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Proteins Associated with *Culex nigripalpus* Nucleopolyhedrovirus Occluded Virions▽

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Occlusion-derived virions (ODVs) of the nucleopolyhedrovirus of *Culex nigripalpus* (CuniNPV) were purified by Ludox density gradient ultracentrifugation, and the proteins were separated by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Proteins were identified by using Edman sequencing, matrix-assisted laser desorption ionization–time of flight mass spectrometry, nanoelectrospray quadrupole time-of-flight mass spectrometry, or a combination of these methods. Half of the 44 polypeptide sequences identified in this analysis were unique open reading frames (ORFs) encoded by the CuniNPV genome and did not show similarity to any other sequences present in protein databases. Of the 22 polypeptides that showed similarities to other baculovirus-encoded proteins, only 17 sequences have previously been identified as structural proteins. The newly identified CuniNPV structural proteins cun058, cun059, cun087, cun106, and cun109 are homologues of *Autographa californica* nucleopolyhedrovirus (AcMNPV) ORFs 68, 62, 98, 81, and 2, respectively. The products of four genes, namely, lef-1 (cun045), alkaline exonuclease (cun054), helicase (cun089), and DNA polymerase (cun091), were not detected in the CuniNPV ODV preparations. These four genes are conserved among all annotated baculovirus genomes, and their homologues have been detected in the ODV of AcMNPV.

Baculoviruses that infect mosquitoes are of growing interest since they are currently believed to represent a separate branch within the *Baculoviridae* that existed prior to the split of lepidopteran nucleopolyhedroviruses and granuloviruses (8, 15, 19). These baculoviruses may also be basal to the baculoviruses from hymenoptera, which form a branch distinct from the lepidopteran baculoviruses (11).

Culex nigripalpus nucleopolyhedrovirus (CuniNPV) is highly pathogenic to *Culex* sp. mosquitoes, which are important vectors of the West Nile, St. Louis, and Eastern encephalitis viruses. This virus is responsible for epizootics in field populations of *C. nigripalpus* larvae (6). CuniNPV development is restricted to the nuclei of midgut epithelial cells in the gastric ceca and posterior stomach (6, 22). It has two virion phenotypes: the occlusion-derived virion (ODV) that initiates infection in the midgut epithelial cells and a budded form that spreads the infection within the midgut (22). The genome of CuniNPV contains 109 open reading frames (ORFs), and only 36 of these show similarities to the ORFs of other nucleopolyhedroviruses (2).

Transmission of CuniNPV ODVs to mosquito larvae is facilitated by divalent cations: magnesium is essential for horizontal transmission, whereas the presence of calcium inhibits virus transmission (6). Cascading events are required for infection of a host with a baculovirus. A typical sequence consists of the following: (i) the virions are released from occlusion bodies (OBs) in the insect gut, (ii) the virions attach to and cross the peritrophic matrix, and (iii) the virions attach and enter midgut epithelial cells and nuclei where replication occurs. Envelope proteins of the ODV are of particular importance in this process, since they interact with the mosquito peritrophic matrix, as well as with the plasma membrane of the midgut epithelial cells. Identifying the structural proteins of the CuniNPV ODV with particular emphasis on the envelope proteins will provide insight into the biology of CuniNPV and will allow investigation of host specificity and virulence (9).

One widely used method for resolving proteins from complex mixtures for subsequent identification by mass spectrometry (MS) is two-dimensional polyacrylamide gel electrophoresis (PAGE), wherein proteins are separated by their molecular weights and isoelectric points (29). However, this technique has proven to be challenging and time-consuming in terms of method development and is limited due to the under-representation of certain protein components such as membrane and extremely acidic or basic proteins (24). An alternate method for rapid identification of proteins from complex mixtures is the use of liquid chromatography (LC) separation techniques in conjunction with tandem MS (MS/MS); this approach is commonly known as multidimensional protein identification technology. This method involves processing a sample by enzymatic digestion of the protein mixture, separation of the corresponding peptides via two-dimensional LC (typically, strong cation-exchange and reversed-phase high-pressure LC [HPLC]), and subsequent MS/MS analysis. Protein identity is ultimately determined by searching the MS data against protein sequence databases using various database search algo-

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rithms (20, 29). Another common approach for the identification of proteins from complex mixtures uses one-dimensional gel electrophoresis, followed by nanoelectrospray MS/MS analysis of trypsin-digested regions derived from the one-dimensional-gel-separated mixtures (GeLC-MS/MS). This particular method was utilized to identify the composition of proteins involved with the ODVs from CuniNPV. The present study begins the systematic analysis of the structural proteins of CuniNPV ODVs with emphasis on those localized to the envelope.

MATERIALS AND METHODS

Virus preparations and purification. Viral OB preparations were carried out as described in Moser et al. (22). Briefly, late-second-instar *Culex quinquifasciatus* larvae were exposed to infected larvae or purified OBs. Larvae were collected 48 h after inoculation by straining them through a nylon mesh and rinsing them with deionized water to remove food particles. Collected larvae were homogenized in a glass jar using a blender. The homogenate was filtered through a 5-μm low-protein binding Durapore disk (Millipore) to eliminate cuticular debris. The filtrate was layered on top of a 30% HS-40 Ludox (Dupont) gradient and centrifuged at 15,000 × *g* for 30 min in a Sorvall high-speed centrifuge (model RC-5B) using a swinging-bucket rotor. The OB band formed at a density of approximately 1.14 to 1.18 g/ml and was carefully removed by using a Pasteur pipette and washed twice with 0.1 mM NaOH solution, followed by pelleting with centrifugation at 10,000 × *g*. Alkaline release of ODVs was achieved by adding 0.5 M NaOH to an OB suspension to achieve 10 to 15 mM final concentration. Transformation of the turbid OB suspension into a translucent viscous solution indicated the breakdown of OB and the release of ODVs. Gradient centrifugation of the samples immediately after the release of ODVs led to banding of the ODVs at a density of 1.06 ± 0.01 ($n = 11$) in continuous HS-40 Ludox gradients. Negatively stained ODV preparations were examined by transmission electron microscopy to determine the success of ODV purification, and the viability of ODVs was tested by larval bioassays.

PAGE and protein blots. Purified ODVs were denatured by resuspending them in 10 mM Tris-HCl containing β-mercaptoethanol and sodium dodecyl sulfate (SDS), followed by heating at 95°C for 10 min. Denatured ODVs were separated by SDS-PAGE on an 8 to 16% gel (18) at 25 V for 15 min, followed by 100 V for 1 h by using the Protean Mini-gel system (Bio-Rad, Hercules, CA). Gels were washed for 20 min in distilled water and stained with Coomassie blue R250 (Bio-Rad) according to the manufacturer's instructions. Gels were destained for 45 min with two changes of destaining solution (40% [vol/vol] methanol, 7% [vol/vol] acetic acid).

Blots for N-terminal sequencing were made by equilibrating freshly run SDS-PAGE protein gels in transfer buffer (10 mM $NaHCO_3$, 3 mM Na_2CO_3, 20% methanol, 0.1% SDS; pH 9.9) for 15 min and electroblotting them onto polyvinylidene difluoride (PVDF) membranes (Immobilon-P^{SQ}; Millipore, Bedford, MA) by using a Transblot mini apparatus (Bio-Rad). Electroblotting was carried out at constant amperage of 350 mA for 2 h. PVDF membranes were stained with Coomassie blue R250 (Bio-Rad) by using standard protocols.

Protein identification. All protein analyses were carried out at the Proteomics Core Facility at the Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, Florida. The proteins blotted on the PVDF membranes were excised and subjected to sequence analysis by the Edman degradation method. Protein bands that were analyzed by matrix-assisted laser desorption/ionization time-of-flight MS or the GeLC-MS/MS technique using nanoelectrospray quadrupole time-of-flight MS were excised from Coomassie blue-stained polyacrylamide gels and digested in-gel with trypsin (20, 29). Capillary reversed-phase HPLC separation of protein digests was performed on a 15-cm-by-75-μm (inner diameter) TARGA C18 column (Higgins Analytical, Mountain View, CA) in combination with an Ultimate Capillary HPLC system (LC Packings, Sunnyvale, CA) operated at a flow rate of 200 nl/min. MS/MS analysis was accomplished by a hybrid quadrupole time-of-flight MS instrument (QSTAR; Applied Biosystems, Foster City, CA) equipped with a nanoelectrospray source. MS/MS data generated by the QSTAR were searched against the National Center for Biotechnology Information nr sequence database using the Mascot (Matrix Science, Boston, MA) database search algorithm. Proteins identified by Mascot were considered significant if individual ion scores exceeded the threshold value calculated for identity or extensive homology ($P \leq 0.05$). The percent coverage of each protein by peptide fragments identified by one or more techniques was calculated using only the unique peptide fragments with 100% amino acid identity (i.e., amino acid sequences from recurring peptide fragments or overlapping amino acid sequences were counted only once).

Informatics. Protein sequence analyses were performed by using the MacVector 7.5 software package (Accelrys, San Diego, CA). Database searches (GenBank, EMBL, SWISS-PROT, and PIR) for protein comparisons were carried out by using BLAST and PSI-BLAST (3, 4, 27). Online software packages on the ExPASy (12; http://www.expasy.org/), SignalP 3.0 (7; http://www.cbs.dtu.dk/services/SignalP/), TMPred (16; http://www.ch.embnet.org/software/TMPRED_form.html), and Swiss EMBNet Node (www.ch.embnet.org) servers were used for the prediction of secondary structures, to search for domains and transmembrane regions, and for subcellular localization (28).

RESULTS AND DISCUSSION

To characterize the proteins associated with CuniNPV ODVs, OBs obtained from infected mosquito larvae were purified, and the virions were released and isolated by Ludox density centrifugation. Transmission electron microscopy, as well as larval bioassays, of ODVs confirmed their purity and viability. SDS-PAGE of the virion band and the protein-containing top layer (mostly OB proteins) recovered from the Ludox gradient indicated that some of the protein bands were present in both fractions, although in different proportions (data not shown). Therefore, we assumed that either contamination of ODV bands with proteins from denatured OBs or proteins of the virion structure were released from the virions during processing. However, we made no attempt to determine the specific location of the novel proteins identified in the present study. The lack of mosquito cell lines that could propagate CuniNPV has restricted the production of ODVs to density gradient centrifugation of alkaline-denatured OBs produced in mosquito larvae.

An electron micrograph of negatively stained ODVs from a typical purification is shown in Fig. 1A. The arrows indicate two of the ODVs. The denaturing polyacrylamide gel used to separate the protein bands for GeLC-MS/MS analysis is shown in Fig. 1B. The 13 gel regions used in the analysis and the approximate molecular masses (in kilodaltons) are indicated on each side of Fig. 1B. The CuniNPV structural proteins associated with the ODV, the methods of identification, the percent coverage of each protein identified by various techniques, and the proteins identified in each gel band (shown in Fig. 1B) by GeLC-MS/MS technique are presented in Table 1. It should be noted that many proteins were detected in more than one gel band, often in bands of molecular mass range different from those calculated using the translated CuniNPV ORFs. We postulated that this was a result of incomplete denaturation of ODV, the breakdown of protein complexes, or protein processing and not because the actual proteins differed in size from those predicted by the ORFs. A summary of CuniNPV structural proteins and their conserved counterparts, when applicable, from a few selected NPVs is presented in Table 2. A total of 45 proteins, including the previously characterized major OB protein (cun085 [26]), were identified by one or more of the techniques used here. The total number of structural proteins identified is comparable to that of *Autographa californica* nucleopolyhedrovirus (AcMNPV) (9). Of the 44 proteins identified, 22 were derived from unique ORFs encoded by the CuniNPV genome, whereas the remainder were conserved among all sequenced baculovirus genomes available to date. Of these 22 conserved proteins, 5 had not been previously been found to be associated with ODV. These

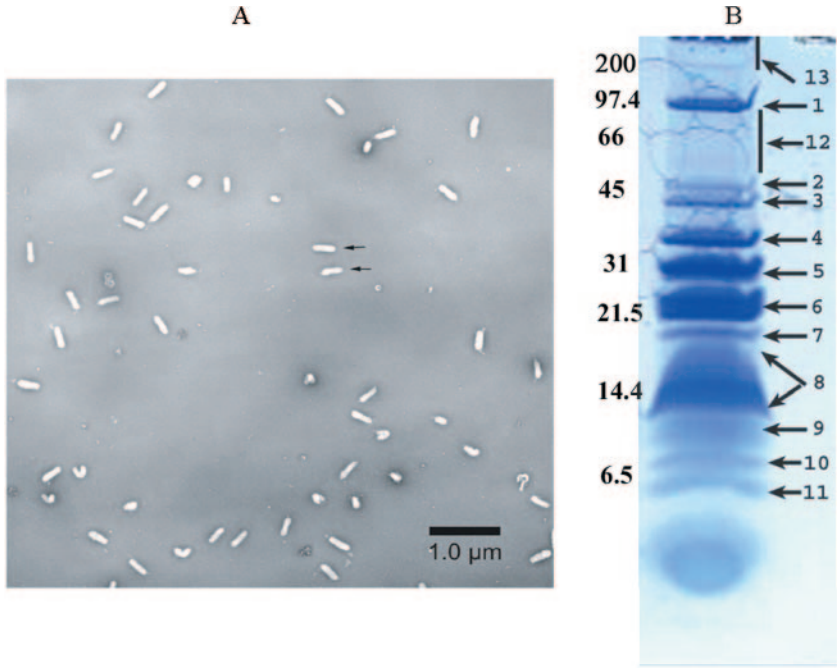

FIG. 1. (A) Electron micrograph of negatively stained ODVs with arrows pointing to two of the ODVs. (B) SDS-PAGE of purified ODV proteins used for GeLC-MS/MS analysis. This gel contained more ODV proteins and was run for a shorter duration than the gel in Fig. 1A. Molecular masses of Bio-Rad broad-range marker (in kilodaltons) are indicated on the left side of the gel. The gel bands used in the analysis are numbered and indicated by the arrows.

five proteins—cun058, cun059, cun087, cun106, and cun109—and, by homology, their counterparts in other baculoviruses can be considered structural components of the OB or ODV. These ORFs show amino acid sequence similarity to ORFs 68, 62, 98, 81, and 2 of AcMNPV, respectively (Table 2).

The majority of CuniNPV structural genes appeared to be organized into two gene clusters. Thirteen structural genes were clustered between cun008 and cun024 (with the exception of cun009, cun016, cun017, and cun020), and a second cluster of 10 genes was found between cun029 to cun038. The two clusters contained 76 and 100% structural genes, respectively, of the total number of genes located within the clusters and include 56% of all of the CuniNPV structural genes identified to date. Of the 13 genes in the first cluster of structural genes, 9 were unique to CuniNPV. The second cluster contains genes encoding for proteins such as *pif*-1, *pif*-2, *odv-ec*27, *gp*41, and *vp*91 that have been localized to ODV of AcMNPV (9). If cun046 (homolog of AcMNPV *pif*-3 (23), cun048, and cun049 (structural genes of unknown function) were combined with the above two gene clusters, the 26 structural genes identified from this region would account for 62% of all of the genes present in this stretch of the CuniNPV genome and for 59% of all of the structural genes identified from CuniNPV. Although other baculovirus genomes contain some structural genes in close proximity, clustering of structural gene at the densities found in CuniNPV has not been found in any other published baculovirus genome sequence.

Bioinformatic analyses predicted that 19 of the 44 CuniNPV structural proteins contained one or more transmembrane regions. In addition, 12 sequences were predicted to contain transmembrane domains and a signal or signal anchor sequences with significant probabilities ($P > 0.8$), while 4 others contained strong nuclear localization signals (Table 2). SignalP and TMPred domain predictions of AcMNPV proteins homologous to CuniNPV structural proteins containing functional domains (transmembrane and/or signal/anchor) revealed that all but AcMNPV ORF 54 (*vp*1054) contained corresponding functional domains in their amino acid sequences. However, *vp*1054 homologs of the nucleopolyhedroviruses of *Helicoverpa zea*, *Lymantria dispar*, *Neodiprion lecontei*, and *Neodiprion sertifer* contained predicted transmembrane domains, whereas other NPVs listed in Table 2 did not.

Four of the CuniNPV genes—cun045, cun054, cun089, and cun091—that showed amino acid sequence similarity to AcMNPV ORFs 14 (*lef*-1), 133 (*alk exo*), 95 (*helicase*), and 65 (*DNA polymerase*), respectively, were not detected in the present study. All four of these sequences are conserved among all baculovirus genomes sequenced to date and have been identified as ODV components of AcMNPV (9). The sizes of CuniNPV homologs of these polypeptides ranged from 235 to 1,332 amino acids in length. In the present study, the GeLC-MS/MS method allowed for detection of proteins that were present in relatively low quantities and with sizes as small as 65 amino acids. Therefore, we conclude that either these four proteins are not components of the ODVs of CuniNPV or were present in quantities below the detection capabilities of the techniques used in the present study. In addition, seven conserved proteins (cun046, cun058, cun059, cun087, cun090, cun106, and cun109) that were not identified as structural components by Braunagel et al. (9) were identified in the present study. Homologs of two of the seven proteins (cun046

TABLE 1. Structural proteins identified from CuniNPV[a]

CuniNPV ORF (AcMNPV ORF)[b]	Length of peptide (amino acids)	Gel band(s)	Identity or localization if known	Method(s) of identification[c]	% Coverage
2	223	7–11		G	18.83
8 (54)	329	6	*vp*1054, virion	G	22.19
10	68	9–11		M, G	100.00
11	546	1–10, 12, 13		E, G	61.90
12	237	1–9, 11–13		E, G	65.40
13*	147	9,10		G	6.80
14 (92)	370	5, 6	p33	G	28.10
15	232	1–13		G	46.98
18 (77)	358	5	*vif*-1	G	13.97
19	88	9, 10		G	32.95
21	101	10, 11		G	42.57
22	382	2–5, 12, 13		G	71.99
23 (100)	65	8, 9	p6.9, DNA binding	G	12.31
24 (89)	289	1–9, 11–13	*p*39-capsid	G	81.66
29 (119)	523	3, 4, 8	*pif*-1	G	34.61
30 (142)	474	1–5, 7–9, 12, 13	49 kDa	M, G	73.21
31	76	8–11		G	47.37
32 (144)	268	3, 6–9	*Odv-ec*27	G	69.78
33 (80)	286	1–13	gp41, tegument	M, G	63.64
34	108	8, 9		G	52.78
35 (83)	741	2–5, 10, 12	*p*96/*vp*91, virion protein	M, G	55.47
36	212	4, 5, 8		G	13.21
37*	143	2, 12		G	6.99
38 (22)	403	4, 5	*pif*-2	G	13.39
46 (115)	203	7–9	*pif*-3	G	31.53
48	117	7–10		G	61.54
49	107	6–11		G	71.96
55*	386	5, 6		G	4.15
57*	410	8, 9		G	3.17
58 (68)	137	8, 9		G	54.01
59* (62)	590	9, 10	lef-9, transcription	G	3.39
64	114	9		G	12.28
69 (109)	409	1–9, 12, 13	*odv-ec*43	G	75.55
70	66	9–11		G	43.93
74 (138)	681	2, 3, 9	p74, envelope	G	24.82
85	822	1, 2, 4, 8, 9, 12, 13	Occlusion body	E, M, G	74.72
87 (98)	303	6, 7		G	24.09
90 (96)	202	7, 8		G	50.99
92	1769	1–3, 12, 13		G	56.30
98	248	4–8, 12, 13		G	28.22
102 (148)	361	1–8, 12, 13	*odv-e*56, envelope	G	49.86
103*	305	5, 6		G	6.23
104* (23)	587	8, 9	*ld*130, fusion protein	G	2.21
106 (81)	186	8, 9		G	31.72
109* (2)	490	8, 9	*bro,* ATP-GTP binding	G	2.04

[a] The ORF number of CuniNPV, the predicted length of the ORF, the identity and/or location of the proteins (if known), the methods of identification, and the percent coverage of each protein by peptide fragments identified by one or more methods are shown. For each conserved baculovirus protein indicated in column 1, the AcMNPV ORF number is given in parentheses.

[b] *, Proteins with less than 10% coverage.

[c] E, Edman degradation; M, matrix-assisted laser desorption ionization–time of flight mass spectrometry; G, GeLC-MS/MS.

and cun090) have recently been confirmed as components of the ODV (23, 30).

Eight of the CuniNPV proteins identified in the present study had less than 10% amino acid coverage as determined by GeLC-MS/MS analysis (Table 1). Although these proteins had only one to three unique peptide fragments, these peptide fragments were identified in more than one gel band and were considered components of bona fide structural proteins. It should be noted that all of these peptide fragments were identified from gel bands that contained proteins of lower molecular weight than that calculated from the CuniNPV ORFs. Therefore, we postulated that these peptide fragments were products of proteins degraded during the release of ODVs by alkaline treatment.

The identification of structural proteins in CuniNPV provided the opportunity to compare and contrast various baculovirus proteomes. Although the total number of structural proteins in CuniNPV is comparable to that of AcMNPV, 50% of the CuniNPV structural proteins are unique. The remaining 50% of the CuniNPV structural proteins accounted for 61% of the total number of conserved baculovirus proteins ($n = 36$) identified in the CuniNPV genome (2). The existence of similar domains between CuniNPV and AcMNPV proteins with low similarities may indicate functional conservation among

TABLE 2. ORF comparison of CuniNPV proteins identified in this study to other baculoviruses[a]

ORF[b]	Mol mass (kDa)[c]	pI[d]	Domain(s)[e]	Name (function)[f]	Homologous ORFs in other NPVs[g]										
					AcMNPV	BmMNPV	OpMNPV	LdMNPV	SpltMNPV	MacoNPV	HzNPV	NeseNPV	NeleNPV	CpGV	XcGV
2	25	8.25	S, TM		x	x	x	x	x	x	x	x	x	x	x
8	37.5	9.28	TM	*vp*1054 (virion assembly)	54	43	58	57	49	133	27	85	83	138	175
10	7.9	9.57			x	x	x	x	x	x	x	x	x	x	x
11	60.5	4.59	TM		x	x	x	x	x	x	x	x	x	x	x
12	27.7	8.07	NLS		x	x	x	x	x	x	x	x	x	x	x
13	16.4	4.81	TM		x	x	x	x	x	x	x	x	x	x	x
14	42.3	9.09	TM	*p*33	92	75	93	94	83	96	83	24	16	93	101
15	25.1	4.87	A, TM		x	x	x	x	x	x	x	x	x		x
18	41.4	9.82	TM	*vlf*-1 (late gene expression)	77	63	80	86	74	106	73	45	42	106	123
19	9.3	4.4	TM		x	x	x	x	x	x	x	x	x	x	x
21	11.3	4.38	S, TM		x	x	x	x	x	x	x	x	x	x	x
22	42.2	9.26			x	x	x	x	x	x	x	x	x	x	x
23	7.5	13.46	NLS	*p*6.9 (DNA binding)	100	84	101	101	90	86	91	36	28	86	94
24	32.2	6.61		*vp*39 (capsid, late coat protein)	89	72	90	92	81	99	81	89	88	96	111
29	58.4	5.83	S, TM	*pif*-1 (per os infectivity factor 1)	119	97	119	155	124	33	114	79	76	75	84
30	54.2	6.58	TM	49 kDa	142	118	139	20	11	167	9	63	60	15	13
31	7.8	8.67	A, TM		x	x	x	x	x	x	x	x	x	x	x
32	30	5.98		*odv-ec*27 (ODV envelope)	144	120	141	18	13	165	11	66	63	97	112
33	31.9	9.3	TM	*gp*41 (tegument)	80	66	83	88	76	104	75	47	44	104	121
34	11.4	4.56	TM		x	x	x	x	x	x	x	x	x	x	x
35	84.2	4.57	A, TM	*vp*91 (capsid)	83	69	86	91	79	101	78	84	82	101	118
36	23.8	9.37			x	x	x	x	x	x	x	x	x	x	x
37	16.1	9.28			x	x	x	x	x	x	x	x	x	x	x
38	45.2	5.44	S, TM	*pif*-2 (per os infectivity factor 2)	22	13	20	119	135	48	136	55	52	48	45
46	22.4	7.39	S, TM	*pif*-3 (per os infectivity factor 3)	115	95	115	143	107	68	101	69	66	35	32
48	13.1	4.14	S, TM		x	x	x	x	x	x	x	x	x	x	x
49	11.9	4.24	S, TM		x	x	x	x	x	x	x	x	x	x	x
55	44.7	8.13	TM		x	x	x	x	x	x	x	x	x	x	x
57	46.9	6.3			x	x	x	x	x	x	x	x	x	x	x
58*	15.6	8.4	TM		68	56	73	80	66	112	66	41	38	114	135
59*	65.5	6.53	TM	*lef-9* (transcription)	62	59	65	64	59	124	56	40	37	117	139
64	12.9	4.92			x	x	x	x	x	x	x	x	x		x
69	46.3	9.32	TM	*odv-ec*43 (occluded virion)	109	92	109	107	96	80	97	70	67	55	53
70	7.3	7.16	TM		x	x	x	x	x	x	x	x	x		x
74	76.7	5.16	TM	*p*74 (envelope)	138	115	134	27	21	160	19	50	47	60	77
85	93.2		TM	OB (occlusion body)	x	x	x	x	x	x	x	x	x	x	x
87*	34.9	6.06		38K/hydrolase	98	82	99	99	88	88	89	59	56	88	96
90	23.1	5.92	S, TM		96	79	97	98	87	92	88	60	57	89	97
92	202.3	5.25	NLS		x	x	x	x	x	x	x	x	x	x	x
98	29.8	11.73	NLS		x	x	x	x	x	x	x	x	x	x	x
102	39.4	6.27	TM	*odv-e*56 (envelope)	148	124	146	14	17	6	15	38	23	18	15
103	34.78	7.36			x	x	x	x	x	x	x	x	x	x	x
104*	66.59	8.06	S, TM	*Ld-130* (fusion protein)	23	14	21	130	136	9	137	x	x	31	27
106	21.6	9.13	TM		81	67	84	89	77	103	76	48	45	103	120
109*	56.81	6.37	TM	*bro* (ATP-ase?)	2	131	116	32 Bro-a	120 Bro-a	59 Bro-a	x	x	63-truncated	60	60

[a] The molecular mass, isoelectric point, and various structural domains predicted from deduced amino acid sequence are presented. References for abbreviated baculovirus names are given below. Abbreviations (reference; GenBank accession bumber): AcMNPV (5; L22858); BmNPV, *Bombyx mori* nucleopolyhedrovirus (13; L33180); CpGV, *Cydia pomonella* granulovirus (21; U53466); CuniNPV (2; AF403738); HzSNPV, *Helicoverpa zea* nucleopolyhedrovirus (10; AF334030); LdMNPV, *Lymantria dispar* nucleopolyhedrovirus (17; AF081810); MacoNPV, *Mamestra configurata* nucleopolyhedrovirus (19a; NC003529); NeleNPV, *Neodiprion lecontei* nucleopolyhedrovirus (19; AY349019); NeseNPV, *Neodiprion sertifer* nucleopolyhedrovirus (11; AY430810); OpMNPV, *Orgyia pseudotsugata* nucleopolyhedrovirus (1; U75930); SpltMNPV, *Spodoptera litura* nucleopolyhedrovirus (25; AF325155); XcGV, *Xestia c-nigrum* granulovirus (14; AF162221).

[b] *, Conserved baculovirus proteins newly identified as structural components in this study.

[c] Molecular mass calculated from the amino acid sequence.

[d] Isoelectric point calculated from the amino acid sequence.

[e] Blank fields indicate that no identifiable peptide domains were present in the proteins.

[f] Blank fields indicate that no function or name was assigned to the proteins at this point.

[g] x, No sequence homologues were identified.

structural proteins of highly divergent baculovirus species. In the present study, five conserved baculovirus proteins were also identified for the first time as proteins associated with the OB or ODV. These results will provide the groundwork for understanding the function of critical structural proteins of CuniNPV and their potential role in key biological features such as infectivity, host range, and virulence. In addition, the identification of the major structural proteins of CuniNPV will facilitate comparative studies with other baculoviruses adapted to aquatic hosts and environments when they become available.

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