Biochemical and immunological studies of proteins from polydnavirus *Chelonus* sp. near *curvimaculatus*

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Polydnavirus from *Chelonus* sp. *curvimaculatus* (CcV) was purified and 18 structural polypeptides associated with CcV were identified by silver staining. Antibodies were raised against CcV protein and used in testing for the presence of the virus in different tissues of the wasp ovary and in stung eggs. It was also established that the virus does not enter during the first 5 s of oviposition. Furthermore, no degradation of the virus proteins was detected inside the egg within 2 h after oviposition. The glycoprotein nature of virus proteins was also determined by concanavalin A/horseradish peroxidase staining. The amino acid compositions of the most highly abundant peptides (41K, 33K, 21K, 17K and 13K) were determined, as was the N-terminal amino acid sequence of the 41K protein. The latter did not show similarity with any reported protein sequences.

**Introduction**

The life cycle of some parasitic hymenopterous insects requires, as an integral component, the participation of polydnaviruses which replicate in the ovary of the female wasp. Two morphologically distinct groups of viruses are recognized as belonging to the polydnavirus family. In braconid wasps, the polydnaviruses are cylindrical nucleocapsids of variable length surrounded either individually or as groups by a unit membrane envelope (Stoltz & Vinson, 1979). Polydnavirus from *Chelonus* species are rod-shaped nucleocapsids and have a distinct envelope tail with variable length (Jones *et al.*, 1986a). Polydnaviruses found in ichneumonid wasps have fusiform nucleocapsids which contain an electron-dense core surrounded by a more translucent zone; this is again surrounded by a thin electron-dense outer layer (Krell, 1987). However, the virus genome is organized as double-stranded, circular genomic segments in both groups (Stoltz *et al.*, 1984).

All polydnaviruses appear to share a common life cycle in that virus replication occurs in the nuclei of calyx epithelial cells of the ovary and virus particles eventually enter the lumen as components of the calyx fluid (Stoltz & Vinson, 1979). Female wasps inject virus particles with the parasite egg during oviposition. The fate of the virus subsequent to oviposition was examined in *Manduca sexta* after parasitization by *Cotesia congregata* (Stoltz & Vinson, 1979). Virions were first seen in the cytoplasm 1 h after oviposition and within 2 h after oviposition viral DNA entered the host nuclei. Recent evidence suggests that the wasp venom promotes uncoating of the virus (Stoltz *et al.*, 1988).

Some physiological and immunological effects on parasitized larvae have been attributed to the presence of polydnavirus. For example, inhibition of phenoloxidase activity has been shown in parasitized larvae of *Trichoplusia ni* (Stoltz & Cook, 1983). Edson *et al.* (1981) reported that the virus component of the calyx fluid of *Campoplexis sonorensis* is capable of inhibiting encapsulation of parasite eggs by the host *Heliothis virescens*. Feddersan *et al.* (1986) reported an alternative mechanism in which a protein of virus-like capsids from *Venturia canescens* can potentially provide a protective coat which will prevent the egg from being recognized as foreign tissue by the host (*Ephestia kuehniella*). However, the actual biochemical basis by which the polydnaviruses suppress the host immune system is still unclear. In order to distinguish between the above and other mechanisms which may operate in a given system it is necessary to characterize the virus-associated proteins.

The most pertinent studies on characterization of polydnavirus proteins reported in the literature are those concerning wasps in the family Ichneumonidae. Different numbers of polypeptides have been detected by SDS-PAGE analysis within purified virions of different species. Acrylamide gel analysis of virion proteins purified from *Apanteles melanoscelus*, *Hyposoter exiguae* and *C. sonorensis* showed 18, 20 and 25 different sized polypeptides respectively (Krell & Stoltz, 1979, 1980; Krell *et al.*, 1982). One of the peptides from virus isolated from the ichneumonid, *C. sonorensis*, was determined to
be a glycoprotein. Serological studies on these virus proteins have also been carried out in which antibodies raised against the virus proteins were used not only to examine the relationships among viruses (Cook & Stoltz, 1983), but also to determine the antigenic similarities between the virus and host tissue (Feddersen et al., 1986).

There have been no reports of analysis of virion proteins beyond this level.

We have been studying polydnavirus from *Chelonus* sp. near *curvimaculatus* (CcV). The parasitic wasp injects its egg into the host egg along with other regulatory materials such as calyx fluid, virus particles and venom. Host metamorphic development is redirected after the host eggs are stung by the wasp (Jones et al., 1986b). Viral mRNA transcripts and a parasitization protein have also been detected in *Trichoplusia ni* after oviposition by this wasp (Jones et al., 1986a; D. Jones, unpublished result). We present here data on biochemical and immunological characterization of CcV structural proteins.

**Methods**

*Insects.* Host insects, *T. ni* (Huebner: Noctuidae) and parasitic wasps *C. near curvimaculatus* (Braconid) were reared as described by Jones (1986).

*Chemicals.* 125I-label anti-rabbit IgG from goat serum was prepared as described elsewhere (Tejedor & Ballesta, 1982). Acetic acid, formaldehyde, glutaraldehyde, hydrochloric acid and sodium chloride were obtained from Fisher Scientific Co. All other chemicals, including bovine serum albumin (BSA), were procured from Sigma.

*Virus purification.* Ovaries of wasps were dissected in 10 mM-Tris-aceetate/2 mM-EDTA (TAE) pH 7.4. The ovaries were homogenized with a pestle and the debris was pelleted at 10,000 g for 3 min. The supernatant (200 ml) was layered onto a 4.4 ml 10 to 50% (w/w) sucrose gradient and centrifuged at 100,000 g for 60 min at 5 °C in a Beckman SW60 rotor (Krell et al., 1982). Sucrose gradient fractions (400 μl each) above the virus band and the virus fraction itself were collected and stored at −70 °C until further use.

*Preparation of antibodies against CcV structural proteins.* Three hypoimmune intradermal injections of virus proteins were made into a rabbit at 3 month intervals. Each injection consisted of 200 μl of virus protein solution isolated from 200 wasp ovaries. The first injection was made with Freund's complete adjuvant, and the remaining two with incomplete adjuvant. The first serum was collected 10 days after the first injection. Subsequent serum collection was performed 10 days after each booster injection containing the same amount of virus proteins.

*Analysis of virus in host eggs.* Each host egg was stung a single time by a female wasp. The stung eggs were harvested from the egg sheet and homogenized in buffer (0.1 M-Tris-HCl, 5 mM-EDTA, 150 mM-NaCl, 0.1% trypsin inhibitor, 0.1% SDS, 0.02% sodium azide pH 7.5). In other experiments oviposition was interrupted after 5 and the incompletely stung eggs were collected and processed as above. Proteins from stung and non-stung eggs were subjected to SDS–PAGE and in some cases were then used for immunoblotting.

**Electrophoretic methods.** SDS–PAGE was done on 20 x 20 cm slab gels according to Laemmli (1970). Proteins in SDS–PAGE gels were silver-stained as described (Morrissey, 1981).

**Immunoblotting.** Immunoblotting was carried out according to procedures described elsewhere (Burnette, 1981). After electrophoretic transfer to nitrocellulose, the sheet was incubated in blocking solution which contained 20% horse serum and 5% BSA in buffer (20 mM-Tris-HCl pH 7.5 with 0.9% NaCl). The sheet was incubated with anti-virus rabbit serum (1:200) in the same buffer overnight at room temperature. After washing out the excess rabbit IgG with Tris buffer containing 0.2% SDS, 0.5% Triton X-100 and 0.5% milk powder, the sheet was placed in 125I-labelled anti-rabbit IgG in blocking solution (2 x 107 c.p.m. in 20 ml) for 3 h at room temperature. The excess 125I-anti-rabbit IgG was then washed off and the nitrocellulose sheet was air-dried and exposed to Kodak X-ray film at −70 °C for 1 to 2 days.

**Carbohydrate staining.** Separated proteins were electrophoretically transferred to nitrocellulose and the sheet was air-dried and then incubated with concanavalin A (Con A) peroxidase solution (0.01%) for 30 min in 25 mM-Tris-HCl pH 7.6, containing 1 mM-MgCl2, 1 mM-CaCl2, 0.5 mM-NaCl and 0.02%, sodium azide. After washing off the excess Con A the sheet was stained with 4-chloro-1-naphthol according to the method described by Lin (1986).

**Amino acid composition and N terminus sequencing.** Virus proteins were separated by SDS–PAGE and transferred to Immobilon (polyvinylidene difluoride) from which amino acid compositions and the N terminus sequence were determined (Matsudaira, 1987).

**Results**

**Virus purification**

The centrifugation of total ovary homogenate supernatant onto a sucrose gradient concentrated the virus particles into a thin white–blue band. Polyacrylamide gel electrophoresis of virus prepared on a sucrose gradient resolved at least 18 different polypeptides (Fig. 1a).

**Antibodies to CcV proteins**

In order to do additional quantitative and qualitative analysis of virus structural proteins, a more sensitive technique was employed. Antiserum was raised against proteins from purified CcV and was used as primary antibody in immunoblot analysis. Following sucrose gradient fractionation of ovarial preparations, duplicate aliquots from gradient fractions were subjected in parallel to 5 to 20% gradient SDS–PAGE. One was stained for proteins with silver nitrate (Fig. 1b) and the other was subjected to immunological analysis (Fig. 1c). The antibodies reacted with at least 10 polypeptides in the fraction corresponding to purified virus (Fig. 1c), of which those of 13K, 17K, 23K and 41K showed the strongest reactions.

The strong signals obtained for the four lower Mr, proteins prompted additional analysis. The relative abundance of each of the four was determined by
densitometric analysis of silver-stained gels. These measurements were compared with the relative abundance as indicated by densitometric scanning of immunoblots. The four appeared to yield much stronger signals than would be predicted on the basis of the abundance of each protein. It may be that these proteins are much more immunogenic than the higher Mr proteins of the virus.

**Immunological localization of the virus**

The antibodies were used in an initial test of the sites of synthesis of CcV proteins within the ovary (Fig. 2). The strongly reactive bands of 13K, 17K, 23K and 41K are present in both the calyx fluid and oviduct from which the calyx fluid was extruded (Fig. 2, lanes 1 and 3). This result makes physiological sense as we would expect that proteins produced in the calyx cells, prior to entry into the calyx lumen, will be immunologically detectable in preparations from the calyx lumen and calyx tissue. However, none of the reactive polypeptides was detected in the protein sample from ovarioles (Fig. 2, lane 2), a region farther away from the common oviduct.

**Detection of the virus proteins in stung eggs**

As these antibodies can be used to detect the virus protein in tissue samples, detection of virus proteins present in stung eggs was performed. As shown in Fig. 3, the antibodies easily detected the four most strongly reactive CcV protein bands (13K, 17K, 23K and 41K) in the sample of purified CcV (Fig. 3, lane 1). The 13K and 17K bands were the most easily detected in completely stung eggs (lane 3). Furthermore, the lack of detection of CcV protein in eggs stung for 5 s suggests that the virus is either absent, or is present in undetectable quantities, in eggs stung for 5 s and in non-stung eggs (Fig. 3, lanes 2 and 4). The fate of the virus proteins in the egg (2 h after oviposition) was also examined. When these eggs were subjected to the same treatment as above no change in the reactive polypeptide profile was detected (Fig. 4, lanes 2 and 3). This result indicates that the detected proteins.
virus proteins are stable inside the egg for up to 2 h after the oviposition.

Detection of glycoproteins in the virus preparation

Staining of total virus proteins for glycoproteins indicated that several polypeptides are relatively strongly glycosylated (e.g. the 97K and 41K proteins) and several others are less glycosylated (Fig. 5).

Amino acid composition and sequence of the virus proteins

The amino acid compositions of five CcV proteins are shown in Table 1. These proteins appear to be rich in Asx, Glx and Ser and have a low abundance of Met and His. Preliminary isoelectric focusing studies indicate that most of these proteins have an acidic isoelectric point when focused under native conditions (not shown). The N terminus sequence of one of these (41K) proteins was determined as VGILDTVLSSNTIQPH. The sequence is not related to other sequences in the Genetic Sequence Data Bank (GenBank) nor to the proteins deduced from sequencing of another polydnavirus genome (Blissard et al., 1987; 1989).

Discussion

The interaction between parasites in the genus Cheilinus and their lepidopteran host, *T. ni*, appears to be a useful model system for understanding the mechanisms of parasite regulation of host development and physiology. In this system, the ovipositing female is the source of the
regulatory material which redirects host physiology and metamorphosis (Jones et al., 1986b; Jones, 1987). In addition, it has been shown that the venom participates in the escape of the parasitic larvae from the host immune responses (Leluk et al., 1988; T. Taylor & D. Jones, unpublished results). However, material from the female in addition to venom is also necessary for the complete effect (Jones, 1987) and this material enters the host at a similar time as the CcV.

Comparing the virus electrophoretic profile of CcV proteins and its immunoblot counterpart indicates that lower Mr major protein components carry antigenic components. However, the different intensity signals shown among the virus-associated peptides in the immunoblot may be explained by the fact that some peptides are more immunogenic than others. When two additional fractions, collected just above the virus band from the sucrose gradient, were analysed along with the virus fractions some higher Mr proteins stained with silver. There were no corresponding signals on the parallel immunoblot. It may be concluded that antibodies used in this study gave specific reactions to virus-associated proteins.

Since the CcV is produced in the calyx cells of the ovary, it would not be possible to remove all CcV proteins from an ovary homogenate preparation merely by squeezing out the extracellular virus particles from the calyx lumen. Also, for logistic reasons, it is not possible completely to squeeze out all components in the calyx lumen. Hence, we detected CcV proteins in the ovary preparation after extruding the bulk of the calyx lumen components. However, the immunoblot results related to localization of the virus are in agreement with previous electron microscopy findings (Jones et al., 1986a) that
The virus do not enter the egg during the first 5 s of oviposition. Leluk & Jones (1989) used antibodies to the venom proteins and showed that most of the venom is injected into fully stung eggs. However, polydnavirus proteins. We report here that a number of the CcV proteins are glycosylated. In preliminary studies, the CcV antibodies did not cross-react with proteins in the haemolymph of the lepidopteran host, in contrast to the situation in which virus-like particles from V. canescens have immunological cross-reactivity with antigens in the lepidopteran host, E. kuehniella (Feddersen et al., 1986). It is important to determine whether these and other nucleocapsid proteins are a viral gene product or host membrane proteins.

The results of the present study will be helpful in future studies requiring purified virus and monitoring of the virus in both wasp and lepidopteran hosts. Characteristics of virus structural proteins are helpful and important for the study of their origin (e.g. whether they are virus-encoded or acquired from the host), as well as investigations on any roles they may play in alterations of host physiology.

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References


Table 1. Amino acid composition of five major virus proteins

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* Cys and Trp were not determined.
† Percentage occurrence of residue/mol.

CcV replication occurs exclusively in the calyx region and that CcV is present in high concentration in the calyx fluid.

Using the antiserum, we observed immunological detection of virus proteins in stung eggs. The results also indicate that all of the antigenic proteins are injected into the eggs and that no changes in their size occurs during oviposition or within 2 h after injection. Furthermore, the present data suggest that detectable amounts of the virus do not enter the egg during the first 5 s of oviposition. Leluk & Jones (1989) used antibodies to the venom proteins and showed that most of the venom is injected within the first 5 s of oviposition. In that study, no virus proteins injected into fully stung eggs detectably cross-reacted with the antibodies toward the venom. In the present study, we again not detected shared epitopes between the virus structural proteins and venom proteins because virus antibody does not cross-react with venom injected into fully stung eggs. However, polydnavirus and venom from C. sonorensis have common epitopes (B. A. Webb & M. D. Summers, personal communication). The fate of these virus proteins in subsequent days during the egg development can now be tracked using this antiserum. Thus, in this host–parasite interaction, it may be possible to distinguish clearly between entry of these factors. This should expedite analysis of their function in regulation of the host physiology.

The glycoprotein nature of a 25K virus protein isolated from an ichneumonid wasp (C. sonorensis CsV) has been reported (Krell et al., 1982). To our knowledge there are no reported data on the glycosylation state of braconid polydnavirus proteins.
Analysis of polydnavirus proteins


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