Polydnavirus particle proteins with similarities to molecular chaperones, heat-shock protein 70 and calreticulin

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INTRODUCTION

Insect parasitoid wasps produce virus-like particles in specialized calyx cells situated in their ovaries. The particles, known as polydnaviruses (PDVs), contain circular DNA segments excised from the wasp genome during their production and assembly in the calyx cells (Stoltz, 1990). Ichneumonid wasps produce ovoid-shape particles, whereas particles associated with braconid wasps are cylindrical in shape with a tail-like structure (Webb, 1998). At parasitization, the particles are injected into the host larvae together with the parasitoid egg. Inside the host, they enter various host tissue cells, where the packaged genes are expressed. It is known that some of the protein products interfere with host physiology (Beckage, 1998). This includes suppression of the host immune system resulting in the failure of host haemocytes to mount an encapsulation response towards the parasitoid egg. The particle-derived gene expression might be persistent during the course of parasitization (Strand et al., 1992) or transient (Asgari et al., 1996). There is no replication of particle DNA inside the host caterpillar (Webb, 1998).

The general view is that these particles have originated from a pathogenic ancestral virus(es) that has lost some viral properties and become part of the wasp genotype during evolution. Recent discussions on the endosymbiotic relationship of virus and wasps (Whitfield & Asgari, 2002) suggest that PDVs may constitute vehicles for the transfer of genetic information from the wasp to the host. In this scenario, genes encoded by the circular DNA include proteins involved in host manipulation, whereas genes encoding structural particle proteins are not circularized.

The GenBank accession numbers for the sequences reported in this paper are AY150370 for CrCRT and AY150371 for CrHs70.

Fig. 1. Western blot analysis of purified CrPDV particle proteins. Particles were purified from five female wasps and analysed on a Western blot (12% SDS-PAGE) using antibodies raised against the purified particles. Several proteins were detected by the antibodies. The locations of the proteins with similarities to heat-shock protein 70 (CrHs70) and calreticulin (CrCRT) associated with the particles are indicated by arrows. The location of Crp32 described previously is also shown. MW, molecular mass marker.
and are mainly expressed during particle production. To identify the origin of PDVs, it is therefore essential to isolate and characterize proteins that are a structural part of PDVs. The genes expressed from PDV DNA in the host that have been identified so far do not show any similarity to other known viruses.

Since circular DNAs from PDVs appear to lack genes encoding structural virus proteins, we used antibodies against purified PDVs from the wasp Cotesia rubecula to perform immune screens of peptides from cDNA fragments from wasp ovaries. These screens revealed particle proteins from the wasp C. rubecula that had similarities to calreticulin and heat-shock protein 70 (Hsp70) (CrCRT and CrHS70, respectively). The association of chaperones with virus production and assembly has been explored in various systems (Sullivan & Pipas, 2001). Calreticulin, found mainly in the endoplasmic reticulum (ER), is a widely expressed calcium-binding protein that acts as a molecular chaperone (Michalak et al., 1992). However, calreticulin has also been localized in other cellular compartments, such as in the cytoplasm and nucleus, the cell surface and as secreted forms. There is growing evidence suggesting that calreticulin may mediate a broader array of cellular functions.

METHODS

Insect cultures and isolation of C. rubecula PDVs (CrPDVs). The parasitoid C. rubecula and its host were maintained at 25˚C, on a 14/10 h (light:dark) photoperiod. Pieris rapae larvae were reared on cabbage plants. Adult C. rubecula wasps were fed with honey-water solution.

Polydnaviruses were purified from female wasps as described previously (Beckage et al., 1994). Briefly, isolated ovaries were macerated in PBS using micro-scissors followed by centrifugation at 750 g for 5 min to exclude large cellular debris. The homogenate was then passed through a 0.45 µm filter (Ministart; Sartorius) using a 3 ml syringe. Viral particles were pelleted by centrifuging the filtrate at 12 000 g for 20 min and resuspended in PBS.

Western blotting and elution of antibodies. Protein samples were separated by 10 or 12 % SDS-PAGE (Laemmli, 1970) and transferred on to nitrocellulose membranes as described (Sambrook

Fig. 2. For legend see page 1167.
Fig. 2. Nucleotide sequence and deduced amino acid sequence of cDNAs encoding for (A) a calreticulin and (B) a heat-shock protein 70 from C. rubecula ovaries associated with CrPDV particles. The numbers of nucleotides and amino acid residues are shown at the end of each line. In each sequence, a putative initiation codon (ATG) is shown in bold and the stop codon is underlined. The conserved ER retention signal found in calreticulins is boxed in (A). Putative cleavage sites are indicated by arrows in both sequences.
et al., 1989). Anti-CrPDV (1 : 5000) (Asgari & Schmidt, 1994), anti-Hsp70 (see below) and anti-calreticulin (1 : 3000; B. L. Lee, Pusan National University, South Korea) antibodies were used as probes followed by detection with alkaline phosphatase-conjugated anti-rabbit IgG antibodies (1 : 5000).

To obtain specific anti-Hsp70 antibodies, purified phages containing the gene encoding CrHs70 were plated and induced for protein production by overlaying with an IPTG-soaked membrane. The expressed proteins transferred on to the membrane were probed with anti-CrPDV antibodies (1 : 5000) and then detected by a secondary antibody. The anti-Hsp70 antibodies were eluted from the membrane as described (Smith & Fisher, 1984). Eluted antibodies were used (without dilution) in a Western blot containing purified CrPDVs to confirm the presence of Hsp70 in the particles.

**Isolation of RNA and slot blotting.** Total RNA was extracted from ovaries and wasp carcasses devoid of ovaries as described (Chomczynski & Sacchi, 1987). RNA (3 μg) samples were blotted on to a nylon membrane under denaturing conditions according to the manufacturer’s instructions (BioRad). The blot was hybridized with a 32P-labelled PCR product encoding CrCRT for 20 h at 65°C. A P. rapae 18S ribosomal DNA fragment probe (R. Glatz, unpublished) was used as a control.

**Screening a cDNA library using anti-CrPDV antibodies.** A C. rubecula ovary/venom gland expression cDNA library was constructed using a SMART cDNA library construction kit (Clontech) according to the manufacturer’s instructions. Plaques on plates (2.5 × 10⁴ in total) were induced for protein expression by overlaying plates with nitrocellulose membranes soaked in 10 mM IPTG and dried. Induction was carried out at 42°C for 4 h. Membranes were removed and washed in TBST for 5 min. Membranes were then blocked for 1 h in milk blocking solution to which anti-CrPDV antibodies had been added (1:5000). Positive plaques were detected by a secondary antibody and isolated for rescreening. Confirmed positives were recircularized as plasmids according to the manufacturer’s instructions (Clontech). Inserts were sequenced in both directions.

**RESULTS AND DISCUSSION**

Purified particles (PDVs) from parasitoid wasps usually have a complex protein profile when analysed by SDS-PAGE. C. rubecula particles analysed on a Western blot using antibodies raised against the purified particles (Asgari & Schmidt, 1994) revealed an array of proteins that are a structural part of or associated with the particles (Fig. 1). Previously, we identified a 32 kDa protein (Crp32) as the major protein found in C. rubecula particles (Asgari et al., 1998) (see Fig. 1). In order to isolate other proteins from these particles, an expression CDNA library was constructed from venom gland and ovary of C. rubecula female wasps.

![Fig. 3. Amino acid sequence alignment of CrCRT with calreticulins from Galleria mellonella (GenBank accession no. BAB79277) and Anopheles gambiae (accession no. AAL68781). The conserved ER retention signal (HDEL) is shown by asterisks. Sequence similarity of the proteins to CrCRT is 73-8 and 69-2%, respectively.](image-url)
Antibodies raised against purified particles were used as a probe to screen the library (\( \sim 5 \times 10^4 \) plaques). Several positives were obtained, rescreened and sequenced in both directions. Two of these clones were identified as CrCRT and CrHs70.

**Calreticulin**

The longest cDNA obtained encoding CrCRT was 1367 bp in length encoding 403 amino acids. A methionine at position 1 was identified as the putative initiation site (Cavener & Ray, 1991) and a stop codon at position 1210 (Fig. 2A). Using PSORT II (http://psort.nibb.ac.jp/form2.html), a putative cleavage site was found between amino acids 18 and 19, consistent with other calreticulins (Fig. 2A). At the end of the C domain, a conserved HDEL motif was identified (Fig. 3). In mammals, a KDEL sequence is conserved and believed to be an ER retention signal. However, calreticulins isolated so far from insects all have an HDEL sequence instead. Sequence alignment in GenBank showed a high similarity to the Hsp70 and Hsc70 proteins from various organisms. The highest similarities were to *Rivulus marmoratus* (Teleostei, 83.4 %) and *Artemia franciscana* (Crustacea, 84.3 %) (Fig. 5). The predicted size for CrHs70 is 71.4 kDa. Western blot analysis using specific anti-Hsp70 antibodies recognized a protein of approximately 71 kDa in size in purified *C. rubecula* particles (Fig. 4B).

Proteins belonging to the Hsp70 family have been detected in association with several viruses involved in viral assembly and transfer (Sullivan & Pipas, 2001). It has also been shown that members of this family are specifically incorporated in human immunodeficiency virus type 1 (HIV-1) virions (Gurer et al., 2002). Several roles for the incorporated proteins have been suggested, including involvement in

![Fig. 4](image)

**Heat-shock protein 70**

The cDNA obtained for CrHs70 was 2086 bp in length encoding 656 amino acids (Fig. 2B). A putative cleavage site was found between amino acids 16 and 17 using PSORT II (Fig. 2B). Sequence homology searches revealed a high similarity to the Hsp70 and Hsc70 proteins from various organisms. The highest similarities were to *Galleria mellonella* (73.8 %) and *Anopheles gambiae* (69.2 %) calreticulins (Fig. 3). The predicted size for CrCRT is 46.5 kDa with a pI of 4.40, although calreticulin usually runs atypically at 60 kDa on SDS-PAGE (Opas et al., 1996; Spiro et al., 1996). Antibodies raised against *G. mellonella* calreticulin were used as a probe in a Western blot containing purified *C. rubecula* particles. This confirmed that calreticulin is indeed associated with these particles and also runs at 60 kDa (Fig. 4A). Slot blot analysis also showed that CrCRT is highly expressed in the ovaries compared with the rest of the body (Fig. 4C).
assembly, transport to the plasma membrane and in early events during infection of HIV-1, assisting in uncoating of viral capsids. Although the role of CrHs70 in CrPDVs requires further investigation, similar involvements can be envisaged in PDVs.

Apart from mediating correct assembly of proteins, these proteins may also be involved in several other cellular functions, such as protein transport across membranes and vesicle transport along microtubules and virion assembly. The expression of molecular chaperones is usually upregulated during virus infection and replication, probably not as a result of stress due to infection, but rather as a requirement for some viral functions. Certain viruses encode their own specific chaperones, whereas others rely on the host cellular chaperones. More studies are required to

Fig. 5. Amino acid sequence alignment of CrHs70 with Hs70 proteins from *Rivulus marmoratus* (Teleostei, AAF71255) and *Artemia franciscana* (Crustacea, AAL27404). The GenBank accession numbers are in brackets. Sequence similarity of the proteins to CrHs70 is 83-4% and 84-3%, respectively.

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elucidate the functional role of these molecules in polydnavirus biology. However, since PDVs cannot be replicated in vitro (even their in vivo replication is atypical of a virus), a possible role of these proteins in virus production is not possible using traditional assays. Moreover, it remains to be seen how conserved these structural proteins are within the virus families to pinpoint the closest relatives and ancestral origins of these unique particles.

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REFERENCES


