Archives of Insect Biochemistry and Physiology 52:104–113 (2003)

Polydnavirus of *Campoletis chlorideae*: Characterization and Temporal Effect on Host *Helicoverpa armigera* Cellular Immune Response

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Polydnavirus was isolated from oviduct calyx in the parasitoid wasp *Campoletis chlorideae* (Hymenoptera: Ichneumonidae), and termed CcIV. The virus particles consist of fusiform nucleocapsids surrounded by two unit membrane envelopes. The DNAs purified from these viruses were found diversified in molecular weight and existed in nonequimolar concentrations. At least 20 different-sized bands were present after electrophoresis, and they ranged from approximately 3 to 26 kb. Persistence and gene expression of CcIV were examined in parasitized and virus-injected *Helicoverpa armigera* larvae. Viral DNA could be detected in the hemocyte of *H. armigera* at 30 min post-parasitization (p.p.), and persisted for 6 days. While no viral DNA increase was found, CcIV transcripts were first detected in host hemocytes at day 1 p.p. and continued for 5 days. Similar transcripts were observed in hemocytes from larvae that had been injected with calyx fluid or CcIV 24 h earlier. CcIV viral DNAs hybridized only with certain first-strand cDNAs from hemocytes, suggesting that only part of the CcIV genome was expressed in *H. armigera*. The pattern of CcIV gene expression was consistent with that of the inhibition of encapsulation for Sephadex G-10 and parasitoid eggs by host larvae. The recovery of host immune response at day 4 p.p. indicated that CcIV exhibited a partial and temporal effect on the host immune system and the developing parasitoid appeared to avoid encapsulation via different mechanisms. Arch. Insect Biochem. Physiol. 52:104–113, 2003.

KEYWORDS: polydnavirus; calyx fluid; *Campoletis chlorideae; Helicoverpa armigera*

INTRODUCTION

Polydnaviruses (PDVs) have been found only in parasitic Hymenoptera in the families Braconidae and Ichneumonidae (Stoltz and Whitfield, 1992; Stoltz, 1993). These viruses are characterized by double-stranded, superhelical DNA genomes which are heterogeneous in size (Stoltz et al., 1984). The polydnaviruses carried by braconid and ichneumonid wasps differ in their particle morphology, molecular characteristics, and envelope properties. Bracoviruses have more cylindrical nucleocapsids within a single unit membrane envelope and are released by lysis of calyx cells into the ovary. Ichnoviruses have fusiform nucleocapsids, each surrounded by two unit membranes and are extruded by budding from calyx cells within the ovary. Viral replication occurs only in the calyx region of the female wasp with the resulting suspension of polydnavirus and protein

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Contract grant sponsor: Innovation Program of Chinese Academy of Sciences; Contract grant number: KSCX2-SW-105; Contract grant sponsor: Major State Basic Research Project of China; Contract grant number: G2000016208; Contract grant sponsor: National Natural Science Foundation of China: Contract grant number: 30070517

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Received 3 May 2001; Accepted 19 October 2002

DOI: 10.1002/arch.10071
Published online in Wiley InterScience (www.interscience.wiley.com)

referred to as calyx fluid (Stoltz and Vinson, 1979). PDVs are injected along with eggs by parasitic wasps into their lepidopteran hosts during oviposition.

In both PDV genera, viral gene expression in the lepidopteran host could modify some host physiological systems, especially the immune response and endocrine system, for successful development of the endoparasitic wasp (Lavine and Beckage, 1995; Strand and Pech, 1995a). They destroy the hemocytes normally involved in encapsulation. *Microplitis demolitor* PDV (MdBV) enters *Pseudoplusia includens* granulocyte cells and rapidly induces apoptosis and, ultimately, causes the cells to die (Strand and Pech, 1995b). In some cases, PDVs alter host hemocyte behavior and disable them to spread on a substrate and prevent parasitoid eggs from being encapsulated (Davies et al., 1987; Lavine and Beckage, 1995; Strand and Pech, 1995a). In some species, immunosuppression has been reported for the duration of parasitism, whereas in others the effects may be transitory (Li and Webb, 1994; Asgari et al., 1996; Lavine and Beckage, 1996). Recent studies have concentrated on examining viral gene expression accompanied by host hemocyte dysfunction. Expression of a 1.6-kb MdBV occurred in hemocytes from a parasitized host at 2 h p.p. (Strand, 1994), and two MdBV mRNAs expressed in host larvae contained a cysteine-rich domain (Strand et al., 1997). *Cotesia kariyai* PDV was detected in all tested host larvae tissues and the hemocytes contained the most viral DNA and RNA (Hayakawa et al., 1994). In *Heliothis virescens* larvae parasitized by *Campoletis sonorensis*, the polydnavirus genes VHv1.1 and VHv1.4 produced proteins that disrupted the hemocyte cytoskeleton and reduced encapsulation of parasite eggs (Li and Webb, 1994; Cui et al., 1997).

Although the viral persistence and expression in parasitized hosts have been reported, the relationship of time accordance between PDV genes expression and host immunosuppression still remains to be determined. The ichneumonid *Campoletis chlorideae* is a key early larval parasitoid of cotton bollworm *Helicoverpa armigera* in the Huanghe River Valley of China. In the present study, we report that *C. chlorideae* PDV genes expressed abundantly during the first two days p.p. and the high expression was consistent with the parasitoid eggs free of encapsulation. It is suggested that CcIV protect eggs from host immune reactions during the early stages of parasitization, and the developing parasitoid larvae can avoid host immune response via other mechanisms.

MATERIALS AND METHODS

Insect

A colony of *C. chlorideae* was maintained in our laboratory. *H. armigera* larvae as host insects were reared on artificial diets at 27 ± 1°C and a 15-h light (L):9 h dark (D) photoperiod. For *C. chlorideae* oviposition, the late 2nd or early 3rd instar host larvae were stung by mated female wasps one or two times. The parasitized host larvae were kept individually at 27 ± 1°C and a 12 L:12 D photoperiod. After 6 to 7 days, *C. chlorideae* final instar larva emerged from the host and then immediately pupated in cocoon. The adult wasps were fed with a 10% honey-water solution. In the Southern blot hybridization experiment, unparasitized host larvae served as negative controls and adult female wasps served as positive controls.

Calyx Fluid Collection and Virus Purification

The reproductive tracts from 80 to 100 female wasps were dissected in Ringer's solution (128 mM NaCl,18 mM $CaCl_2$,1.3 mM Kcl, 2.3 mM $NaHCO_3$, pH 7.0) by gently pulling the ovipositor from the abdomen. The calyces were punctured with forceps and the contents were allowed to diffuse into Ringer's solution. This preparation was centrifuged (4,000*g*, 3min) to precipitate the eggs and tissue debris, and the supernatant was collected .The pellet was washed twice with Ringer's solution by centrifugation. The supernatant was combined and used as calyx fluid.

As for virus purification, the ovaries were homogenized and the coarse debris was removed by

centrifugation (8,000*g*, 3 min). The supernatant was transferred onto a 25 to 65% (wt/wt) continuous sucrose gradient, and centrifuged at 48,000*g* for 4 h at 4°C (Hitachi, P50). The virus band was collected, resuspended in Ringer's solution, and repelleted by centrifugation at 48,000*g* for 30 min. The resuspended virus pellet was stored at –75°C (Krell et al., 1982; Beckage et al., 1994). Quantities of calyx fluid and virus injected in experiments were based on female equivalents.

Extraction of Viral DNA and Agarose Gel Electrophoresis

Viral DNA was extracted from CcIV using a modified method described by Harwood et al. (1994).

The purified CcIV DNA or DNA digested with *Eco*R I, *Bss*H II, or *Hin*dIII were subjected to 0.7% agarose gels (Fleming et al., 1983; Hayakawa et al., 1994). *Eco*R I + *Hin*dIII fragments of λDNA were used as markers.

Isolation of DNAs and RNAs From *H. armigera* Hemocytes

For specific experiments, early and late 3rd-instar *H. armigera* were used for parasitization and injection, respectively. A dose of 0.5 female equivalent of calyx fluid or 1 equivalent of purified CcIV was used for injection according to the *H. armigera* larval body weight (Davies et al., 1987). An increased amount of purified CcIV was injected relative to calyx fluid to compensate for the potential loss of viable virus during purification.

For analysis of CcIV persistence and gene expression in *H. armigera* hemocytes, cohorts of 20 larvae were selected at designated times, 2 and 4 h, and 1, 2, 3, 4, 5, and 6 days p.p. or 24 h postinjection (Strand, 1994). Hemocytes were collected by bleeding larvae from a cut proleg into anticoagulant (62 mM NaCl,100 mM glucose,10 mM EDTA, 30 mM trisodium citrate, 26 mM citric acid, pH 4.6) and pelleting the cells at 800*g* for 5 min. DNAs were extracted as described for purification of CcIV DNA. Total RNAs were isolated using Trizol RNA extracting kit (Gibco-BRL). Integrity of the RNA samples was confirmed by visualization of ribosomal RNA on 0.8% agarose gels. Purified DNA was stored in TE (pH 8.0) at –20°C and RNA was stored in RNase-free water at –75°C.

cDNA Synthesis

First-strand cDNA were synthesized from RNA isolated from *H. armigera* hemocytes, with a cDNA synthesis kit (ThermoScript™RT – PCR system, Gibco-BRL). About 5 μg of total RNA isolated from *H. armigera* hemocytes were mixed with 1 μl primer (Oligo $(dT)_{20}$) in a 0.5-ml tube and the volume was adjusted to 10 μl with DEPC-treated water. RNA and primer were denatured by incubating at 65°C for 5 min, then placed on ice, 10 μl of master reaction mix was pipetted into each reaction tube. The sample was transferred to a DNA Thermal Cycler (Perkin Elmer 9600) and incubated for 1 h at 55°C. The reaction was terminated by incubating at 85°C for 5 min, then 1 μl RNase H was added, and finally the mixture was incubated for 20 min at 37°C. Synthesized cDNA was visualized in 0.8% agarose gels.

Southern Blot Hybridization

CcIV DNA was digested with *Eco*R I + *Hin*dIII restriction enzymes and labeled with digoxigenin using a DIG High Prime DNA Labeling and Detection Kit (Roche Molecular Biochemicals). cDNAs and 2 μg DNAs isolated from *H. armigera* hemocytes that were digested with *Eco*R I and *Hin*dIII were electrophoresed on a 0.8% agarose gel and transferred to nylon membrane. DIG-labeled CcIV DNA was used as a hybridization probe. Hybridization was done at 55°C in a hybridization solution containing 5 × SSC, 0.1% N-lauroylsarcosine (w/v), 0.02% SDS (w/v), and 1% blocking reagent (Boehringer Mannheim) for 15 h. Filters were washed twice (each 5 min at room temperature) with 2 × SSC, 0.1% SDS, then washed 2 × 15 min with 0.1% SSC, 0.1% SDS at 57°C. Annealed DNAs were detected using anti-digoxigenin alkaline phosphatase conjugate according to the manufacturer's

protocols. Alkaline phosphatase activity was detected by incubating the filters with chemiluminescent substrate CSPD, followed by exposure to X-ray film (Kodak XK-1).

In Vivo Encapsulation Experiments

Larvae were divided into three groups by the time of bead injection: 1 day p.p., 4 day p.p., and nonparasitized larvae that were used as control. Sephadex G-10 beads were stained in a 0.1% Congo Red solution to aid in recovery after injection (Lavine and Beckage, 1996). Three to five Sephadex beads in 2 µl of Ringer's solution were injected into each 3rd-instar host larva mentioned above. Larvae were then dissected 1 day later to assess encapsulation, and verify the presence of parasitoids in the hemocoeles of larvae that had been parasitized 1 and 4 days before bead injection. Twenty-five to 39 larvae were used in each group.

For egg injection, parasitoid eggs from calyx region were washed three times in Ringer's solution, then 2–3 eggs in 2 µl of the Ringer's solution were injected into each normal late 3rd-instar larva and the larva injected with 0.5 wasp equivalent calyx fluid one day earlier. The larvae were dissected on day 1 or day 4 post injection. The number of encapsulated eggs recovered from each larva was recorded. Twenty-three to 25 larvae for each treatment were dissected.

Two-tailed *t*-test was performed for testing the difference between the means of the treatment and the control.

RESULTS

Virus Purification and Characterization of Virus DNA

Sucrose density gradient centrifugation yielded a dense band of purified PDV at a gradient of 45%, and sometimes with a shallow band above the main band (Fig. 1). This shallow band appeared to consist of damaged particles and was not collected with the main band, since it could not be viewed by electron microscopy. Subjected to negative staining and viewed by transmission electron microscopy, the purified virus and calyx fluid showed many homogeneous virions containing one nucleocapsid surrounded by two membranes, and free of contamination (Fig. 2). The agarose gel electrophoretogram of DNA purified from virus particles demonstrated a heterogeneity of bands indicative of a segmented DNA genome similar to those described previously for other polydnaviruses (Fig. 3). At least 20 bands were present, and the

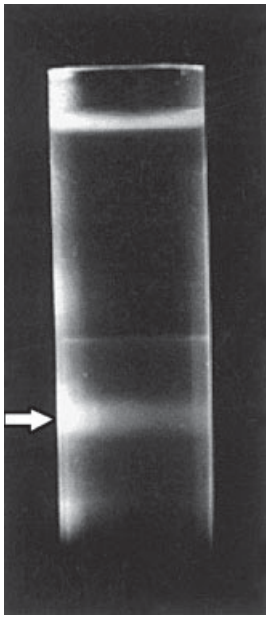

Fig. 1. CcIV band after 25–65% sugar gradient centrifugation. The arrow indicates the main band of CcIV.

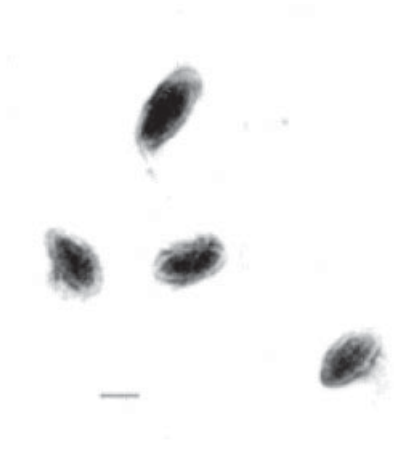

Fig. 2. Negative staining of CcIV purified from 25–65% sucrose gradient centrifugation. Scale bar = 100 nm.

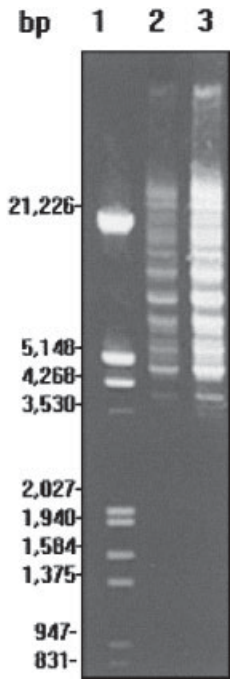

Fig. 3. Gel electrophoretic profiles of undigested CcIV DNA. **Lanes 2, 3:** Increasing quantities of CcIV DNA separated in a 0.7% agarose gel, stained with ethidium bromide, and visualized with UV light. Lane 1, MK; lane 2, 3.5 μg; lane 3, 5.0 μg).

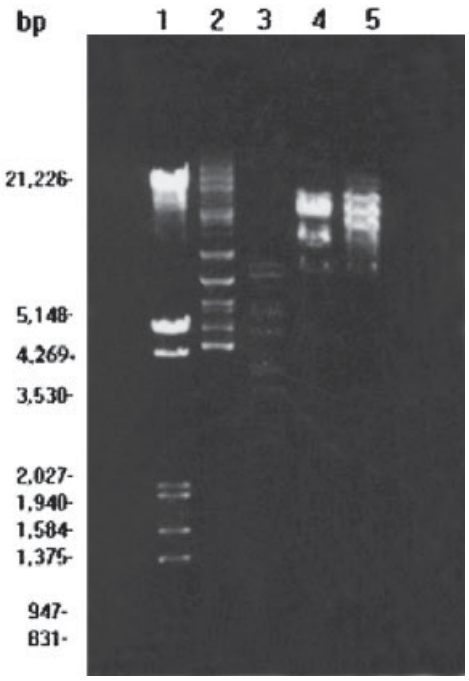

Fig. 4. CcIV DNA digested with restriction enzymes. Each preparation (1 μg) was subjected to 0.8% agarose gels. A mixture of *Eco*RI and *Hin*dIII-digested lambda DNA was used as molecular weight markers. **Lanes 1:** MK; **2:** CcIV-DNA; **3:** *Eco*RI-digested DNA; **4:** *Bss*HII-digested DNA; **5:** *Hin*dIII-digested DNA.

size range of these DNAs was approximately 3–26 kbp. The different sizes of the viral DNAs existed in nonequimolar concentrations. CcIV DNAs digested with *Eco*R I, *Bss*H II, or *Hin*dIII fractionated into approximately 30, 23, and 20 bands, respectively (Fig. 4). Assuming each restriction fragment is unique, the aggregate size of the CcIV genome was estimated to be 128.8 kb for *Eco*R I, 124.9 kb for *Bss*H II, and 133.4 kb for *Hin*dIII fragments.

Persistence of CcIV in *H. armigera* Hemocytes

To determine whether and when CcIV existed in hemocytes of parasitized larvae, DNA was isolated from host larvae hemocytes at various times and hybridized with DIG-labeled CcIV DNA. Southern blot analysis indicated that viral DNA persisted in host hemocyte through the course of parasitism. It showed CcIV infected host hemocyte at 30 min after oviposition by the wasp, and existed in the hemocyte until the parasitoid larvae emerged (Fig. 5). DIG-labeled CcIV DNA did not hybridize to DNA from *H. armigera* hemocyte, but hybridized strongly to DNA from *C. chlorideae* female wasps (Fig. 5). The sizes of hybridized fragments varied among 30 min, 2 h p.p., day 1 p.p., and day 3 p.p, but the DNA levels of hybridization did not increase during the whole course of parasitization., suggesting CcIV DNA did not replicate in its host.

Presence of CcIV Transcripts in *H. armigera* Hemocytes

To determine whether CcIV was genetically expressed in the parasitized host, cDNAs were synthesized from total RNA isolated from parasitized larvae hemocyte at sequential periods, and hybridized with DIG-labeled CcIV DNA. CcIV expression was most abundant at day 1 and day 2 p.p., and sharply declined from day 3 to day 5 p.p.; no hy-

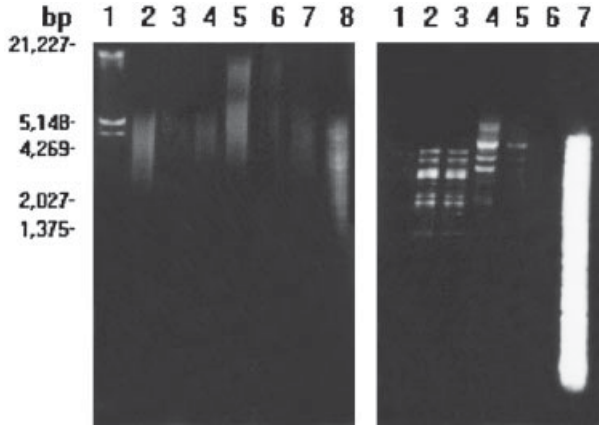

Fig. 5. Southern blot analysis of DNAs extracted from parasitized host hemocytes at various times. **Left:** DNAs (2 μg per lane) were digested with *Eco*RI and *Hin*dIII and separated on a 0.8% agarose gel. **Lanes 1:** MK; **2:** unparasitized host hemocyte DNA; **3:** 30min post-parazitization (p.p.); **4:** 2h p.p.; **5:** 1 d p.p.; **6:** 3 d p.p.; **7:** 6 d p.p.; **8:** female wasp DNA. **Right:** The gel was blotted onto a nylon membrane, and the membrane was hybridized with DIG-labeled CcIV DNA which was digested with *Eco*RI and *Hin*dIII (Right). **Lanes 1:** unparasitized host hemocyte DNA; **2:** 30 min p.p.; **3:** 2 h p.p.; **4:** 1 d p.p.; **5:** 3 d p.p.; **6:** 6 d p.p.; **7:** female wasp DNA.

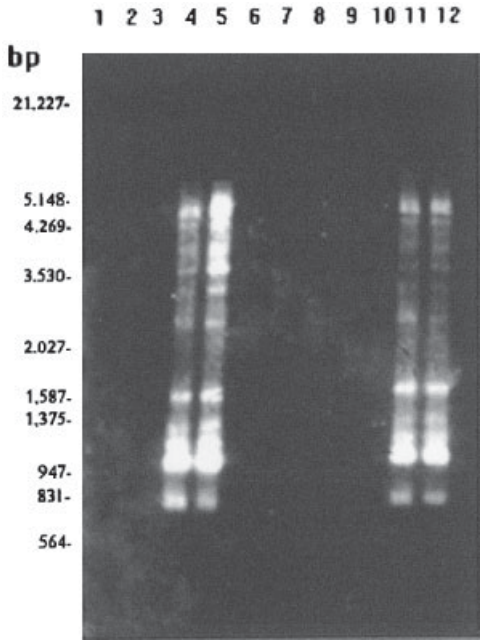

Fig. 6. Hybridization of first-strand cDNAs from hemocytes of parasitized and calyx fluid or CcIV-injected *H. armigera* larvae to CcIV DNA. cDNAs were synthesized from RNA (5 μg) isolated from *H. armigera* hemocytes at various times post parasitism or 24 h post injection of calyx fluid or CcIV. cDNAs were separated in 0.8% agarose gels, transferred to nylon membrane, and hybridized to DIG-labeled CcIV DNA, which was digested with *Eco*RI and *Hin*dIII. **Lanes 1:** unparasitized; **2:** 2 h p.p.; **3:** 4 h p.p.; **4:** 1 d p.p.; **5:** 2 d p.p.; **6:** 3 d p.p.; **7:** 4 d p.p. ; **8:** 5 d p.p.; **9:** 6 d p.p.; **10:** 24 h post Ringer's solution injected; **11:** 24 h post calyx fluid injected; **12:** 24 h post CcIV injected.

bridization signal was observed on day 6 p.p. (Fig. 6). Similar hybridization results were obtained in hemocytes from hosts that had been injected 24 h previously with calyx fluid or purified CcIV (Fig. 6). In the controls, DIG-labeled CcIV DNA did not hybridize to cDNA from unparasitized larvae and larvae injected with Ringer's solution (Fig. 6). The transcripts, therefore, detected with cDNAs synthesized from RNAs of parasitized larvae, were virus specific. Although approximately 32 fragments of CcIV DNA were observed in ethidium bromide-stained *Eco*R I + *Hin*dIII digests, only 11 fragments ranging in size from 0.6 to 7 kb were hybridized with cDNAs from hemocytes of parasitized larvae (Fig. 6).

Encapsulation Responses Following Bead and Egg Injections

Only 4% of Sephadex G-10 beads were encapsulated in *H. armigera* at day 1 p.p. while about 90% beads were encapsulated in day 4 p.p. (Table 1). At day 1 p.p., the encapsulation rate was significantly decreased compared with that in unparasitized larvae ($P < 0.01$); but at day 4 p.p. the encapsulation rate had no significant difference.

TABLE 1. Encapsulation of Injected Sephadexes in Third Instar *H. armigera* Larvae Parasitized by *C. chlorideae* and Examined at 24 H Post-Injection*

Treatment	No. of Sephadex beads injected	Encapsulation rate (%)	Significance
1 d p.p.	24	4.2	$P < 0.01$
4 d p.p.	39	89.7	Ns
Control (unparasitized)	25	100	

*P values are for a two-tailed t-test of significance between the means of the treatments and the control.

TABLE 2. Encapsulation of Washed Eggs Injected With or Without Wasp Calyx Fluid in Third Instar *H. armigera* Larvae*

	Eggs injected with calyx fluid			Eggs injected without calyx fluid		
Treatment	No. eggs injected	Encapsulation rate (%)	Significance	No. eggs injected	Encapsulation rate (%)	Significance
1 d post injection	23	0	$P < 0.01$	51	9.8	$P < 0.01$
4 d post injection	25	40		17	88.2	

**P* values are for a two-tailed *t*-test of significance between two treatments.

When eggs washed three times in Ringer's solution were injected into normal host larvae, about 9.8 and 88% were encapsulated at day 1 and day 4 post-injection, respectively. However, when 0.5 wasp equivalent calyx fluid existed in host larvae, no encapsulation of eggs washed in Ringer's solution was observed at day 1 post injection, and 40% of them were encapsulated at day 4 post injection (Table 2). Whether there is calyx fluid or not, the encapsulation rate increase between larvae of day 1 and day 4 post-injection was significant (Table 2; $P < 0.01$).

DISCUSSION

Polydnaviruses from ichneumonid and braconid endoparasitic wasps may exert multiple effects on wasp hosts, especially on immune and endocrine systems. Although 50 PDV species have been described systematically, CcIV from *C. chlorideae* has not been characterized before. *C. chlorideae* is the main natural enemy of cotton pest *H. armigera* in North China. The immune adaptation of this parasitoid to host mediated by polydnavirus has not been studied yet. Our study has found that the calyx region of *C. chlorideae* contained abundant PDVs, and the morphology and genomic organization of CcIV were similar to that of other ichnoviruses reported previously. Compared with its homogeneous species, *C. sonorensis* PDV, which has been most extensively studied at the molecular level, is comprised of at least 28 DNA fragments (Blissard et al., 1986a), while the genome of CcIV contains 20–21 segmented DNA fragments with a size range of approximately 130 kb.

Thirty minutes after parasitization, CcIV viral DNA existed in host hemocytes and continued for 6 days, but the amount of viral DNA was not increased throughout the time. This suggested that CcIV persist in host larvae without any detectable level of viral replication, just like the results on *C. sonorensis* (Theilmann and Summers, 1986), *M. demolitor* (Strand et al., 1992), and *C. kariyai* (Hayakawa et al.,1994) previously reported. The sizes of hybridized fragments that varied between 30 min, 2 h p.p. and day 1, day 3 p.p. probably suggest viral DNA was integrated into *H. armigera* genomic DNA after parasitization for 1 day and 3 days. The restriction enzymatic sites of DNAs isolated from host hemocytes at various times p.p. were different between integrated and nonintegrated genomes when digested with *Eco*R I and *Hin*dIII. It has been reported that polydnavirus genome from the braconid *Glyptapanteles indiensis* (GiBV) integrated in vitro into the genome of cells derived from the natural host *Lymantria dispar* (McKelvey et al., 1996; Gundersen-Rindal and Dougherty, 2000). Whether CcIV DNA could integrate into host chromosome remains to be determined.

It is documented that polydnavirus-induced significant changes in host cellular immune system. Since viral transcription has been observed in host after parasitization (Blissard et al., 1986a,b; Strand et al., 1992; Hayakawa et al., 1994; Trudeau and Strand, 1998; Cui et al., 2000), the expression time and pattern vary in many polydnavirus-host systems. For CsIV, *C. sonorensis* developed endoparasitically and at least 12 viral mRNAs were detected (Fleming et al., 1983; Blissard et al., 1986b). CsIV mRNAs were most abundant from 12 to 48 h p.p. The number of Cs IV mRNAs detected varied at the different time points (Blissard et al., 1986b). A 1.6-kb mRNA was the predominant transcript. During the times examined from 2 h to day 9 p.p. most viral mRNAs appeared to be present in the same relative quantities (Blissard et al., 1986b). Here we report that in *H. armigera* hemocytes, CcIV expression was most abundant in the first 2 days of parasitism and continued for 5

days, but the expression declined sharply after day 2 p.p. The most abundant CcIV mRNAs expressed were almost the same at the times examined. The 0.9-kb mRNA was most abundantly expressed. This may suggest the nesting of the segments in the polydnavirus genomes (Webb and Cui, 1998). At least 11 viral mRNAs can be detected on day 1 and day 2 p.p. Similar transcripts were also observed in hemocytes of host injected with calyx fluid or purified virus 24 h earlier, indicating that viral activity was independent of the presence of a developing parasitoid. Hybridization of cDNAs from host hemocytes with CcIV restriction fragments also indicated that only part of the CcIV genes was expressed in the parasitized host and was involved in the alteration of host physiology after parasitization. Although in many host tissues polydnavirus has been detected, the main target that the virus infected is hemocyte. It has been reported that the high levels of MdBV expression were correlated with the disruption of host hemocyte spreading, and might suppress host encapsulation response (Strand, 1994; Trudeau and Strand, 1998). We found that CcIV expression was most abundant in the first two days of parasitism, and this was consistent with the results of encapsulation of Sephadex after parasitization. At 24 h after parasitization, *H. armigera* larva showed a strong suppression of their encapsulation response to Sephadex G-10 beads, while the beads were encapsulated in hosts parasitized for 4 days. Thus, we suggest that the effect of CcIV on host immunity is temporal and limited. CcIV may suppress host immune system at early stages p.p. to protect parasitoid eggs from encapsulation. When the larva has emerged in the host, the parasitoid perhaps escapes from encapsulation by other mechanisms. It has been proved Braconids *C. congregata* and *Cardiochiles nigriceps* secrete proteins to modulate the host immune response (Beckage, 1993; Vinson et al., 1994). Calyx fluid (0.1 or 0.5 female equivalent) injected 24 h earlier failed to suppress the encapsulation of transplanted Sephadex, but could successfully suppress the encapsulation of washed eggs. This implies that CcIV suppressed host immune response through a co-operation with other factors, such as surface molecules of the parasitoid eggs.

It is clear that PDV is required to protect parasitoid eggs by suppressing the host immune system. Other factors such as venom (Tanaka, 1987) and ovarian proteins (Webb and Luckhart, 1994, 1996; Luckhart and Webb, 1996) may also be required in some species in the very earliest stages before viral genes are expressed. However, the temporal effect of PDV on host immune response and the partial recovery of host immune function have been demonstrated here and elsewhere (Stoltz and Guzo, 1986; Lavine and Beckage, 1996). *Hyposoter fugitivus* ichnovirus suppresses cellular immunity in *Malacosoma disstria*, but a degree of cellular immunity returns in 1 to 2 days after hatching of the parasitoid egg and the resurgent encapsulation response appears to be selective, since parasitoid larvae remain unaffected (Stoltz, 1993). Similar examples of this type of interaction were also found by Vinson (1972). Apart from the cellular immunity, the phenoloxidase (PO) activity was also affected after parasitism and injection with calyx fluid. These effects were consistent with CcIV gene expression. PO activity in hemolymph of *H. armigera* was strongly depressed at the early stages of parasitization and slightly recovered at the late stage (Yin et al., 2001). Since exposure of host insects to a pathogen has a detrimental effect on parasitoid survival (Brooks, 1993; Lavine and Beckage, 1996), a partial return of the host immune response would ensure the *C. chlorideae* of developing successfully in the host hemocoele.

ACKNOWLEDGMENTS

We express our appreciation to Prof. Aaron Weinberg of Case Western Reserve University and Prof. He Gong for reviewing the manuscript. We thank Dr. Zhimin Feng for helpful discussion, Shengjiang Tan for the assistance of the molecular experiment, and Junfeng Dong for help in maintaining the parasitoid colony. This work was supported by grants from the Innovation Program of Chinese Academy of Sciences (KSCX2-SW-105), the Major State Basic Research Program of China (G2000016208), and National Natural Science Foundation of China (30070517) to C.W.

LITERATURE CITED

Asgari S, Hellers M, Schmidt O. 1996. Host hemocyte inactivation by an insect parasitoid: Transient expression of a polydnavirus gene. J Gen Virol 77:2653–2662.

Beckage NE. 1993. Games parasites play: the dynamic roles of proteins and peptides in the relationship between parasite and host. In: Beckage NE, Thompson SN, Federici BA, editors. Parasites and pathogens of insects. New York: Academic Press. p 25–57.

Beckage NE, Tan FF, Schleifer KW, Lane RD, Cherubin LI. 1994. Characterization and biological effects of *Cotesia congregata* polydnavirus on host larvae of the tobacco hornworm, *Manduca sexta*. Arch Insect Biochem Physiol 26:165–195.

Blissard GW, Fleming JGW, Vinson SB, Summers MD. 1986a. *Campoletis sonorensis* virus: expression in *Heliothis virescens* and identification of expressed sequences. J Insect Physiol 32:351–359.

Blissard GW, Vinson SB, Summers MD. 1986b. Identification, mapping and in vitro translation of *Campoletis sonorensis* virus, mRNAs from parasitized *Heliothis virescens* larvae. J Virol 57:318–327.

Brooks W.M. 1993. Host-parasitoid-pathogen interactions. In: Beckage NE, Thompson SN, Federici BA, editors. Parasites and pathogens of insects. New York: Academic Press. p 231–272.

Cui LW, Soldevila AI, Webb BA. 1997. Expression and hemocyte-targeting of a *Campoletis sonorensis* polydnavirus cysteine-rich gene in *Heliothis virescens* larvae. Arch Insect Biochem Physiol 36:251–271.

Cui LW, Soldevila AI, Webb BA. 2000. Relationships between polydnavirus gene expression and host range of the parasitoid wasp *Campoletis sonorensis*. J Insect Physiol 46:1379–11407.

Davies DH, Strand MR, Vinson SB. 1987. Changes in differential hemocyte count and in vitro behaviour of plasmatocytes from host *Heliothis virescens* caused by *Campoletis sonorensis* polydnavirus. J Insect Physiol 33:143–153.

Fleming JGW, Blissard GW, Summers MD, Vinson SB. 1983. Expression of *Campoletis sonorensis* virus in the parasitized host, *Heliothis virescens*. J Virol 48:74–78.

Gundersen-Rindal D, Dougherty EM. 2000. Evidence for integration of *Glyptapanteles indientis* polydnavirus DNA into the chromosome of *Lymantria dispar* in vitro. Virus Res 66:27–37.

Harwood SH, Grosovsky AJ, Cowles EA, Davis JW, Beckage NE. 1994. An abundantly expressed hemolymph glycoprotein isolated from newly parasitized *Manduca sexta* larvae is a polydnavirus gene product. Virology 205:381–392.

Hayakawa Y, Yazaki K, Yamanaka A, Tanaka T. 1994. Expression of polydnavirus genes from the parasitoid wasp *Cotesia kariyai* in two noctuid hosts. Insect Mol Biol 3:97–103.

Krell P J, Summers M D, Vinson S B. 1982. Virus with a multipartite superhelical DNA genome from the Ichneumonid parasitoid *Campoletis sonorensis*. J Virol 43:859– 870.

Lavine MD, Beckage NE. 1995. Polydnaviruses: potent mediators of host insect immune dysfunction. Parasitol Today 11:368–378.

Lavine MD, Beckage NE. 1996. Temporal pattern of parasitism-induced immunosuppression in *Manduca sexta* larvae parasitized by *Cotesia congregata*. J Insect Physiol 42:41–51.

Li X, Webb BA. 1994. Apparent functional role for a cysteine-rich polydnavirus protein in suppression of the insect cellular immune response. J Virol 68:7482–7489.

Luckhart S, Webb BA. 1996. Interaction of a wasp ovarian protein and polydnavirus in host immune suppression. Dev Comp Immunol 20:1–21.

McKelvey TA, Lynn DE, Gundersen-Rindal D, Guzo D, Stoltz DA, Guthrie KP, Taylor PB, Dougherty EM. 1996. Transformation of Gypsy Moth (*Lymantria dispar*) cell lines by infected with *Glyptapanteles indiensis* polydnavirus. Biochem Biophys Res Commun 225:764–770.

Stoltz DB. 1993. The polydnavirus life cycle. In: Beckage NE, Thompson SN, Federici BA, editors. Parasites and pathogens of insects. New York: Academic Press. p 167–187.

Stoltz DB, Guzo D. 1986. Apparent haemocytic transformations associated with parasitoid induced inhibition of immunity in *Malacosoma disstria* larvae. J Insect Physiol 32:377–388.

Stoltz DB, Vinson SB. 1979. Viruses and parasitism in insects. Adv Virus Res 24:125–171.

Stoltz DB, Whitfield JB. 1992. Viruses and virus-like entities in the parasitic hymenoptera. J Hymenoptera Res 1:125–139.

Stoltz DB, Krell PJ, Summers MD, Vinson SB. 1984. Polydnaviridae: A proposed new family of insect viruses with segmented, double-stranded, circular DNA genomes. Intervirology 21:1–4.

Strand MR. 1994. *Microplitis demolitor* polydnavirus infects and expresses in specific morphotypes of *Pseudoplusia includens* hemocytes. J Gen Virol 75:3007–3020.

Strand MR, Pech LL. 1995a. Immunological basis for compatibility in parasitoid-host relationships. Ann Rev Entomol 40:31–56.

Strand MR, Pech LL. 1995b. *Microplitis demolitor* polydnavirus induces apoptosis of a specific hemocyte morphotype in *Pseudoplusia includens*. J Gen Virol 76:283–291.

Strand MR, Mackenzie DI, Grassl V, Dover BA, Aiken JM. 1992. Persistence and expression of *Microplitis demolitor* polydnavirus in *Pseudoplusia includens*. J Gen Virol 73: 1627–1635.

Strand MR, Witherell RA, Trudeau D. 1997. Two *Microplitis demolitor* polydnavirus mRNAs expressed in hemocytes of *Pseudoplusia includens* contain a common cysteine-rich domain. J Virol 71:2146–2156.

Tanaka T. 1987. Effect of the venom of the endoparasitoid, *Apanteles kariyai*, Watanbe, on the cellular defense reaction of the host, *Pseudaletia separata* Walker. J Insect Physiol 33:413–420.

Theilmann DA, Summers MD. 1986. Molecular analysis of *Campoletis sonorensis* virus DNA in the lepidopteran host *Heliothis virescens*. J Gen Virol 67:1961–1969.

Trudeau D, Strand MR. 1998. A limited role in parasitism for *Microplitis demolitor* polydnavirus. J Insect Physiol 44: 795–805.

Vinson SB. 1972. Factors involved in successful attack on *Heliothis virescens* by the parasitoid *Cardiiochiles nigriceps.* J Invertebr Pathol 20:118–123.

Vinson SB, Mourad AK, Sebesta DK. 1994. Sources of possible host regulatory factors in *Cardiochiles nigriceps* (Hymenoptera: Braconidae). Arch Insect Biochem Physiol 26:197–209.

Webb BA, Cui LW. 1998. Relationships between polydnavirus genomes and viral gene expression. J Insect Physiol 44:785–793.

Webb BA, Luckhart S. 1994. Evidence for an early immunosuppressive role for related *Campoletis sonorensis* venom and ovarian proteins in *Heliothis virescens*. Arch Insect Biochem Physiol 26:147–163.

Webb BA, Luckhart S, 1996. Factors mediating short- and long-term immune suppression in a parasitized insect. J Insect Physiol 42:33–40.

Yin LH, Wang CZ, Qin JD. 2001. Effect of the endoparasitoid *Campoletis chloridiae* on phenoloxidase activity in *Helicoverpa armigera* hemolymph. Chin Sci Bull 46:1303–1306.