were combined and centrifuged at 100 000 rpm for 15 min in a TL-100 Ultracentrifuge (rotor TLA 100.2) and the pellet resuspended in 100:1 of deionised water.

Formvar-coated carbon-copper grids were exposed to a drop of the homogenate for 30 s. These grids were then stained with 2% phosphotungstic acid (PTA) for 60 s. In both cases filter paper was used to remove excess water and PTA respectively. Samples were examined as described previously.

Results

TEM observation of the poison apparatus revealed the presence of spheric virus-like particles in both the poison gland and poison sac. Spherical VLP's appeared within cytoplasmic vesicles of secretory cells (Fig. 1) where they presumably replicated. VLP's were also observed free within the cytoplasm (Fig. 2), sometimes in heavily diseased cells (Fig. 3). In that case, VLP's invariably appeared associated to small round vesicles and rod shaped structures, presumably final products from viral infection, and corresponding to old membranes and proteins, respectively. Free VLP's were also observed within the lumen of the gland (Fig. 4).

TEM of negative-stained tissue homogenates demonstrated the occurrence of two different VLP's. Particles from one type had a double shelled icosahedral structure 72 nm in diameter, with external projections 12 nm long and 8 nm wide located at the vertices (Fig. 5). The inner shell, 61 nm in diameter, presented hollow surface spikes (7 nm in length and 18 nm wide) at the vertices (Fig. 6), similar to Reovirus-like particles, RVLP (Hukuhara & Bonami, 1991). The other type (Fig. 7) appeared pleomorphic (33 to 56 nm in diameter) and exhibited an envelope with club-shaped projections 15 nm in length (Fig. 8), similar to those present in Coronaviridae (Cavanagh *et al.*, 1995). Notwithstanding, the complete morphology of these VLP's does not fully correspond to any known virus insect family described so far.

Discussion

Non-pathogenic reovirus-like particles have been isolated from the gut of many invertebrates, even in hosts of *O. concolor*, such as *C. capitata* (Plus *et al.*, 1981; Plus & Cavalloro, 1983) and *B. oleae* (Manousis, Koliais & Moore, 1987). Hamm, Styer & Steiner (1994) have also described reovirus-like particles in gut epithelial cells of the braconid *Microplitis croceipes*, replicating in vesicles that look very much like the ones we have found in *O. concolor*, but reoviruses are typically isolated from the gastrointestinal tracts of insects (Tyler & Fields, 1991).

VLP's from the venom apparatus of different braconid wasps have already been reported previously. Particles described by Edson (1981) and Edson *et al.* (1982) from *Meteorus leviventris* (Wesmael) closely resemble those found in *O. concolor*, but interestingly those found both by Edson *et al.* (1982) and Lawrence & Akin (1988) in the closer related species

Fig 1. Infected secretory cell from the poison gland filaments, showing a cytoplasmic vesicle containing virus-like particles (VLP) (arrowheads). Rough endoplasmic reticulum (double arrowhead) and vesicles (arrow) were very abundant in this type of cell. (Transmission electron microscopy).

Fig 2. Free VLP's (arrowheads) within the cytoplasm of a secretory cell. Splitting of vesicles containing VLP'S could account for the high concentration of particles usually found within cells (tem).

Fig 3. A presumed long-time infected cell containing VLP's (arrowheads) associated to small round vesicles and rod shaped structures (arrows), probably final products from viral infection, corresponding to old membranes and proteins, respectively. Lysis of diseased cells could account for virus spreading among neighboring cells. (tem).

Fig 4. Small vesicle containing VLP's near the lumen of the gland (arrowhead), and free VLP's within the lumen (double arrowheads), likely indicating release by exocytosis. (tem).

Diachasmimorpha (= *Biosteres*) *longicaudata* (Ash.), a parasitoid of the Caribbean fruit fly *Anastrepha suspensa* (Loew), are completely different. Two different VLP's were found in the venom glands of *D. longicaudata*: rod-shaped ones and spherical ones, resembling rhabdo- and entomopoxvirus, respectively. It is noteworthy that both *O. concolor* and *D. longicaudata* belong to the same subfamily within Braconidae: Opiinae, and share hosts belonging to the same subtribe within Tephritidae although they come from different biogeographical areas, whereas *M. leviventris* belongs to another subfamily: Meteorinae

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Fig. 5. Reovirus-like particle (RVLP) exhibiting external projections resembling characteristic RVLP A-spikes (arrowheads) on the vertices. Transmission electron microscope (tem) of negative-stained (with phosphotungstic acid) gland filament homogenate.

Fig. 6. Core of a RVLP showing hollow surface spikes resembling typical RVLP B-spikes on its vertices. Tem of negative-stained gland filament homogenate.

Fig. 7. A core of RVLP (arrowhead) and pleomorphic virus-like particles (arrows). Tem of negative-stained gland filament homogenate.

Fig. 8. Pleomorphic virus-like particles showing envelope and club-shaped projections on the periphery. Tem of negative-stained gland filament homogenate.

(usually included in Euphorinae) and is a parasitoid of lepidopterans. Apparently relatedness among species does not preclude relatedness among associated viruses.

Acknowledgements

The authors would like to thank A Fraile and F García-Arenal from the Department of Biotechnology, ETSI Agrónomos de Madrid and A. Fernández, Centro de Microscopía Electrónica, Universidad Complutense de Madrid, for their advice.

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(Received 17 September 1996)