The Structural Proteins and RNA Components of a Cytoplasmic Polyhedrosis Virus from Nymphalis io (Lepidoptera: Nymphalidae)

By C. C. PAYNE AND T. W. TINSLEY
NERC Unit of Invertebrate Virology, 5 South Parks Road, Oxford OX1 3UB, U.K.
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SUMMARY

The proteins of purified polyhedra of the cytoplasmic polyhedrosis virus (CPV) of Nymphalis io were examined by electrophoresis in polyacrylamide gels containing SDS. The major polypeptide in polyhedra, and in inclusion body protein (polyhedral protein) had a mol. wt. of 37,000, and stained positively for carbohydrate. Purified virus particles contained three polypeptides, with mol. wt. of 116,000, 109,000 and 30,000. RNA extracted from the virus particles had a melting profile characteristic of double-stranded RNA. Nine bands were resolved when this RNA was electrophoresed through 3% polyacrylamide gels. A comparison of the molar proportions of these segments suggests that there are 10 pieces of RNA, which form a genome with a mol. wt. of 14.4 x 10^6. There was good agreement between the sizes of the structural polypeptides, and the estimated coding capacity of four of the RNA segments. CPV virus particles share features in common with reovirus and, in particular, with the ‘core’ particles obtained by the enzymic digestion of intact reovirus particles.

INTRODUCTION

Virus particles of cytoplasmic polyhedrosis viruses (CPVs) resemble reovirus and some other vertebrate and plant viruses in possessing a segmented genome composed of double-stranded RNA and a virus-associated RNA polymerase (Miura et al. 1968; Kalmakoff, Lewandowski & Black, 1969; Lewandowski, Kalmakoff & Tanada, 1969). During replication, many virus particles are occluded within a para-crystalline lattice of protein (polyhedral protein) to form inclusion bodies or ‘polyhedra’. Viruses of this type have been reported in many insect species (Aruga & Tanada, 1971).

Virus particles of the CPV isolated from Bombyx mori (L.) contain five structural polypeptides, while the polyhedral protein consists of two major components (Lewandowski & Traynor, 1972). However, serological differences have been reported between this CPV and those isolated from some other insect species (Cunningham & Longworth, 1968; Krywienczyk, Hayashi & Bird, 1969). In addition, Hayashi & Krywienczyk (1972) have reported differences between the mol. wt. of the RNA segments of B. mori CPV and those of a CPV of Malacosoma disstria (Hubner). We have characterized the structural components of the CPV of Nymphalis io (L.) as part of a study to compare the properties of a number of these viruses.
METHODS

Purification of polyhedra. Polyhedra were extracted from the infected midgut epithelium of fifth-instar larvae of *Nymphalis io*. The infected tissue was removed, placed in 0.03 M-tris-HCl, pH 7.5, 0.025 M-KCl (TK buffer) and washed to remove most of the food material (Hayashi & Bird, 1970). After trituration in a ground-glass tissue grinder, polyhedra were pelleted by sedimentation at 4000 g for 15 min, washed twice in TK buffer and finally resuspended in 0.5% sodium deoxycholate (DOC). The polyhedral suspension was re-centrifuged at 4000 g after 30 min at 20°C, washed to remove excess DOC and further purified on a discontinuous sucrose gradient as described by Hayashi & Bird (1970). The final preparation of polyhedra was standardized by a measurement of protein concentration using a modified Folin test (Bailey, 1967).

Purification of virus particles. Virus particles were extracted from polyhedra by a modification of the method of Cunningham & Longworth (1968). Polyhedra were resuspended in 0.2 M-sodium carbonate-sodium bicarbonate buffer, pH 10.8, at a concentration of 3.5 mg polyhedra per ml buffer. After 3 min, 8 vol. of distilled water were added and the mixture was clarified by low-speed sedimentation. Virus particles were collected from the supernatant fluid by sedimentation at 77000 g for 90 min. The virus pellet was resuspended in TK buffer and purified by density gradient sedimentation on 15 ml, 10 to 50% (w/v) sucrose gradients at 49000 g for 1 h. Virus particle-containing fractions were recovered from the middle of the gradient. After dilution with TK buffer, the virus particles were pelleted by sedimentation at 77000 g for 90 min. Using this procedure, it was possible to extract up to 2 mg virus particles from 100 mg polyhedra.

Preparation of polyhedral protein. In preliminary experiments, it was found that polyhedral protein was degraded during exposure to alkali. To minimize this degradation, polyhedral protein was obtained by dissolving polyhedra for 30 s in 0.2 M-sodium carbonate-sodium bicarbonate, pH 10.8; 30 s was the minimum time required for the suspension of polyhedra to clarify. Molar HCl was added to reduce the pH to 6.0, at which point the polyhedral protein precipitated. The precipitate was collected by low-speed sedimentation and washed three times with 0.1 M-sodium phosphate, pH 6.0, to remove any virus particles which had been trapped within the precipitate. The final precipitate was dissolved in an appropriate sample buffer for polyacrylamide gel electrophoresis.

Polyacrylamide gel electrophoresis of virus proteins. The method used was a modification of that described by Lesnaw & Reichmann (1970). Five % gels were prepared containing 5% (w/v) acrylamide, 0.13% *N,N*-methylene bisacrylamide (bis), 0.1% SDS, 0.06% tetramethylethylenediamine (TEMED), 0.075% ammonium persulphate, 0.1 M-sodium phosphate, pH 7.8; 7 and 10% gels were prepared using the same proportion of acrylamide to bis. Gels were pre-run at 5 mA/gel for 30 min in the electrode buffer (0.1 M-sodium phosphate, pH 7.8; 0.1% SDS; 0.1% 2-mercaptoethanol).

Virus protein samples were prepared by dissolving 20 to 100 μg protein in 50 to 100 μl of 0.1 M-sodium phosphate, pH 7.8, containing 8 M-urea, 1% (w/v) SDS and 1% (v/v) 2-mercaptoethanol (USM buffer). This buffer was sometimes modified by the omission of urea and/or 2-mercaptoethanol. Before use the samples were boiled for one minute.

Electrophoresis was carried out in electrode buffer, at 2 mA/gel for 20 min, followed by 8 mA/gel for a further 5 to 12 h. Gels were fixed and stained as described by Maizel (1966) and scanned at 550 nm in a Pye-Unicam SP 1800 spectrophotometer. Some gels were stained for carbohydrate using the method of Clarke (1964).

Mol. wt. determinations of the virus polypeptides were made in 7%, gels using the method
of Shapiro, Vinuela & Maizel (1967). Transferrin, bovine serum albumin, ovalbumin, lactic dehydrogenase, alpha-chymotrypsinogen A, carbonic anhydrase, myoglobin and cytochrome c (Sigma Chemical Co. Ltd.) were used as standards.

**Extraction of RNA.** RNA was extracted from purified virus particles using a hot phenol-SDS method described by Scherrer (1969). Virus particles were resuspended in 0.01 M-sodium acetate, pH 5.0, 0.05 M-sodium chloride, 1% SDS, and were extracted twice with 90% (v/v) phenol at 60°C. Excess phenol was removed from the aqueous phase by extraction with diethyl ether. The virus RNA was precipitated with 2 vol. of absolute ethanol at −20°C, and washed with ethanol and ether before use in melting point determinations, or for polyacrylamide gel electrophoresis. Alternatively, the RNA was readily released from the virus particle by incubation in 1% SDS at 60°C for 30 min. This was frequently used as a means of preparing a sample for gel electrophoresis.

**Melting-point determinations.** Purified RNA samples were dissolved in SSC (0.15 M-sodium chloride; 0.015 M-sodium citrate, pH 7.0). E260 was measured in 10 mm quartz cells in a Pye-Unicam SP 800 spectrophotometer. A temperature increase of 1°C/min was automatically controlled by a temperature programmer and electrically heated cell holder.

**Polyacrylamide gel electrophoresis of RNA.** Three % polyacrylamide gels were prepared containing 2.83% (w/v) acrylamide, 0.17% bis, 0.15% ammonium persulphate, 0.06% TEMED, 0.1 M-tris-HCl, pH 7.6, 0.02 M-sodium acetate, 0.001 M-EDTA and 0.1% SDS. RNA samples were dissolved in 0.01 M-tris-HCl, pH 7.6, 0.02 M-sodium acetate, 0.001 M-EDTA, and 10% (w/v) sucrose. Electrophoresis was at 3 to 4 mA/gel for up to 24 h in 0.01 M-tris-HCl, 0.02 M-sodium acetate, 0.001 M-EDTA. When the period of electrophoresis exceeded 24 h the electrode buffer was renewed.

Gels were scanned at 260 nm and peak areas of the RNA components were estimated by cutting out and weighing the peaks from the densitometer trace. The areas were converted to molar proportions by dividing the peak area by the mol. wt. of the appropriate segment. The mol. wt. of the RNA segments from *Nymphalis io* CPV were calculated by comparison with the segments of known mol. wt. from *Bombyx mori* CPV (Fujii-Kawata, Miura & Fuke, 1970) run in a parallel gel.

**RESULTS**

**Polypeptide components of polyhedra**

When 100 µg samples of SDS-disrupted polyhedra were fractionated by polyacrylamide gel electrophoresis, five components (P1 to P5) were resolved in 5% gels (Fig. 1a). Average mol. wt. from eight determinations are shown in Table 1. No differences were detected in the number or relative mobilities of the components when 2-mercaptoethanol and/or urea were omitted from sample and electrophoresis buffers or when samples were fractionated on 7 or 10% gels.

In an attempt to assess the purity of polyhedra, a small sample was suspended for 24 h at room temperature in 1% SDS, 0.1 M-phosphate, pH 7.8. Under these conditions, the polyhedra remained apparently intact, and were removed from the suspension by low-speed sedimentation. When the supernatant fluid was electrophoresed on 5% gels, a single, faint band was observed with the mobility of polypeptide P5. This protein is the major component of polyhedra, and has been identified (see below) with polyhedral protein. The traces of this polypeptide in the supernatant fluid could be explained by slight solubilization of the protein on the outside of polyhedra. The absence of any other polypeptides suggested that there were no major external proteinaceous contaminants.
Table 1. Molecular weights of the polypeptide components of Nymphalis io CPV polyhedra

<table>
<thead>
<tr>
<th>Band</th>
<th>Mol. wt.</th>
<th>Standard deviation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>115 800</td>
<td>± 3400</td>
</tr>
<tr>
<td>P2</td>
<td>108 500</td>
<td>± 2700</td>
</tr>
<tr>
<td>P3</td>
<td>87 800</td>
<td>± 4200</td>
</tr>
<tr>
<td>P4</td>
<td>61 500</td>
<td>± 3300</td>
</tr>
<tr>
<td>P5</td>
<td>37 100</td>
<td>± 1700</td>
</tr>
</tbody>
</table>

* Calculated from eight determinations.

Fig. 1. SDS-polyacrylamide gel electrophoresis (5 % gels) of the polypeptides of Nymphalis io CPV polyhedra, virus particles and polyhedral protein (a) polyhedra. (b) 1, polyhedra; 2, virus particles extracted from polyhedra by alkaline dissolution. (c) Polyhedral protein prepared by dissolving polyhedra in alkali for 30 s.

Polypeptide components of virus particles

Virus particle proteins were fractionated into two major and one minor components after dissociation in USM buffer (Fig. 1b). The two major bands migrated at the same rates as polypeptides P1 and P2 from polyhedra. The minor component, referred to as polypeptide P6 (with a mol. wt. of 30 300) was not observed in gels of SDS-treated polyhedra. This protein in polyhedra was present in insufficient quantity to be detected by staining or was masked by the large quantity of polypeptide P5.
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Fig. 2. Alkali-degradation of the polypeptide components of polyhedra. (a) From polyhedra untreated with alkali; (b) from polyhedra exposed to alkali for 2 h; (a) and (b) stained with Coomassie brilliant blue; (c) from polyhedra exposed to alkali for 2 h, stained with the periodic acid-Schiff's reagent.

Polypeptide components of polyhedral protein

When purified polyhedral protein was prepared by dissolving the polyhedra in alkali for 30 s, at least four bands were observed after electrophoresis of 100 µg samples (Fig. 1c). These polypeptides had the same mol. wt. as polypeptides P2 to P5. Polypeptide PI was never observed. However, minor components were frequently visible, with mol. wt. greater than that of P1 (one of these can be seen in Fig. 1c). It is likely from the evidence considered below, that these proteins were multiple aggregates of P5.

When polyhedra were exposed to alkali for a prolonged period, the components of polyhedral protein were degraded. Thus, when polyhedra were dissolved for 2 h in 0.2 M-sodium carbonate-sodium bicarbonate, pH 10.8, and dialysed against USM buffer before electrophoresis, the profile shown in Fig. 2 was obtained. Three fragments with mol. wt. lower than 20000 were observed, plus the two major virus particle polypeptides P1 and P2. This treatment also revealed a diffuse band migrating between the position of P4 and P5 (Fig. 2b). This component (P4a) was absent from virus particles (Fig. 1b), and from purified polyhedral protein degraded with alkali. The possible significance of this material is considered later (see Discussion).

A polypeptide with the mobility of P2 was present in virus particles, and its occurrence in purified polyhedral protein therefore suggested that this was contaminated with virus particle protein. However, when polyhedral protein was purified and then exposed to alkali for 2 h before electrophoresis, the low mol. wt. fragments were observed, but neither of the
high mol. wt. virus particle proteins. This result suggested that component P2 of polyhedra consisted of: (1) a virus particle polypeptide, which was not degraded by alkali, and (2) a component of polyhedral protein which was alkali-degradable.

The marked alkali-lability of all the components of polyhedral protein suggested that they had very similar properties. We considered the possibility that they represented a series of aggregates, despite the observation that the calculated mol. wt. for P2 to P5 did not fit well into a polymeric series based on P5.

Parallel polyacrylamide gels were run, containing SDS-solubilized samples of polyhedral protein. After electrophoresis, one gel was fixed in 15% (w/v) trichloroacetic acid which precipitated and made visible the major protein bands. When bands P5 and P4 were located, the comparable regions of the remaining gels were cut out, the proteins eluted and re-run on 5% gels. The results are shown in Fig. 3. The extracted P5 band contained components
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Fig. 4. The polypeptides of polyhedra (a) stained with Coomassie brilliant blue; (b) stained with the periodic acid–Schiff’s reagent.

with the mobility of P5, P4 and P3, while the major polypeptide in the extracted P4 band had the mobility of P5. This result suggested that P4 was an aggregate (probably a dimer) of P5, which disaggregated after further treatment with USM buffer, and that a small proportion of P5 re-aggregated during elution to produce components P4 and P3.

Additional evidence for the similar nature of these polypeptides was obtained from gels stained with the periodic acid–Schiff’s reagent for carbohydrate. P5, P4 and P3 all stained positively (Fig. 4). However, after alkali-degradation, the carbohydrate became detached from the protein and migrated to the bottom of the gel (Fig. 2c). This detachment of the carbohydrate in the presence of alkali may be a significant feature of the degradation of polyhedral protein.

Virus RNA

Purified RNA extracted from Nymphalis io CPV virus particles had a melting profile characteristic of a double-stranded nucleic acid, with an abrupt increase in absorbance between 88 and 100 °C, and a \( T_m \) of 93 °C (Fig. 5). The small increase in extinction at lower temperatures, similar to that observed by Howell & Verwoerd (1971) in preparations of bluetongue virus RNA, may be attributable to contaminating single-stranded RNA, or to a single-stranded virus component.

When the RNA was fractionated on 3% polyacrylamide gels, nine components were resolved (Fig. 6), with mol. wt. ranging between 0·55 and 2·29 \( \times 10^6 \) (Table 2). When electrophoresis was continued for 40 h, components 1 to 3 were more clearly resolved (Fig. 7), and it was evident from the calculated molar proportions that component 1 consisted of two
Fig. 5. Thermal denaturation profile in SSC of RNA extracted from *Nymphalis io* CPV virus particles.

Fig. 6. Polyacrylamide gel electrophoresis of the RNA components of CPV's. Samples were electrophoresed in 3% gels for 18 h at 3 mA/gel, and scanned at 260 nm. (a) *Nymphalis io* CPV RNA; (b) *Bombyx mori* CPV RNA.
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Table 2. The RNA segments of *Nymphalis io* CPV: molecular weights and molar proportions

<table>
<thead>
<tr>
<th>Segment</th>
<th>Mol. wt. ((\times 10^4))*</th>
<th>Molar proportions ((a))†</th>
<th>Molar proportions ((b))†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(2.29 \pm 0.03)†</td>
<td>3.85</td>
<td>1.94</td>
</tr>
<tr>
<td>2</td>
<td>(2.16 \pm 0.03)†</td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>(2.06 \pm 0.03)†</td>
<td>1.09</td>
<td>1.08</td>
</tr>
<tr>
<td>4</td>
<td>(1.25 \pm 0.03)</td>
<td>1.01</td>
<td>0.91</td>
</tr>
<tr>
<td>5</td>
<td>(1.09 \pm 0.03)†</td>
<td>0.98</td>
<td>0.88</td>
</tr>
<tr>
<td>6</td>
<td>(1.01 \pm 0.03)</td>
<td></td>
<td>0.91</td>
</tr>
<tr>
<td>7</td>
<td>(0.88 \pm 0.03)</td>
<td>1.15</td>
<td>0.78</td>
</tr>
<tr>
<td>8</td>
<td>(0.78 \pm 0.02)</td>
<td>1.14</td>
<td>0.55</td>
</tr>
<tr>
<td>9</td>
<td>(0.55 \pm 0.02)†</td>
<td></td>
<td>0.98</td>
</tr>
<tr>
<td>Total</td>
<td>(14.36)§</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Determined by comparison with the mol. wt. of *Bombyx mori* CPV RNA segments (Fujii-Kawata et al. 1970).
† Molar proportions \((a)\) measured from samples electrophoresed through gels for 18 h, and normalized to segment 7; \((b)\) measured from samples electrophoresed for 40 h and normalized to segment 2.
‡ Standard deviation calculated from eight measurements.
§ This total mol. wt. was obtained by including two segments with a size of \(2.29 \times 10^6\), in agreement with the molar proportion data.

**Fig. 7.** Prolonged electrophoresis (40 h) of the RNA components of (a) *Nymphalis io* CPV; (b) *Bombyx mori* CPV.

RNA segments, of identical or closely similar mol. wt., which had not been resolved. Components 2 to 9 were present in equimolar proportions. If one copy of each segment is present within a virus particle (as in reovirus and *Bombyx mori* CPV) then the genome of *Nymphalis io* CPV contains 10 segments with a total mol. wt. of \(14.36 \times 10^6\). Although *B. mori* CPV also contains 10 segments of RNA (Fujii-Kawata et al. 1970), these were quite distinct from those of *N. io* CPV (Figs. 6, 7).

These calculations were made with the assumption that all the RNA segments observed in gels were double-stranded, and that any single-stranded RNA was not detected. This seems justified, as no bands were observed in such gels when RNA extracts from uninfected...
Table 3. The relationship between the coding capacity of the individual RNA segments of Nymphalis io CPV and the structural polypeptides of polyhedra

<table>
<thead>
<tr>
<th>RNA Segment</th>
<th>Mol. wt. (x 10^-8)</th>
<th>Size of protein*</th>
<th>Virus particle</th>
<th>Polyhedral protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>2.29</td>
<td>115000</td>
<td>115800</td>
<td>—</td>
</tr>
<tr>
<td>1b</td>
<td>2.29</td>
<td>115000</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>2.16</td>
<td>108000</td>
<td>—</td>
<td>108500</td>
</tr>
<tr>
<td>3</td>
<td>2.06</td>
<td>103000</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>1.25</td>
<td>63000</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>1.09</td>
<td>55000</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>1.01</td>
<td>51000</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>0.88</td>
<td>44000</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>0.78</td>
<td>39000</td>
<td>—</td>
<td>37100</td>
</tr>
<tr>
<td>9</td>
<td>0.55</td>
<td>28000</td>
<td>30000</td>
<td>—</td>
</tr>
</tbody>
</table>

* This theoretical protein size was calculated assuming that the messenger RNA transcribed from the virus RNA has a mol. wt. half that of the parental RNA, and that average mol. wt. for nucleotides and amino acids are 330 and 100, respectively.

Nymphalis io or Bombyx mori larvae (known to contain large amounts of ribosomal and other single-stranded RNAs) were electrophoresed for periods of 18 to 40 h.

It has been shown for reovirus and Bombyx mori CPV (Smith, Zweerink & Joklik, 1969; Lewandowski & Traynor, 1972) that a strong correlation exists between the size of the RNA segments of the virus genome, and the size of the virus polypeptides. Similarly for Nymphalis io CPV (Table 3) there is good agreement between the theoretical and experimentally determined mol. wt. of the structural proteins of virus particles and polyhedral protein, assuming that P3 and P4 are aggregates of P5.

**DISCUSSION**

The structural polypeptides detected in samples of Nymphalis io CPV polyhedra were (with the exception of polypeptide 4a) accounted for as components of either virus particles or polyhedral protein. We have suggested that the multiple bands observed in samples of polyhedral protein represent aggregates of one major polypeptide with a mol. wt. of 37000. The evidence for this came from three observations: (1) All components were degraded by alkali. (2) Components P3 to P5 stained for carbohydrate, and may be glycoproteins (P2 may have been insufficiently concentrated to stain). (3) Polypeptides P3 to P5 were detected in purified samples of P4 and P5 re-run on polyacrylamide gels.

The estimated mol. wt. of these ‘polymers’ were not in good agreement with an oligomeric series with a monomer of 37000. However, certain protein polymers migrate at a rate significantly faster than expected from their theoretical mol. wt. (Griffith, 1972). More stringent reducing conditions than 1% 2-mercaptoethanol may eliminate these apparent polymeric forms.

The possibility that carbohydrate is associated with polyhedral protein has been confirmed by the incorporation of radioactive glucosamine in polyhedral protein of Bombyx mori CPV (C. C. Payne & J. Kalmakoff, unpublished results). With Nymphalis io CPV, the detachment of carbohydrate could explain the degradation observed after prolonged exposure to alkali. Thus, if several carbohydrate moieties were attached by an O-glycosidic linkage to seryl or threonyl residues, mild alkaline hydrolysis could remove the carbohydrate
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by β-elimination. Further hydrolysis of the dehydropeptide formed would result in the cleavage of the protein chain (Witkop, 1961), and the appearance of lower mol. wt. components such as those observed in gels.

Lewandowski & Traynor (1972) reported that traces of polypeptides in polyhedra of Bombyx mori CPV were neither components of virus particles nor of polyhedral protein. In Nymphalis io CPV, polypeptide P4a falls into this category. Its diffuse appearance suggests a mixture of polypeptides rather than a single protein. As suggested for B. mori CPV, such components could be non-specifically trapped within polyhedra during their formation and may be virus non-structural proteins, or host-derived proteins.

Although the genomes of Bombyx mori CPV and Nymphalis io CPV are very similar in size (mol. wt. approx \(15 \times 10^6\)), the individual RNA components are not. It is therefore not surprising that the mol. wt. of structural proteins of N. io CPV virus particles and polyhedra differ from those of B. mori CPV (Lewandowski & Traynor, 1972). However, there also appear to be different numbers of structural proteins in the two viruses. Three polypeptides were observed in N. io CPV virus particles, compared with five in B. mori CPV (Lewandowski & Traynor, 1972). In addition, the polyhedral protein of N. io CPV appears to consist of a single kind of polypeptide whereas B. mori CPV contains two.

The relationship between the size of the structural proteins of Nymphalis io CPV and the coding capacity of certain RNA segments is in good agreement with theoretical expectations assuming that each RNA segment is monocistronic. Four segments can at present be accounted for in terms of structural proteins, three for virus particle proteins, and one for the 37000 polyhedral protein.

Cytoplasmic polyhedrosis viruses possess several of the characteristics of reovirus, including a double-stranded RNA genome consisting of discrete segments (Shatkin & Sipe, 1968; Kalmakoff et al. 1969; Fujii-Kawata et al. 1970) and a nucleoside triphosphatase activity (Kapuler et al. 1970; Storer, Shepherd & Kalmakoff, 1974). As observed by Payne (1971) and Lewandowski & Traynor (1972), the similarities are closest between CPV virus particles and the subviral particles or ‘cores’ of reovirus type 3 which are produced by chymotrypsin digestion of the outer capsid (Smith et al. 1969). These cores contain four polypeptides, two of which, like the structural proteins of Nymphalis io CPV virus particles, are of high mol. wt. Bombyx mori CPV virus particles and reovirus ‘cores’ also have similar buoyant densities (Joklik, Skehel & Zweerink, 1970; Lewandowski & Millward, 1971), are resistant to digestion by chymotrypsin (Hayashi & Bird, 1968; Shatkin & Sipe, 1968), contain RNA polymerase and nucleoside triphosphatase activities requiring no activation, and ten segments of double-stranded RNA (Lewandowski et al. 1969). These similarities between CPV virus particles and the single-capsid ‘cores’ of reovirus imply that the complex double-capsid structure proposed for CPVs (Hosaka & Aizawa, 1964) is unsatisfactory and that the actual structure more closely resembles the model proposed by Lewandowski & Traynor (1972).

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REFERENCES


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