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Characterization of viral proteins of *Oryctes* baculovirus and comparison between two geographical isolates

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Summary. Bacilliform *Oryctes* baculovirus particles have been visualized in electron micrographs of midgut sections from virus infected *Oryctes rhinoceros* beetles. Morphologically the Indian isolate (*Oryctes* baculovirus, KI) resembled the previously reported *Oryctes* baculovirus, isolate PV505. The constituent proteins of baculovirus KI have been analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and by Western blots using polyclonal antibodies raised against the complete viral particles, as probes. A total of forty eight viral proteins have been identified. Fourteen viral proteins were located on the viral envelope. Among the proteins constituting the nucleocapsid, three were located internally within the capsid. A 23.5 kDa protein was tightly associated with viral DNA in the nucleocapsid core. Two envelope and seven capsid proteins stained positive for glycosylation. Comparison between the viral proteins of KI and PV505 revealed differences in SDS-PAGE profiles and glycosylation patterns. Immunoblotting of KI and PV505 proteins with anti KI antiserum demonstrated antigenic differences between the two viral isolates.

Introduction

Oryctes baculovirus was first isolated in Malaysia by Huger [15] from diseased larvae of rhinoceros beetle, *Oryctes rhinoceros* L., a major insect pest of coconut and oil palm throughout the tropics. The virus has been spectacularly successful in the biological suppression of the rhinoceros beetle in the South Pacific islands, where it was introduced as a biocidal agent for the insect pest [2, 8]. This virus isolate has been classified as a subgroup C baculovirus in the family *Baculoviridae*, and is distinguished from the other subgroups by the absence of the matrix protein polyhedrin or granulin [21]. Another *Oryctes* baculovirus strain has been isolated from the wild population of the insect pest (rhinoceros beetle) in Kerala state, India [23]. This isolate has also proved to be successful in the biological control of rhinoceros beetles in field trials [Mohan and Pillai, unpublished observations] and is of considerable economic importance. *Oryctes* baculovirus has also been isolated from the wild population of *O. rhinoceros* in Seychelles and the islands of Indonesia and the Philippines [11, 33].

The genomes of twelve geographical isolates of *Oryctes* baculovirus, including the Indian isolate (KI) have been compared by restriction endonuclease analysis by Crawford et al. [11]. All isolates showed very similar restriction endonuclease fragment profiles, with slight variations involving small insertions or deletions of DNA fragments.

The present paper describes the characterization of viral proteins of the Indian isolate (KI) and its comparison with *Oryctes* baculovirus PV505 (Philippines origin), previously reported [10].

Materials and methods

Viruses

Oryctes baculovirus (KI) was isolated from the wild population of *O. rhinoceros* [illegible] Kerala state, India [23]. Strain PV505, originally isolated from Philippines, was obtained from Allan Crawford, DSIR, Auckland, New Zealand. Both virus isolates were routinely propagated and multiplied in *O. rhinoceros* adults. For electron microscopic confirmation of the virus infection, slices of infected midgut of *O. rhinoceros* beetles were fixed in 2% glutaraldehyde followed by staining with 1% OsO_4 and embedded in Epon-araldite. Sections were examined in a transmission electron microscope. For the large scale isolation of virus particles, laboratory bred male and female beetles (a week after emergence from pupae) were infected with *Oryctes* virus. To prepare the virus inoculum, three to four midguts of virus infected *Oryctes* beetles or grubs were triturated in cold 50 mM phosphate buffer (pH 8.0), clarified by centrifugation at 5,[illegible] rpm for 10 min and passed through 0.45 µ pore size membrane filter. For infection the virus inoculum in 10% sucrose was fed to *O. rhinoceros* beetles by placing drops on the mouth parts of upturned beetles. Seven days later the infected beetles were placed in a plastic tray containing 50 mM phosphate buffer (pH 8) and antibiotics (streptomycin and neomycin 100 mg/l each; benzyl penicillin 50,000 units/l) to a depth of 6 mm. Virus excreted into the buffer was purified by centrifugation on sucrose gradients made in 50 mM phosphate buffer, pH 8 (once through a 10–50% w/v gradient at 45,000 g for 50 min and two cycles of 25–50% w/w sucrose gradient at 100,000 g for 1 h). Alternatively virus from infected guts of *Oryctes* beetles was purified by chromatography on controlled pore glass (Sigma Chem. Co., MO, U.S.A.; mesh size 120–200, pore diameter 700 Å) [1, 32]. The virus emerging in the void volume of the column was further purified by sucrose gradient centrifugation as described above. The banded virus particles from sucrose gradients were dialysed against Tris-HCl buffer (50 mM, pH 8.0) and used for protein characterization.

Purification of nucleocapsid

Removal of the envelope was carried out by treatment of the virus with detergent followed by banding on sucrose gradients [26]. The virus particles (0.5 mg protein/ml) were incubated with 1% NP40 in 50 mM Tris-HCl buffer, pH 7.5 for 1 h at 30 °C with gentle shaking. The NP40 treated virus was layered over 10–50% (w/v) sucrose gradient in Tris buffer, pH 7.5 and centrifuged at 45,000 g for 45 min. The nucleocapsid band was recovered and the nucleocapsids were washed by pelleting [illegible] Tris buffer, pH 7.5 at 100,000 g for 1 h.

Polyacrylamide gel electrophoresis (PAGE) of virus proteins

Purified virus samples were disrupted in SDS-PAGE sample buffer and subjected to electrophoresis on a 7–15% gradient polyacrylamide gel using the discontinuous buffer system [18]. The gels were stained with Coomasie blue G250-silver double staining methods [12]. Molecular weight (M_r) computations of viral proteins were made using the standard low and high M_r markers (Sigma, Dalton Mark VII). The viral glycoproteins were identified on 7–15% gradient SDS-PAGE gels using the improved periodic acid-Schiff (PAS) method [17]. The glycoprotein bands were stained deep pink.

Antisera

Antisera to the baculovirus isolates were raised in rabbits by first injecting intramuscularly and in foot pads, 1.5 ml of purified KI or PV505 (200 µg/ml) emulsified with an equal volume of Freund's complete adjuvant. Subsequently the rabbits were injected with 200 µg of virus emulsified in incomplete Freund's adjuvant for the next three weeks at weekly intervals. Serum was collected 3 weeks after the final injection and stored at −20 °C in aliquots after determining the titer by ELISA.

Immunoaffinity purification of KI and PV505

Immunoaffinity matrix was prepared by coupling the antibodies (against KI or PV505) to CNBr-activated Sepharose 4B (Pharmacia, Sweden) as per the instructions of the manufacturer. KI or PV505 viral particles were passed through the respective immunoaffinity columns and the adsorbed virus was eluted with a salt gradient (50–750 mM NaCl in 50 mM Tris-HCl, pH 8). KI was eluted at 230 mM and PV505 at 245 mM NaCl concentration. The emerging virus particles ($A_{260,\ 280\ nm}$ peak fractions) were pooled and pelleted by centrifugation at 100,000 g for 1 h.

Radioiodination of KI viral proteins

Iodination of enveloped KI virus isolate and its nucleocapsid preparation was carried out to differentiate the viral proteins located on the envelope and nucleocapsid. Since the integrity of virions during labelling was critical, only freshly purified virus bands from sucrose gradients were used for this purpose. Proteins located internally in the capsid were iodinated after disruption of the nucleocapsids in 1 M NaCl in 0.1 M Tris-HCl (pH 7.5) for 10 h at 37 °C on a shaker. Iodination was carried out by the IODO-GEN method [14, 19], using 200 µCi of ^{125}I for 15 µg of virus protein. Free ^{125}I was removed from the proteins by passing through a Sephadex G-15 column (10 ml bed volume) and the viral proteins/virus in the void volume were immunoprecipitated with anti KI serum and *Staphylococcus aureus* cells ('Pansorbin', Calbiochem, U.S.A.). The immunoprecipitates were subjected to (7–15% gradient) SDS-PAGE. The gels were dried and exposed to Kodak X-Omat films at −80 °C.

Salt extraction of KI viral proteins

Viral particles were disrupted in high salt conditions to identify proteins associated with DNA in the nucleoprotein core. Aliquots of purified KI were incubated in varying NaCl concentrations (1, 2, and 4 M, in 50 mM Tris-HCl, pH 7.5, 1 mM EDTA and 1% NP40) for 12 h at 37 °C in a shaker. The disrupted virus was layered over 30% w/v sucrose (in 50 mM Tris-HCl, pH 7.5, 1 mM EDTA) and centrifuged for 45 min at 100,000 g. The pellet was resuspended in TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA). The supernatant was dialysed first against TE, followed by water and lyophilised. The pellet and supernatant samples were subjected to SDS-PAGE. The protein binding to genomic viral DNA was checked by examining the retardation in mobility of the DNA samples in the pellet by electrophoresis in 0.4% agarose gels. The pellet samples were also analysed after treatment with 0.2% SDS at 60 °C for 30 min to denature and dissociate the DNA binding proteins before electrophoresis. Phenol-extracted, purified KI DNA was included as reference.

Western blotting of KI and PV505 viral proteins

For Western blots, the modified procedure of Moriearity [24] was adopted. The viral proteins of KI and PV 505 were resolved on a 7–15% gradient SDS-PAGE and electroblotted on to nitrocellulose membranes. The blots were probed with anti KI serum (diluted 25 fold). The protein bands in the blot were visualised by reacting with goat-anti rabbit IgG (affinity purified, Pel Freez biologicals, U.S.A.) coupled to horse radish peroxidase, and staining for enzyme activity.

Results

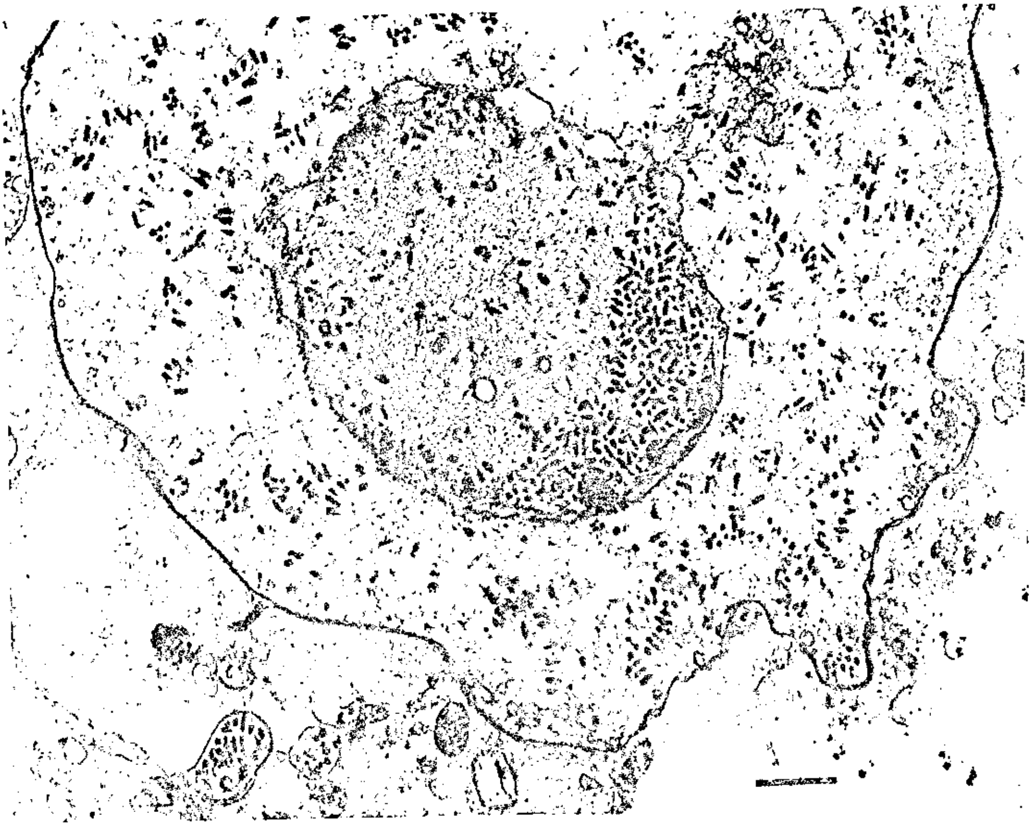
Large number of bacilliform viral particles measuring 220–240 × 80–110 nm could be seen in the cytoplasm and nucleus of infected midgut cells from *Oryctes rhinoceros* (Fig. 1 a, b). Mature nucleocapsids with a unit membrane were seen aggregated on the inner side of the nuclear membrane (Fig. 1 a). The nucleus of the infected cell (Fig. 1 b) also showed spherical vesicles in different stages of acquiring the electron dense core (indicated by arrows). Replication and assembly of viral particles in the nucleus is a characteristic feature of DNA-viruses in the family *Baculoviridae*.

Virus particles were purified from the homogenates of infected midguts. Since the excreta of infected beetles contained large number of virus particles, this also proved to be a good source of virus.

Viral proteins

The electrophoretic pattern of the structural proteins of *Oryctes* baculovirus (KI) is shown in Fig. 2a. The pattern resembled other baculoviruses [28]. Careful scrutiny revealed the presence of 43 proteins ranging from 9.9 to 133 kDa. To facilitate the identification of all the high and low M_r proteins, several gradient gels were run with combinations of higher percentage of acrylamide (10–20%), different electrophoresis run times and by loading the gel with varying amounts of viral proteins. The structural proteins of the virus as deduced from several of these gels are listed in Table 1. When the total viral proteins separated by SDS-PAGE were electroblotted onto nitrocellulose membranes and probed with antibodies raised against the total viral particles, the majority of the viral proteins were revealed as stained bands (see Fig. 6b and later sections). Five additional viral proteins were identified consistently in immunoblots, which were not apparent in SDS-PAGE gels.

Fig. 1. Electron micrographs of *Oryctes* baculovirus. Section of midgut epithelial cell of the beetle, *Oryctes rhinoceros*, infected with baculovirus. **a** Bacilliform virus particles are seen in the nucleus and cytoplasm (magnification × 12,500, bar represents 1 µm). **b** Baculovirus particles in the nucleus under higher magnification (× 65,000, bar represents 100 nm). Various stages of assembly of viral particles within vesicles (arrows) can be seen



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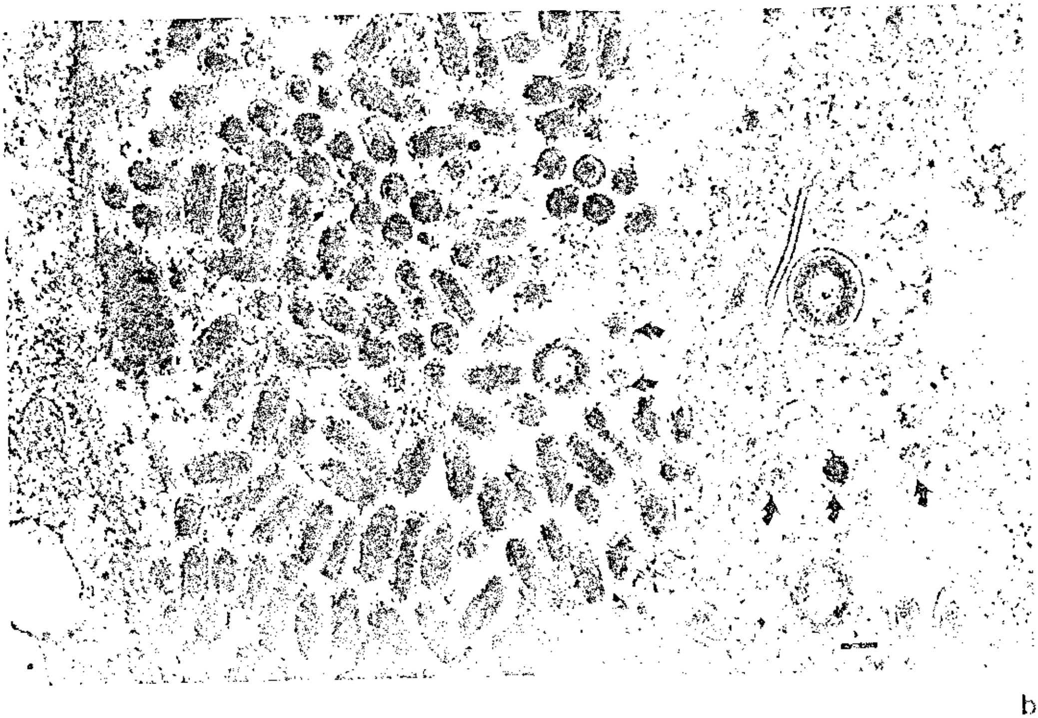

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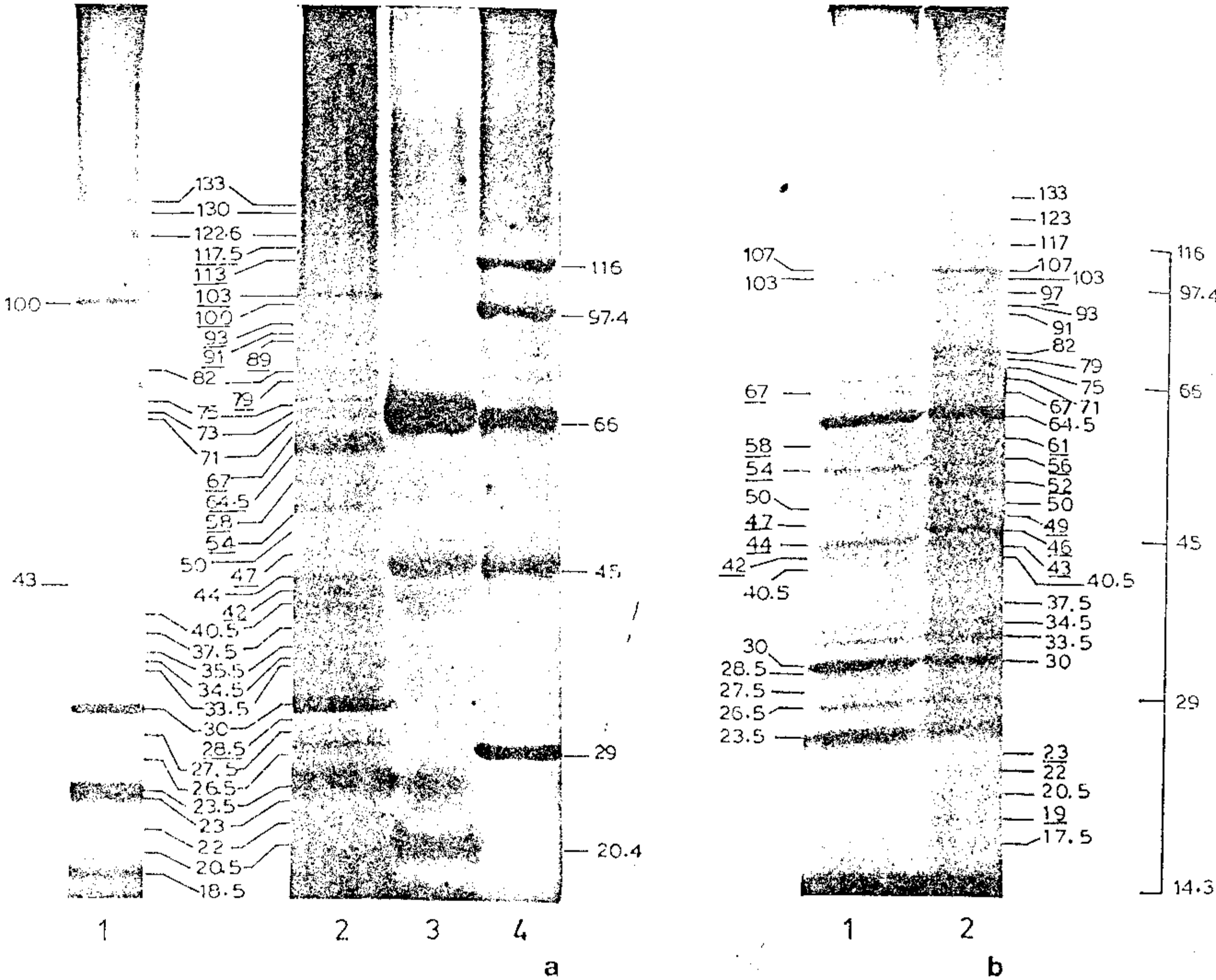

Fig. 2. Characterization of viral proteins. Proteins of the baculovirus isolates were separated on a 7–15% gradient SDS-PAGE and stained with Coomassie blue-silver, double staining method. **a** *Oryctes* baculovirus, KI. *1* Nucleocapsid, *2* enveloped virus, *3*, *4* standard M_r markers (kDa). Viral proteins found only in enveloped virus are underlined. **b** Comparison between *Oryctes* baculovirus isolates KI and PV505. *1* KI, *2* PV505. Viral proteins specific to either to KI or PV505 are underlined

The nucleocapsid preparations of KI (envelope removed by treatment with NP40) showed 29 proteins (including VP18.5) spread over a similar range of M_r (Fig. 2a), suggesting that the other 14 proteins were located on the viral envelope (Table 1). Consistently the protein VP103 and 44 underwent a slight reduction in M_r (by 3 and 1 kDa) in the nucleocapsid preparation relative to the counterpart proteins in the enveloped virus lane. Also, an additional protein band of 18.5 kDa was consistently seen in KI nucleocapsid, not detected in the enveloped virus lane. The observed banding pattern of viral proteins was reproducible with the exception of two high M_r proteins VP217 and 205 and

Table 1. Total viral proteins of enveloped KI and the nucleocapsid

Enveloped virus proteins	Nucleo-capsid	Enveloped virus proteins	Nucleo-capsid
VP133	+	VP42	−
VP130	+	VP40.5	+
VP122.5	+	VP37.5	+
VP117.5	−	VP35.5	+
VP113	−	VP34.5	+
VP103	+	VP33.5	+
VP100	−	VP30	+
VP93	−	VP28.5	−
VP91	−	VP27.5	+
VP89	−	VP26.5	+
VP82	+	VP23.5	+
VP79	−	VP23	+
VP75	+	VP22	+
VP73	+	VP20.5	+
VP71	+	−	VP18.5*
VP67	−	VP17.5	+
VP64.5	−	VP16.5	+
VP58	−	VP15	+
VP54	−	VP13.3	+
VP50	+	VP11.7	+
VP47	−	VP9.9	+
VP44	+		

VP107 from lane 1 of Fig. 2b

* VP18.5 was seen only in nucleocapsid (NP40 treated samples)

\+ Presence or − absence of protein in the sample

VP155, 150, 143, 78.5, 77 were additionally identified from immunoblots

hence they were not taken into account. These proteins could have arisen due to aggregation of viral proteins under certain conditions. Such aggregations have been reported for nuclear polyhedrosis viruses (NPVs) [28].

Comparison of viral proteins of the two isolates, KI and PV505

Analysis of PV505 viral proteins by SDS-PAGE showed a total of 39 proteins. This was deduced from a number of gels with different running conditions (Fig. 2b shows only 35 proteins). The virus protein profiles of KI and PV505 (Fig. 2b) showed distinct differences. These were of two types: i) difference in staining intensity of protein bands having same mobility in the virus isolates (e.g., VP107 and 103) and ii) presence of virus specific proteins. For instance

PV505 exhibited nine specific proteins VP97, 61, 56, 52, 49, 46, 43, 23, and 19 not observed in KI lane. Similarly, KI specific proteins VP58, 54, 47, 44, 42 and 16.5 were not seen in PV505.

Radioiodination of KI viral proteins

Iodination of enveloped KI with ^{125}I resulted in the labelling of VP79, 64.5, 58, 54 (Fig. 3). The major envelope protein VP64.5 was the most intensely labelled. A few faintly labelled capsid proteins were also detectable. When intact nucleocapsids were iodinated VP44, 34.5, 33.5, 30, and 19 were labelled. The envelope protein VP64.5 could be faintly (relative to whole virus) detected in the nucleocapsid lane, probably as a contaminent. Iodination of salt-disrupted KI nucleocapsids resulted in the labelling of three additional proteins (VP50, 17.5, and 11.7) not seen in the nucleocapsid lane. These three proteins are presumably located internally within the nucleocapsid. Among them VP50 and 17.5 were prominently labelled. Also VP30 in salt disrupted nucleocapsid (Fig. 3, lane 3) was strongly labelled relative to VP30 in the nucleocapsid (Fig. 3, lane 2). This could be due to better accessibility to sites in VP30 for iodination, when the nucleocapsid was disrupted with salt.

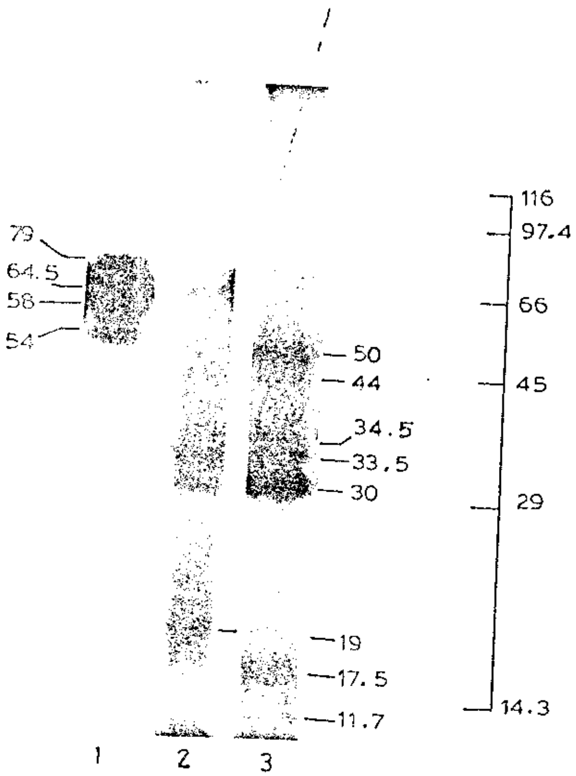

Fig. 3. Envelope and capsid proteins of baculovirus KI. The purified virus particles were labelled with ^{125}I and immunoprecipitated with polyclonal antibodies raised against KI (for details, see text). The immunoprecipitated proteins were resolved on a 7–15% gradient SDS-PAGE. *1* Enveloped virus, *2* nucleocapsid, *3* NaCl disrupted nucleocapsid. Positions of standard M_r markers are given on the right hand margin

Effect of salt concentration on the nucleoprotein core of KI

Treatment of the nucleocapsid with NaCl concentrations varying from 1 M to 4 M resulted in the disruption of viral particle. On pelleting the nucleoprotein core through 30% w/v sucrose, VP 23.5 was found abundantly in the pellet along with DNA at all the concentrations of NaCl used (Fig. 4). In 4 M NaCl the pellet consisted of only DNA and VP 23.5. All other structural proteins of the virus isolate could be recovered in the supernatant (Fig. 4, lane 4) along with a small amount of VP 23.5. Exposure of KI to 5 mM each of EDTA and EGTA resulted in poor disruption of virions. Inclusion of these chelators with NaCl did not enhance the disruption of virions. These chelators have been shown to cause dissociation of capsid in polyoma virus [3, 4].

Protein binding usually results in retardation of mobility of DNA in gel electrophoresis. Therefore, a gel retardation assay was done to check the mobility difference between DNA in the pelleted samples compared to phenol extracted purified DNA. No differences in mobility were detectable in 0.4% agarose gels. The failure to detect any significant retardation of the nucleoprotein in the pellet could be due to the limited resolution of the large genomic DNA (≃123 kbp) in agarose gels.

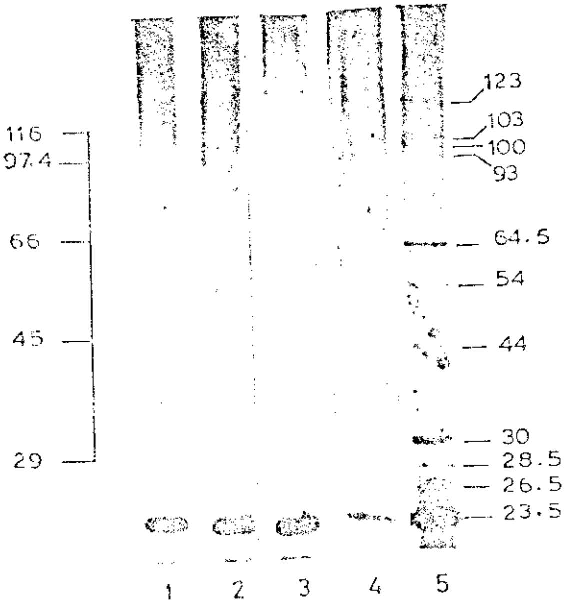

Fig. 4. Disruption of KI virus with salt. Purified virus was disrupted in 1, 2, or 4 M NaCl and 1% NP40 for 12 h at 37 °C. The disrupted virus was layered over 30% w/v sucrose and centrifuged at 100,000 g for 45 min. The pellet constituting the nucleoprotein core was resuspended in TE buffer. The supernatant was dialysed against TE followed by water and lyophilized. The pellet and supernatant were analysed in 10% SDS-PAGE. *1–3* Pellets obtained after treatment with 1, 2, and 4 M NaCl respectively; *4* supernatant from 4 M NaCl treatment; *5* KI baculovirus. Positions of M_r markers are indicated on the left hand margin

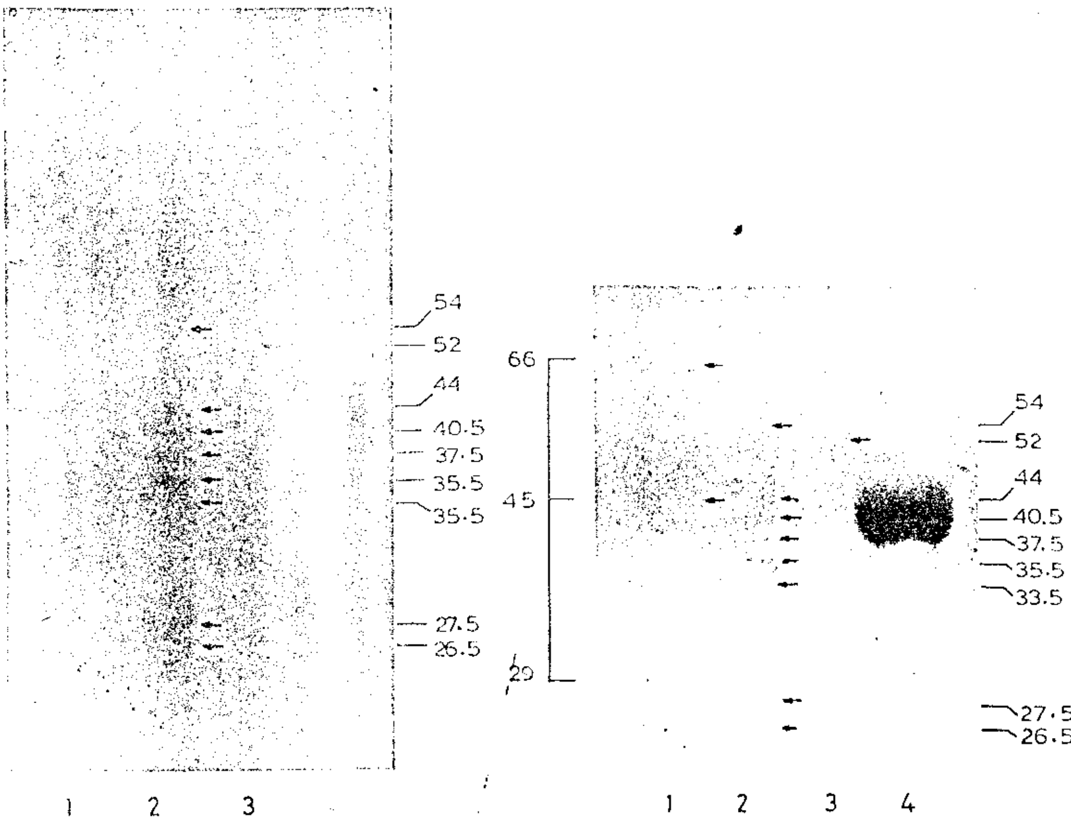

Fig. 5. Glycoproteins of KI an[illegible] V505. Viral proteins were resolved in a 7–15% gradient SDS-PAGE and stained by t[illegible] eriodic acid Schiff (PAS) method. *1* M_r marker, only bovine serum albumin (66 kDa [illegible] d ovalbumin (45 kDa) were stained for glycoproteins in the marker lane; *2* KI; *3* PV50[illegible] horse radish peroxidase (as a positive control). Positions of standard M_r markers are gi[illegible] on the left hand margin in kDa. 120 µg of virus protein was loaded per lane. [illegible] ions of glycoproteins are marked with arrows

[illegible] glycoproteins

The KI protein bands st[illegible] sitive for glycoproteins corresponded to the envelope protein VP54 a[illegible] proteins VP44, 40.5, 37.5, 35.5, 33.5, 27.5, and 26.5 (Fig. 5). In con[illegible] a prominent single band corresponding to V[illegible] was stained posit[illegible] coprotein in PV505. However, VP64.5 was [illegible] faintly in both vi[illegible] many PAS stained gels but is not apparent in Fig. 5.

Western blotting of [illegible] 505 proteins detected by anti KI serum

In order to identify the v[illegible] evoking strong antibody response, Western [illegible] KI protein[illegible] using different dilutions of anti KI serum [illegible] VP30 was the [illegible] stained protein at a 1/1,000 dilution of [illegible] serum although [illegible] 3, were stained faintly. Additional viral

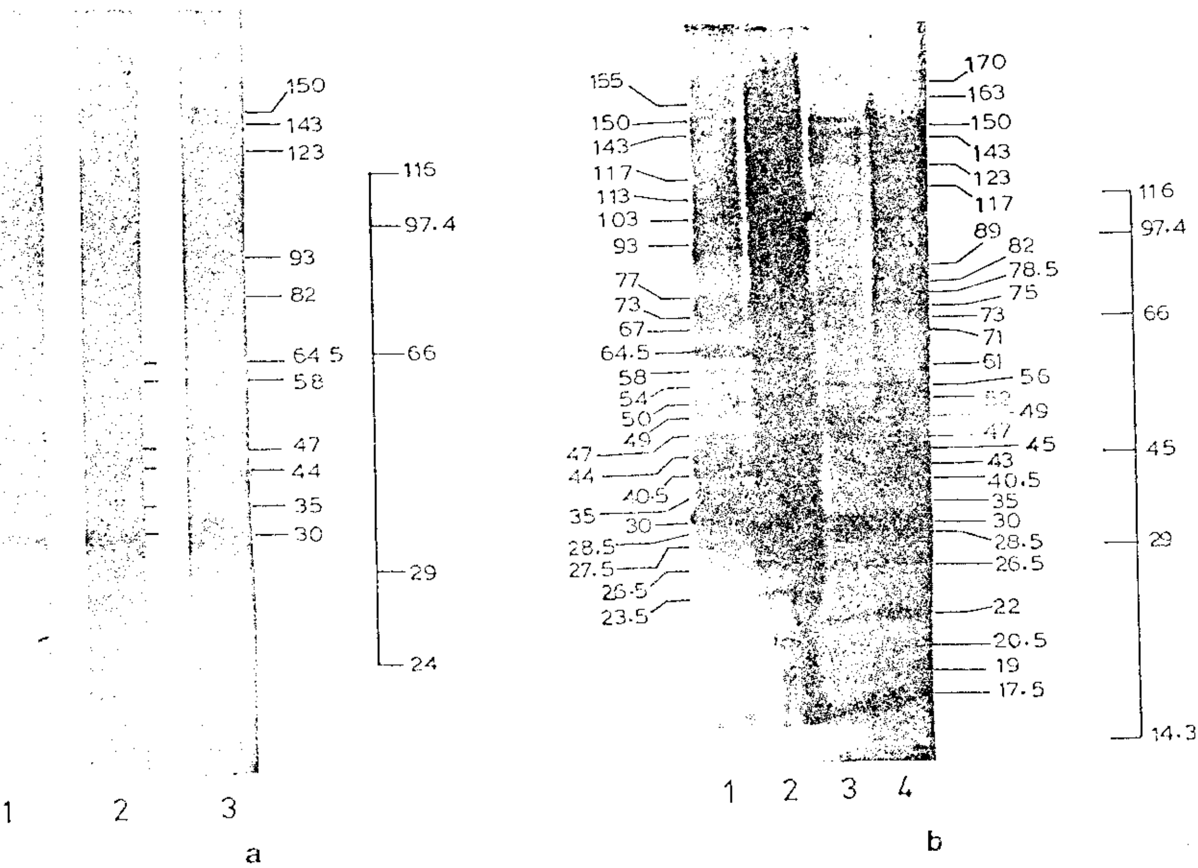

Fig. 6. Western blotting of viral proteins. KI and PV505 proteins were resolved in a 7–15% gradient SDS-PAGE and electroblotted onto nitrocellulose membrane. a The blots of KI proteins were probed with anti KI serum at dilutions indicated. *1*, × 1,000; *2*, × 500; *3*, × 100. b The blot was probed with anti KI serum (25 × diluted). *1*, *2* KI purified on sucrose gradient and immunoaffinity column, respectively; *3*, *4* PV505 purified on immunoaffinity column and sucrose gradient, respectively. Each lane was loaded with 60 µg of virus. Positions of standard M_r markers in kDa are indicated on the right hand margin

proteins as VP64.5, 58, 47, 44, and 35 were detected at 1/500 dilution and VP150, 143, 123, 93, and 82 at 1/100 dilution of the antiserum.

No differences were seen in the immunoblots between the sucrose gradient and immunoaffinity purified viruses (Fig. 6b). A total of 33 KI proteins were detected with homologous (anti KI) antiserum at 1/25 dilution. As anticipated, the overall staining intensity of KI proteins was more pronounced than PV505 protein bands, when detected with anti KI serum.

The Western blots have revealed the presence of many viral proteins not observed in silver stained gels, albeit some of low staining intensity. These were VP150, 143, 78.5, and 77 (common to KI and PV505) and individual virus isolate specific proteins VP155 of KI and VP163 and 170 of PV505.

Differences in the immunoblot profiles were noted between KI and PV505 when incubated with anti KI serum (Fig. 6b). For instances, anti KI serum reacted strongly with KI VP93, 27.5, and 23.5 but not with the counterparts of these proteins, present in PV505. Another significant difference was the intense staining of the major envelope protein of KI, VP64.5 with anti KI serum as against a barely visible band in PV505 with the same antiserum. In addition, anti KI serum reacted strongly with KI proteins VP103, 77, 73, 40.5, 30, and 26.5. These proteins were weakly detected in PV505. Certain PV505 specific proteins like VP61, 56, 52, 46, and 43 (see Fig. 2b) could be detected in immunoblots of PV505 incubated with anti KI serum. The staining intensities of these PV505 specific proteins were faint, with the exception of VP56. But other PV505 specific proteins like VP23 and 97 were not detectable in immunoblots with anti KI serum. Although VP49 is a PV505 specific protein (Fig. 2b) a strongly staining band (Fig. 6b) co-migrating with VP49 of PV505 was seen in KI immunoblot. Likewise, VP47 was thought to be KI specific virus protein (Fig. 2b) but a strongly reacting band corresponding to VP47 could be seen in PV505 lane (Fig. 6b).

Discussion

Oryctes baculovirus (KI) replicates and matures within the nucleus of the infected cell. A nucleocapsid shell with a membrane could be seen in the nucleus during the assembly of the virion. The shell is subsequently filled with an electron dense core containing DNA. Crawford and Sheehan, [10] observed similar empty nucleocapsid shells with unit membrane, 12 h post-infection in *Heteronychus arator* cell line. In contrast, subgroups A and B baculoviruses form as naked nucleocapsids and acquire envelopes either during budding from the plasma membrane or before occlusion into polyhedra or granules [29].

The presence of a large number of viral proteins in *Oryctes* baculovirus was an expected manifestation of its large genome (123–127 kbp). The Coomassie blue-silver double staining technique has revealed 43 viral proteins in the KI strain. Five additional proteins have been identified in Western blots. Payne et al. [24] had earlier reported 12 proteins with Coomassie blue stain and Crawford and Sheehan [10] reported 27 proteins using silver stain in *Oryctes* baculovirus PV505. In the present studies, we could detect 39 protein bands in PV505 due to better resolution and increased sensitivity in protein staining procedure.

The altered mobilities of viral proteins between virus isolates KI and PV505 ([illegible]) could be either due to intrinsic differences in protein sizes as a consequence of additions/deletions in the genome [11] or due to the extent of post translational modifications (e.g., glycosylation). For instance, in Fig. 2b VP52 in PV505 and VP54 in KI could be the same protein (judging from protein [illegible]) but show altered mobilities because of modifications. Another [illegible] is VP46 in PV505, and VP44 in KI. Treatment of KI with NP40 [illegible] the envelope, specifically reduced the M_r of VP103 and 44 by 3 [illegible] respectively. A similar observation has been reported when the [illegible] granulosis virus (GV) and a NPV of *Bombyx mori*, were removed by NP40 treatment [5]. Unmasking of a protease by NP40 is suggested as the possible cause. The concomitant appearance of a protein (VP18.5) only in the KI nucleocapsid lane after treatment with NP40 could be due to a similar mechanism. The presence of VP18.5 in nucleocapsids of PV505 treated with NP40, has been previously reported [10].

Based on the differences in the profiles of iodinated proteins in enveloped KI virus and its nucleocapsid, VP79, 64.5, 58, and 54 were inferred to be on the envelope. This is in agreement with the protein profiles observed by silver staining of SDS-PAGE gels (Fig. 2a). Weak iodination of a few capsid proteins in the iodinated (enveloped) virus lane implied damage to viral envelopes in some particles. Of the fourteen envelope proteins in KI (Fig. 2a) only four were prominently iodinated, presumably for the following reasons: (i) limited abundance of the other envelope proteins, (ii) the relative proportion of tyrosine in those proteins is too low to permit detection by iodination, and (iii) those envelope proteins are located in the inner of the two envelopes known to be present in *Oryctes* baculovirus [10] and hence are not accessible for iodination. The inner envelope is acquired in the nucleus during viral assembly and the second while budding from the plasma membrane. From the iodination pattern of viral proteins in salt disrupted nucleocapsids it is clear that VP50, 17.5, and 11.7 are located internally in the capsid, accessible for iodination only when the capsid was disrupted with NaCl. Notable was the absence of labelling of the major nucleocapsid protein VP23.5 in the salt disrupted nucleocapsids. This could possibly be due to its tight association with viral DNA.

Tweeten et al. [30] observed under electron microscope the release of naked DNA as long thin strands from nucleocapsids of GV exposed to 1 M NaCl. The liberation of nucleoprotein core leaving behind empty capsids has also been reported [16] when *Heliothis zea* NPV nucleocapsid was disrupted in 2.5 M NaCl. In the present study we have used dissociation conditions upto 4 M NaCl concentration. The presence of a large proportion of VP23.5 and the absence of other capsid proteins after extraction with 4 M NaCl implied association of DNA and VP23.5 in the nucleoprotein core, rather than a mere contamination of DNA with intact empty capsids. Inclusion of 5 mM EDTA and EGTA along with salt did not enhance disruption of the nucleoprotein core. The differences in the patterns of glycosylated viral proteins between KI and PV505 appear significant and are probably reflected in the antigenic make up of the viral isolates [22] Viral proteins VP40.5, 37.5, 33.5, 27.5, and 26.5 though common to both viral isolates (Fig. 2b), were stained positive for glycoproteins only in KI virus. This could be due to low degree of glycosylation in these PV505 proteins because the staining intensity with PAS is proportional to the extent of glycosylation.

Immunoblotting of the total virus proteins of KI and PV505 revealed the presence of proteins not observed by the Coomassie blue-silver double staining method. The KI capsid protein VP30 was the most immunogenic, followed by envelope protein VP64.5. The striking differences in staining intensities of proteins between KI and PV505 with anti KI serum, point to the overall differences in antigenicity of proteins between the two isolates.

Total antigenic distinctness was found in three KI proteins. Viz. VP93, 27.5, and 23.5 with no crossreactivity with the corresponding proteins in PV505. Many viral proteins common to both isolates Viz. VP103, 77, 73, 64.5, 40.5, 30, and 26.5 were detected by anti KI serum, reflecting the sharing of epitopes on these common proteins. The low staining intensity of many PV505 proteins, however suggests that not all epitopes on the PV505 protein are shared by the corresponding KI protein. The pronounced difference in staining intensity of VP64.5 between KI and PV505 probably represents one end of the scale marking the minimum sharing of epitopes on VP64.5 between the isolates. The antigenic differences seen (in terms of difference in staining intensity in Western blots) among proteins common to both isolates is possibly due to post-translational modifications. Differences in the glycosylation of viral proteins between KI and PV505 have been shown (Fig. 5). Post-translational modifications of baculovirus proteins have been widely reported [9, 13, 20, 28]. The glycoproteins of lymphocytic choriomeningitis viruses have been reported to share identical epitopes [6] due to evolution of these glycoproteins from common precursors [7]. Similarly, cross-reactions between two phenotypes of *Autographa californica* NPV have been attributed to sharing of viral proteins in different modified versions [31].

The detection of many PV505 specific proteins by anti KI serum further substantiates sharing of epitopes, even between proteins that are not common to both isolates. This is possible when proteins in either virus isolate undergo increase or decrease in M_r in response to additions and deletions in the genome, as has been reported in KI genome [11]. In such an event majority of epitopes on the protein would remain unchanged. Closer scrutiny of Fig. 2b shows that viral proteins specific to KI actually differ from the higher or lower M_r PV505 specific proteins by 2–3 kDa. This strengthens the assumption that small additions or deletions in the viral proteins had occurred resulting in altered mobility but with extensive sharing of epitopes. Crawford et al. [11] have located two deletions of −100, −40, and two additions of +70, +70 bp in the KI genome relative to PV505. The PV505 specific proteins, VP97 and 23 (Fig. 2b) were not detected by anti KI serum in Western blots implying that these are totally heterogenous proteins specific to PV505. The antigenic heterogeneity observed between KI and PV505 in indirect ELISA [22] and immunoblots (Fig. 6b) support greater divergence than what could be accounted for by the reported deletions or additions in KI genome [11].

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References

1. Barton RJ (1977) An examination of permeation chromatography on columns of controlled pore glass for routine purification of plant viruses. J Gen Virol 35: 77–87
2. Bedford GO (1980) Biology, ecology and control of palm rhinoceros beetles. Ann Rev Entomol 25: 309–339
3. Brady JN, Winston VD, Consigli RA (1977) Dissociation of polyoma virus by the chelation of calcium ions found associated with purified virions. J Virol 23: 717–724
4. Brady JN, Winston VD, Consigli RA (1978) Characterization of a DNA protein complex and capsomere subunits derived from polyoma virus by treatment with ethylene glycol bis-N,N′-tetraacetic acid and dithiothreitol. J Virol 27: 193–204
5. Brown DA, Harriet MB, Kelly DC (1977) Biophysical properties of the structural components of a granulosis virus isolated from the cabbage white butterfly (*Pieris brassicae*). Virology 81: 317–327
6. Bruns M, Peralta LM, Lehmann-Grube F (1983) Lymphocytic choriomeningitis virus. III. Structural proteins of the virion. J Gen Virol 64: 599–611
7. Buchmeier MJ, Lewicki HA, Tomori O, Oldstone MBA (1981) Monoclonal antibodies to lymphocytic choriomeningitis and Pichinde viruses: generation, characterization and cross-reactivity with other arenaviruses. Virology 113: 73–85
8. Caltagirone LE (1981) Land mark examples in the classical biological control. Ann Rev Entomol 26: 213–232
9. Carstens EB, Tjia ST, Doerfler W (1979) Infection of *Spodoptera frugiperda* cells with *Autographa californica* nuclear polyhedrosis virus. I. Synthesis of intracellular proteins after virus infection. Virology 99: 386–398
10. Crawford AM, Sheehan C (1985) Replication of *Oryctes* baculovirus in cell culture: viral morphogenesis, infectivity and protein synthesis. J Gen Virol 66: 529–539
11. Crawford AM, Zelazny B, Alfiler AR (1986) Genotypic variation in geographical isolates of *Oryctes* baculovirus. J Gen Virol 67: 949–952
12. De Moreno MR, Smith JF, Smith RV (1985) Silver staining of proteins in polyacrylamide gels: increased sensitivity through a combined Coomassie blue-silver stain procedure. Anal Biochem 151: 466–470
13. Dobos P, Cochran MA (1980) Protein synthesis in cells infected by *Autographa californica* nuclear polyhedrosis virus (Ac-NPV): the effect of cytosine arabinoside. Virology 103: 446–464
14. Fraker PJ, Speck JC (1978) Protein and cell membrane iodinations with a sparingly soluble chloroamide, 1,3,4,6-tetrachloro-3,6-diphenyl-glycoluril. Biochem Biophys Res Commun 80: 849–857
15. Huger AM (1966) A virus disease of the Indian rhinoceros beetle, *Oryctes rhinoceros* (Linnaeus), caused by a new type of insect virus, *Rhabdionvirus oryctes*, gen. n., sp. n. J Invertebr Pathol 8: 38–51
16. Kelly DC, Brown DA, Ayres MD, Allen CJ, Walker IO (1983) Properties of the major nucleocapsid proteins of *Heliothis zea* single enveloped nuclear polyhedrosis virus. J Gen Virol 64: 399–408
17. Konat G, Offner H, Mellah J (1984) Improved sensitivity for detection and quantitation of glycoproteins on polyacrylamide gels. Experientia 40: 303–304
18. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685
19. Leslie H, Hay FC (1980) Immunochemicals. In: Practical immunology, 2nd edn. Blackwell, London, pp 240–242
20. Maruniak JE, Summers MD (1981) *Autographa californica* nuclear polyhedrosis virus phosphoproteins and synthesis of intracellular proteins after virus infection. Virology 109: 25–34

21. Matthews REF (1982) Classification and nomenclature of viruses. Fourth report of the International Committee on Taxonomy of Viruses. Intervirology 17: 1–199
22. Mohan KS, Gopinathan KP (1989) Quantitation of serological cross-reactivity between two geographical isolates of *Oryctes* baculovirus by a modified ELISA. J Virol Methods 24: 203–214
23. Mohan KS, Jayapal SP, Pillai GB (1983) Baculovirus disease in *Oryctes rhinoceros* populations in Kerala. J Plant Crops 11: 154–161
24. Moriearity PL (1984) Characterization of antigens by Western blotting. In: Morel CM (ed) Genes and antigens of parasites: a laboratory manual, 2nd edn. Fundacao Oswaldo Cruz, Rio de Janeiro
25. Payne CC (1974) The isolation and characterization of a virus from *Oryctes rhinoceros*. J Gen Virol 25: 105–116
26. Payne CC, Compson D, de Looze SM (1977) Properties of the nucleocapsids of a virus isolated from *Oryctes rhinoceros*. Virology 77: 269–280
27. Stiles B, Wood HA (1983) A study of the glycoproteins of *Autographa californica* nuclear polyhedrosis virus (Ac-NPV). Virology 131: 230–241
28. Summers MD, Smith GE (1978) Baculovirus structural polypeptides. Virology 84: 390–402
29. Summers MD, Volkman LE (1976) Comparison of biophysical and morphological properties of occluded and extracellular nonoccluded baculovirus from in vivo and in vitro host systems. J Virol 17: 962–972
30. Tweeten KA, Bulla LA Jr, Consigli RA (1980) Structural polypeptides of the granulosis virus of *Plodia interpunctella*. J Virol 33: 877–886
31. Volkman LE (1983) Occluded and budded *Autographa californica* nuclear polyhedrosis virus: immunological relatedness of structural proteins. J Virol 46: 221–229
32. Zelazny B, Alfiler A, Mohamed NA (1985) Glass permeation chromatography for purification of the baculovirus of *Oryctes rhinoceros* (Coleoptera: Scarabaeidae). J Econ Entomol 78: 992–994
33. Zelazny B (1977) Occurrence of the baculovirus disease of the coconut palm rhinoceros beetle in the Philippines and in Indonesia. FAO Plant Prot Bull 25: 73–77

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