Venom Promotes Uncoating in vitro and Persistence in vivo of DNA from a Braconid Polydnavirus

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SUMMARY

Earlier studies have suggested that successful parasitism by certain braconid parasitoids may depend on the presence in host insect larvae of both polydnavirus and venom. We have shown that venom from the braconid parasitoid, Cotesia melanoscela, was required for in vivo persistence of polydnavirus DNA in host larvae. In parallel studies using an in vitro system, we observed that in the presence of venom nucleocapsids were released into the cytoplasm and subsequently uncoated at nuclear pores; in the absence of venom, this sequence of events was not observed.

The braconid polydnaviruses constitute one of two major groups of insect viruses having polydisperse, dsDNA genomes (Stoltz & Vinson, 1979; Stoltz et al., 1984). These viruses differ from the ichneumonid polydnaviruses primarily in terms of particle morphology. Whereas ichneumonid isolates have quasi-cylindrical nucleocapsids surrounded by two envelopes, braconid polydnaviruses have cylindrical nucleocapsids of variable length surrounded by a single unit membrane (Stoltz & Vinson, 1979). During oviposition both types of viruses are injected into host larvae, enter host tissue and appear to promote successful parasitism by means of an immunosuppressive activity (Edson et al., 1981; Guzo & Stoltz, 1985; Stoltz, 1986; Stoltz & Guzo, 1986). In the case of at least some braconid polydnaviruses, the presence of virus alone is not sufficient to induce a state of immunosuppression in the host, venom being also required (Kitano, 1982; Guzo & Stoltz, 1985). At present, however, the nature of this apparent synergism is poorly understood. We report here that venom is probably required during some early stage in virus uptake by susceptible host cells.

In previous work, we showed that polydnavirus DNA from the braconid parasitoid Cotesia melanoscela could persist for several days in the naturally parasitized host larvae (Stoltz et al., 1986); this occurs in the apparent absence of viral replication (unpublished data). Since it was already known that venom was involved in this system and required for successful parasitism (Guzo & Stoltz, 1985), the present study focused on the question of whether venom might affect the uncoating and subsequent persistence of viral DNA. To that end, virus in either the presence or the absence of venom was manually injected into larvae of the tussock moth Orgyia leucostigma and viral DNA in host tissues was subsequently analysed by Southern blot hybridization. Larvae were injected with virus and then with venom at various intervals (Fig. 1). Viral DNA could be detected in these larvae provided venom had been injected within 6 to 12 h of the original injection of virus. In a parallel experiment, we attempted to find out how long the virus would remain viable in the absence of venom. As shown in Fig. 1, virus had to be injected within 3 to 6 h after injection of venom if persistence of viral DNA was to be observed. Each of the above experimental protocols was repeated twice, with equivalent results in each case. Preliminary experiments using an alternative host (Lymantria dispar) have yielded equivalent results (not shown).
Fig. 1. Requirement for venom in the persistence of braconid polydnavirus DNA \textit{in vivo}. Samples consisted either of 0.5 μg virus DNA (lane 1), or about 50 μg of total nucleic acid extracted in each case from 10 third-instar \textit{O. leucostigma} larvae 24 h after treatment (lanes 2 to 8). Injected materials were in phosphate-buffered saline (pH 7.0), at concentrations equivalent to the total amount of product estimated to be present in 0.1 female parasitoid. Virus and venom were used as unpurified extracts dissected from parasitoid ovaries. DNA samples were digested with EcoRI, electrophoresed in 0.8% agarose and blotted onto a nitrocellulose filter. The blots shown were probed with a 3.7 kb clone (insert only) derived from a partial viral genomic library. This sequence hybridizes primarily to a single large (approx. 40 kb) relaxed circular molecule in uncut viral DNA profiles (not shown). (a) Lane 1, viral DNA; lane 2, larvae parasitized naturally by female \textit{C. melanoscela} wasps; lane 3, larvae injected with virus alone; lanes 4 to 8, larvae injected with virus, followed by injection of venom at 1, 3, 6, 12 and 24 h after the initial injection. (b) as in (a), except that manual injection of virus and venom together replaced natural parasitism (lane 2) and in lanes 4 to 8 larvae were injected with venom, followed by virus at 1, 3, 6, 12 and 24 h after the initial injection. Animals were sacrificed 24 h after the final injection.

Considered \textit{in toto}, the observations suggested that venom might be required at an early stage of virus penetration and/or uncoating and it was felt that an electron microscopic study might confirm this. Initially, we attempted to follow the infection pathway \textit{in vivo}, but considerable difficulty was experienced in routinely locating areas of tissue containing sufficient virus for analysis, especially in the case of experiments involving manual injection. This problem is at present unresolved and remains perplexing because no such difficulties were encountered during the course of an earlier study in which virus penetration was examined following natural parasitization (Stoltz & Vinson, 1977). The virus must presumably be taken up following manual injection, but we suspect that virus particles may be localized primarily in certain, as yet unidentified, areas of the host animal. If such areas existed, they could easily be missed by random dissection and sectioning protocols.
We were interested in eventually developing an \textit{in vitro} system and so concentrated on the gypsy moth cell line IPLB-LD-652Y which was originally established from pupal ovaries (R. H. Goodwin, personal communication). In preliminary studies, it was found that these cells supported polydnavirus transcription, but not replication. In addition, the spectrum of transcripts appeared to approximate that obtained \textit{in vivo} (Fig. 2). For these reasons, we considered that this cell line might have potential for use in experiments aimed at elucidating the early stages in polydnavirus infection. Using this cell line, we were readily able to observe the uptake of virus particles, and the effect of venom on this process.

No physical particle counts were carried out during the course of these experiments, nor is there a plaque assay for polydnaviruses (since they do not replicate \textit{in vitro}). Instead, conditions were established such that out of 100 cells exposed to virus plus venom, 90 would show at least one viral nucleocapsid at the nuclear envelope in sectioned material. Observation at the ultrastructural level strongly suggested that a large proportion of the polydnavirus particles were initially taken up by phagocytosis when venom was present (Fig. 3a). It seems likely that viral envelopes then fused with endosomal membranes thus releasing nucleocapsids directly into the cytoplasm. In some cases, nucleocapsids were observed near the cell periphery (Fig. 3b), suggesting that membrane fusion may also occur with the plasma membrane prior to phagocytosis. Uncoating subsequently occurred at nuclear pores (Fig. 3c to e). This pathway of infection is very similar to that known to occur \textit{in vivo}, with the exception that most insect tissues are not phagocytic, so membrane fusion presumably occurs most often at the cell surface (Stoltz & Vinson, 1977). In the absence of \textit{C. melanoscela} venom, nucleocapsids were never observed free in the cytoplasm or attached to nuclear pores, despite the fact that virus was readily endocytosed (not shown). These observations would appear to be in accord with those made using the \textit{in vivo} system from which we conclude that, in the absence of venom, viral DNA does not persist in host cells. The tissue culture studies allow us to suggest that venom may promote the persistence of viral DNA in both cases because it allows that DNA to reach cell nuclei.

The present study provides for the first time some indication of the stage at which venom may act to promote successful parasitism in certain braconid parasitoid/host systems. At present, we assume that venom acts to promote the release of viral nucleocapsids into the cytoplasm, this in turn leading to uncoating at nuclear pores. Further work will, however, be required in order to confirm that what is observed \textit{in vitro} is relevant \textit{in vivo}. Additional studies using these systems could usefully be directed towards an examination of the potential effects of venom on the acidification of the endosomal compartment and on membrane fusion. Further work will also be required in order to draw parallels between these \textit{in vivo} and \textit{in vitro} systems with greater confidence.
Fig. 3. Electron microscopy of virus uptake in IPLB-LD-652Y cells. Calyx fluid (and venom) from three female parasitoids was suspended in 0.5 ml of TC-100 medium and applied to cell monolayers in 30 ml flasks. After 1 h, an additional 2 ml of medium was added. Cells were processed for electron microscopy 2 h after treatment. In (a), virus particles have been endocytosed (arrowheads); bar marker represents 1.0 μm. In (b), several viral nucleocapsids are seen in the peripheral cytoplasm, while two have reached the nuclear envelope (arrowheads). Bar marker represents 0.2 μm. The montage provided in (c) to (e) represents a putative uncoating sequence; salient features are indicated by arrows. In (c), the filamentous nucleocapsid 'tail' structure (Stoltz & Vinson, 1977, 1979) attaches to a nuclear pore. Bar marker represents 0.2 μm. Partial and complete release of viral DNA, presumably through the tail, are shown in (d) and (e). N, nucleus; C, cytoplasm. Bar marker represents 0.2 μm for (d) and (e).
The persistence of polydnavirus DNA in host environments has recently been demonstrated for two different ichneumonids (Theilmann & Summers, 1986; Stoltz et al., 1986) and one braconid parasitoid (Stoltz et al., 1986 and present study). DNA persistence can reasonably be expected to be a prerequisite for the virus-specific transcription observed here and in previous studies on the ichneumonid virus CsV (Fleming et al., 1983; Blissard et al., 1986). It is not known at present whether input DNA persists intact, or whether the level of DNA which persists is determined by low-level turnover and synthesis.

It is important in the present context to point out that although venom may play an important role in at least some braconid parasitoid/host systems (Kitano, 1982; Guzo & Stoltz, 1985, 1987), a necessary function for ichneumonid venoms remains to be elucidated. Indeed, for the two best-studied ichneumonids, venom would appear not to be required for successful parasitism (Stoltz & Guzo, 1986; S. B. Vinson, personal communication). Thus it is at present impossible to ascribe any common function to ichneumonid venoms. What little is known concerning parasitoid venoms would in fact suggest that they consist of a rather complex mixture of polypeptides (see Beckage et al., 1987) and pharmacologically active substances (Beard, 1978) therefore an equivalent functional complexity should perhaps be expected.

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REFERENCES


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