Cytoplasmic Polyhedrosis Virus Classification by Electropherotype; Validation by Serological Analyses and Agarose Gel Electrophoresis

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SUMMARY

Serological analyses of several different cytoplasmic polyhedrosis viruses (CPVs), including two type 1 CPVs from Bombyx mori, type 1 CPV from Dendrolimus spectabilis, type 12 CPV from Autographa gamma, type 2 CPV from Inachis io, type 5 CPV from Orgyia pseudotsugata and type 5 CPV from Heliothis armigera, demonstrated a close correlation between the antigenic properties of the polyhedrin or virus particle structural proteins and the genomic dsRNA electropherotypes. The dsRNAs of these viruses were analysed by electrophoresis in 3% and 10% polyacrylamide gels with a discontinuous Tris–HCl/Tris-glycine buffer system or by 1% agarose gel electrophoresis using a continuous Tris-acetate-EDTA buffer system. Electrophoretic analysis in agarose gels was found to be the most suitable for the classification of CPV isolates into electropherotypes, and the results obtained showed a close correlation with the observed antigenic relationships between different virus isolates. However, electrophoretic analysis in 10% polyacrylamide gels was most sensitive for the detection of intra-type variation and the presence of mixed virus isolates.

INTRODUCTION

Cytoplasmic polyhedrosis viruses (CPVs) collectively form a genus within the Reoviridae (Matthews, 1982), whose host range is apparently confined to the Arthropoda (reviewed by Harrap & Payne, 1979; Payne & Harrap, 1977; Payne & Mertens, 1983). Unlike other members of the Reoviridae, which have a two-layer capsid structure (reviewed by Joklik, 1983; Gorman et al., 1983; Holmes, 1983; Francki & Boccardo, 1983), CPV particles have only a single-shelled capsid and are in many ways morphologically and biochemically comparable to the virus cores of other reoviruses. Since CPV particles do not possess an outer capsid layer, they arguably do not contain proteins that are comparable to the outer capsid proteins of other members of the Reoviridae. The outer capsid components of those members of the Reoviridae which have vertebrate hosts include proteins which are directly involved in interaction with neutralizing antibodies and therefore in the determination of virus serotype. Although CPVs do not possess an outer capsid layer, the virus particles are occluded in large numbers during virus replication in large proteinaceous occlusion bodies, or polyhedra (representing approximately 5% of the total protein content of polyhedra). The matrix of these polyhedra is composed of a single protein (polyhedrin) which is synthesized in large amounts during virus replication.

The genome of CPV is composed of 10 separate segments of dsRNA which, as with other members of the Reoviridae, code for the virus structural and non-structural proteins; in most cases one protein is encoded by each piece of dsRNA (McCrae & Mertens, 1983). In the absence of suitable tissue culture systems for the development of serum neutralization tests for virus

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identification, CPVs are provisionally classified on the basis of distinctive variations in the electrophoretic migration patterns of the genome segments (electropherotypes). Twelve distinct CPV electropherotypes have been defined, which differ from one another in the migration of at least three genome segments during PAGE in a 3% polyacrylamide gel and a Tris–acetate buffer system (Payne & Rivers, 1976; Payne et al., 1977; Matthews, 1982; Payne & Mertens, 1983). Minor ‘intra-type’ variations have been detected as small differences in the electrophoretic mobility of only one or two genome segments in this gel system. Differences within electropherotypes are more easily detected by PAGE using gel concentrations higher than 5% polyacrylamide (Payne & Mertens, 1983; Payne et al., 1978, 1983).

Studies with rotaviruses (Clarke & McCrae, 1982) and orbiviruses (Mertens et al., 1987; Pedley et al., 1988) have shown that variations in the migration rates of genome segments during PAGE do not correlate with variations in near-terminal RNA sequence or in virus serotype, respectively. In addition, changes in the order of migration of individual genome segments of bluetongue virus have been observed with changes in polyacrylamide concentration (Mertens & Sangar, 1985). Based on these and other similar observations, the results of electrophoretic analysis of genomic RNA, particularly by PAGE, are not thought to be suitable for the simple classification of most dsRNA viruses.

However, recent studies have demonstrated that most of the considerable variation observed in both 3% and 10% PAGE genome profiles, between orbivirus isolates within a single serogroup, disappears when the dsRNA is analysed by agarose gel electrophoresis (AGE). This type of analysis produces a uniform genome profile which, in the case of bluetongue viruses, Palyam serogroup viruses and most members of the epizootic haemorrhagic disease of deer serogroup, is characteristic of each serogroup (Kowalik & Li, 1987; Brown et al., 1988; Gonzalez & Knudson, 1988; Knudson, 1986; Mertens et al., 1987; Pedley et al., 1988; M. E. H. Mohamed, W. P. Taylor, P. S. Mellor & P. P. C. Mertens, unpublished results). These results, in contrast to earlier results from PAGE, indicate that the electrophoretic analysis of dsRNA virus genomes by AGE may not only have direct relevance to virus serogroup but also has value for the serogroup analysis of new virus isolates.

The purpose of the present study was to examine the suitability of high (10%) and low (3%) concentration polyacrylamide gels, as well as agarose gels, for the electrophoretic analysis of CPV dsRNA and the electropherotype grouping of CPV isolates. In the absence of suitable serum neutralization tests, the viruses analysed by these methods were also compared serologically by ELISA, allowing an analysis of the antigenic relationships of both the polyhedrins and virus structural proteins of different electropherotypes. The viruses used in this study were selected to allow an analysis of both intra- and inter-electropherotype variations.

**METHODS**

*Viruses.* The virus isolates used in this study are listed in Table 1. Three strains of CPV type 1 were studied; two could be distinguished by the cuboidal (Bm1C) or icosahedral (Bm11) shape of the polyhedra produced during infection (Hukuhara, 1971) and by minor differences in the electrophoretic mobility of a small number of genome segments (C. C. Payne, unpublished observations). Both isolates were grown in larvae of *Bombyx mori*. Purified polyhedra of Bm11 were supplied by Dr Shigetoshi Miyajima (Aichi-ken Agricultural Research Centre, Yazaki, Nagakute, Aichi-ken 580-11, Japan). The third type 1 CPV strain (Dsl) was supplied by Dr Kazumasa Katagiri (Forestry and Forest Products Research Institute, 1 Matsumosato, Kukizaki-mura, Inashiki-gun, Ibaraki-ken, Japan). CPV type 2 (II2) (Payne & Tinsley, 1974) was grown in larvae of *Mamestra brassicae*. Purified polyhedra of type 5 CPV (Op5) were supplied by Dr George Rohrmann (Department of Agricultural Chemistry, Oregon State University, Corvallis, Oregon 97331-6502, U.S.A.). CPV type 5 (Ha5) was the A2 virus strain in which one of the larger RNA genome segments (Mr 2.3 × 10^6) had disappeared on repeated passage, with the corresponding appearance of another much smaller segment (Mr 0.4 × 10^6) (Rubinstein & Harley, 1978). The CPV type 12 strain (Ag12) (Payne & Mertens, 1983) was grown in larvae of *M. brassicae*.

Polyhedra were purified from CPV-infected larvae as described by Payne (1976). Virus particles were extracted from polyhedra by treatment with 0.2 M-sodium carbonate/bicarbonate buffer pH 10.8 and purified by subsequent centrifugation on sucrose gradients, as described by Payne (1976).

*Antiserum.* Antiserum to virus particles of the CPVs, Bm1C, II2 and Ag12 were prepared in rabbits. Samples of 1 ml of purified virus particles (200 μg/ml) were emulsified in Freund’s complete adjuvant and injected intramuscularly. Two further intramuscular injections of the same quantity of antigen emulsified in Freund’s
Table 1. Details of the CPV isolates used

<table>
<thead>
<tr>
<th>Virus electropherotype*</th>
<th>Host†</th>
<th>Abbreviation used in text</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (cuboidal polyhedra)</td>
<td>Bombyx mori</td>
<td>Bm1C</td>
<td>Payne &amp; Rivers (1976)</td>
</tr>
<tr>
<td>1 (icosahedral polyhedra)</td>
<td>Bombyx mori</td>
<td>Bm1I</td>
<td>Hukuhara (1971)</td>
</tr>
<tr>
<td>1</td>
<td>Dendrolimus spectabilis</td>
<td>Ds1</td>
<td>Payne et al. (1978)</td>
</tr>
<tr>
<td>2</td>
<td>Inachis io</td>
<td>Ii2</td>
<td>Payne &amp; Tinsley (1974)</td>
</tr>
<tr>
<td>5</td>
<td>Heliothis armigera</td>
<td>Ha5</td>
<td>Rubinstein &amp; Harley (1978)</td>
</tr>
<tr>
<td>5</td>
<td>Orgyia pseudotsugata</td>
<td>Op5</td>
<td>Rohrmann et al. (1980)</td>
</tr>
<tr>
<td>12</td>
<td>Autographa gamma</td>
<td>Ag12</td>
<td>Payne &amp; Mertens (1983)</td>
</tr>
</tbody>
</table>

* From the provisional classification of CPVs by electropherotype as given in Payne & Rivers (1976) and Payne & Mertens (1983).
† The insect host from which each virus isolate was first recorded.

incomplete adjuvant were made at weekly intervals after the first injections. Control sera were obtained before the first injections and antisera were collected 3 weeks after the final injections. Antiserum to virions of Ha5 was prepared in rabbits following two intramuscular injections, with a 1-week interval between them, of purified virions mixed with an equal volume of Freund's complete adjuvant. Two weeks after the second injection a final injection of purified virus was made. Serum was removed 2 weeks after the final injection. Antiserum to polyhedra of CPVs Bm1C and Ii2 were produced in rabbits, as described by Payne & Churchill (1977). The antiserum to these polyhedra were adsorbed with homologous purified virus particles to remove virion-specific antibodies, by incubating 1 ml antiserum with 1 ml virus particles (200 µg/ml) in phosphate-buffered saline (PBS) pH 7.0 for 1 h at 37 °C. The resulting precipitate was removed by centrifugation (3000 g, 15 min). The supernatant was then adsorbed twice more as above. Rabbit antiserum to the polyhedrin fraction of Op5 polyhedra was prepared using SDS-PAGE-purified polyhedrin and was kindly supplied by Dr G. F. Rohrmann. Antiserum to polyhedrin of Ha5 CPV polyhedra was prepared in rabbits using SDS-PAGE-purified polyhedrin. Antiserum to the synthetic polyribonucleotide poly(rI-rC) was prepared as described by Payne & Kalmakoff (1975).

dsRNA. Genomic dsRNA was extracted from either purified polyhedra or from virus particles as described by Payne & Tinsley (1974). The dsRNA was labelled at the 3' termini with cytidine 3',5'-[5'-32p]bisphosphate and RNA ligase as described by England & Uhlenbeck (1978) and England et al. (1980).

Electrophoresis. CPV genomic dsRNA was analysed in either 3% or 10% polyacrylamide gels and a discontinuous buffer system as described by Laemmli (1970). The RNA bands were detected by autoradiography of the wet gel. RNA samples were also analysed in 1% agarose gels containing a Tris-acetate EDTA buffer system as described by Maniatis et al. (1982). RNA bands were stained with ethidium bromide and photographed under u.v. illumination. RNA genome segments are referred to in the text by number in order of increasing electrophoretic mobility, and the larger segments are at the top in each figure.

Serological comparisons of CPV antigens

Gel diffusion tests. Gel diffusion tests of CPV polyhedrins against homologous and heterologous antisera were carried out in 0-5% agarose gels in PBS. The antigens used were CPV polyhedra dissolved for 15 min in 0-2 M-sodium carbonate/bicarbonate buffer pH 10-8 to give a concentration of 1 mg/ml. Antiserum were used at a dilution of 1 : 10 in PBS. After incubation at room temperature for 48 h the gels were washed in PBS (24 h) and distilled water (24 h) and then photographed.

ELISA. Antigenic relationships between polyhedrin and virus particle antigens were evaluated using the indirect ELISA method described by Crook & Payne (1980). Comparisons between polyhedrins were made using alkali-dissolved CPV polyhedra as antigens. Polyhedra (1 mg/ml) were dissolved in 0-2 M-sodium carbonate/bicarbonate buffer pH 10-8 for 15 min. They were then diluted with 0-05 M-carbonate/bicarbonate buffer pH 9-6 (coating buffer) to concentrations of 0-03 to 1-0 µg/ml. Samples were then allowed to adsorb to the wells of polystyrene Microelisa plates for 16 to 18 h at 6 °C. Purified virus particles were used as antigens at concentrations of 0-1 to 3-0 µg/ml and were diluted in coating buffer before being added to the Microelisa plates. The purification of IgG fractions from antisera and further details of the ELISA methodology used are given in Crook & Payne (1980).
RESULTS

Electropherotype analysis of CPV genomic RNA

Intra-type variation

Electrophoretic comparisons were made of the genomic dsRNAs of CPV isolates listed in Table 1 by 1% AGE, 3% PAGE and 10% PAGE (Fig. 1 to 3). The results of these analyses are summarized in Table 2.

By AGE, no significant differences were detected in the migration of any of the comparable genome segments from the three isolates of electropherotype 1 CPV (BmlC, BmlI and Dsl) (Fig. 1c). When the RNAs of these type 1 viruses were compared by 3% PAGE they also produced essentially similar, although apparently not identical, genome profiles, with the overall pattern of migration of the BmlC genome segments moving slightly ahead of that of BmlI (Fig. 1b). However comigrational analysis by 3% PAGE revealed that the majority of these apparent differences were due to variations between individual lanes of the 3% gel, rather than to actual migrational differences between the comparable genome segments of the type 1 viruses (Fig. 2, lanes 1 to 6). Minor differences were detected in this way in the migration of only two genome segments (number 5 and 8) between the RNA of Dsl and BmlC CPV (as indicated by arrowheads; Fig. 2, lane 4) and no differences were detected between BmlC and BmlI RNAs. These results indicate that comigrational analysis is essential for a valid comparison by 3% PAGE of the genomic RNA of closely related CPV. This concentration of polyacrylamide was originally used to define CPV electropherotypes (Payne & Rivers, 1976) and to classify these three viruses within CPV electropherotype 1 (Payne et al., 1978, 1983; Payne & Mertens, 1983).

In contrast, analysis of electropherotype 1 CPV genomic dsRNAs by 10% PAGE revealed differences between Dsl and either BmlC or BmlI in seven of the resolvable genome segments (although not in the same group of segments in each case) (Fig. 1a; Fig. 3, lanes 2, 3 and co-run in lane 6; Table 2). The BmlC and BmlI virus RNAs (which were indistinguishable by AGE or 3% PAGE) were also found to differ slightly in the migration of four genome segments (3, 4, 9 and 10) by 10% PAGE (Fig. 1a, Table 2), including segment 10 which encodes the polyhedrin protein of electropherotype 1 CPV (McCrae & Mertens, 1983).

Analysis of Dsl genomic dsRNA by 10% PAGE resolved a submolar or minor RNA band in the region of genome segment 7 (indicated by an arrow in Fig. 3, lane 3). Genome segment 5 of BmlI was also resolved into two submolar bands in this gel system (indicated by arrowheads in Fig. 1a). The detection of minor RNA bands is thought to indicate the presence of two closely related but distinguishable virus strains in these virus isolates.

Material was not available to allow comparison of the two isolates of CPV electropherotype 5 (Ha5 and Op5) by AGE or 3% PAGE analysis of their genomic RNA. However, previous studies by 0-75% AGE and 4-5% PAGE demonstrated that these viruses produce very similar genome profiles (Galinski et al., 1983). They were found to differ in the migration of only a single genome segment, due to a major deletion of RNA sequence in genome segment 2 of the Ha5 virus, resulting in the production of an additional small genome segment (indicated by arrows in Fig. 3, lane 7) (Rubinstein & Harley, 1978). When these two isolates of electropherotype 5 CPV were analysed in a 10% PAGE system, migrational differences were detected in all of the genome segments except number 4 (Fig. 3, lanes 7 and 8; Table 2). In addition, several minor bands were resolved in the genome profile of the Op5 virus, most notably in the resolution of three minor bands in the position of genome segment 6 (indicated by arrows in Fig. 1a and Fig. 3, lane 8). In contrast, in the 3% PAGE system, segment 7 of Op5 was resolved into two minor bands (indicated by arrowheads in Fig. 1b), while segment 6 formed a single major band. This suggests that, as has been observed with segments 5 and 6 of bluetongue viruses (Mertens & Sangar, 1985), these segments alter their relative order of migration depending on the concentration of polyacrylamide used for PAGE. The minor bands detected in the genome profiles of the Op5 virus isolate suggest the presence of several closely related but distinct virus strains.
The analysis by electrophoresis of the genomic dsRNA of six different isolates of cytoplasmic polyhedrosis viruses. \(^{32}P\) radiactively labelled CPV dsRNA preparations were analysed in 10% (a) and 3% (b) polyacrylamide gels containing a Laemmli (1970) buffer system. The bands were detected by autoradiography of the wet gels. In (c) unlabelled CPV dsRNA was analysed in a 1% agarose gel system (Maniatis et al., 1982); after electrophoresis the bands were stained with ethidium bromide and photographed under u.v. illumination. Some intensely staining bands contain two or more genome segments which have comigrated. Genomic segments are referred to by number, in order of increasing electrophoretic mobility, as indicated at the sides of each gel for the genome segments of BM1 and I12 CPV. Lanes 1, BM1; lanes 2, BM1C; lanes 3, D51; lanes 4, Ag12; lanes 5, Op5; lanes 6, I12. For details of arrowheads see text.
Fig. 2. Comigrational analysis of electropherotype 1 and electropherotype 12 radiolabelled dsRNA after 3% PAGE. The lanes contained dsRNA from viruses as follows: Bm11, lane 1; Bm1I and Bm1C, lane 2; Bm1C, lane 3; Bm1C and Ds1, lane 4; Ds1, lane 5; Ds1 and Agl2, lane 6; Agl2, lane 7. Evidence of migrational differences between segments, if any, was detected as double bands in those lanes loaded with the RNA of two virus isolates (lanes 2, 4 and 6), as indicated by arrowheads. The RNA bands were detected by autoradiography of the frozen gel.

**Inter-type variations**

When the genomic dsRNAs from Ji2 and Op5 CPV were compared (by AGE, 3% PAGE and 10% PAGE) to each other and to those of either the electropherotype 1 CPV isolates (Bm1C, Bm1I, Ds1) or Agl2 CPV, similarities were detected in the migration of only a single genome segment if at all (Fig. 1; Table 2). The patterns of genome segment migration of these viruses showed no overall similarities, regardless of the gel system used. These results confirm the earlier observations of Payne & Rivers (1976).

In contrast the genome profile of Agl2 CPV does show some overall similarity to those of the electropherotype 1 CPV isolates (Fig. 1 and 2; Table 2). However, significant differences were detected between Agl2 and the electropherotype 1 viruses in the migration of four genome segments during AGE (segments 5, 7, 8 and 9). PAGE (3%) was originally used to compare Agl2 and Bm1C CPV RNAs and to classify them as distinct but related electropherotypes (Payne *et al.*, 1977). When 35 cm long 3% polyacrylamide gels were used in this study, five migrational differences were detected between the genome segments of Agl2 and Ds1 CPV (segments 6 to 10) and six migrational differences were detected between those of Agl2 and either Bm1C or Bm1I CPV (segments 5 to 10) (Fig. 2; Table 2). When the RNAs of these viruses were analysed by 10% PAGE, differences were again detected in the migration of six genome segments between Agl2 and Ds1 CPV (numbers 1, 2, 4, 5, 7 and 9). However, since segments 6, 8 and 10 of these viruses comigrate during 10% PAGE, the differences observed were not in the same segments that were detected as different by AGE or 3% PAGE (Table 2).
Fig. 3. Analysis in 10% polyacrylamide gels of unlabelled genomic dsRNA of electropherotype 1, 12 and 5 CPVs. The lanes contained dsRNA from viruses as follows: Agl2, lane 1; BmlI, lane 2; Dsl, lane 3; co-run of Dsl and Agl2, lane 4; co-run of Agl2 and BmlI, lane 5; co-run of BmlI and Dsl, lane 6; Ha5, lane 7; Op5, lane 8. After electrophoresis, the RNA bands were stained with ethidium bromide, detected and photographed under u.v. illumination. For details of arrows see text.

Table 2. Summary of results obtained by electrophoretic comparison of CPV genomic dsRNAs

<table>
<thead>
<tr>
<th>Virus genomes compared</th>
<th>Genome segments showing evidence of migrational differences*</th>
<th>3% PAGE</th>
<th>10% PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>BmlI-BmlII</td>
<td>None</td>
<td>None</td>
<td>3, 4, 9, 10</td>
</tr>
<tr>
<td>BmlI-Dsl</td>
<td>None</td>
<td>5, 8</td>
<td>3 to 8, 10</td>
</tr>
<tr>
<td>BmlI-Dsl</td>
<td>None</td>
<td>5, 8</td>
<td>3, 5 to 10</td>
</tr>
<tr>
<td>Agl2-Dsl</td>
<td>5, 7, 8, 9</td>
<td>6 to 10</td>
<td>1, 2, 4, 5, 7, 9</td>
</tr>
<tr>
<td>Agl2-BmlI</td>
<td>5, 7, 8, 9</td>
<td>5 to 10</td>
<td>1 to 10</td>
</tr>
<tr>
<td>Agl2-Dsl</td>
<td>5, 7, 8, 9</td>
<td>5 to 10</td>
<td>1 to 10</td>
</tr>
<tr>
<td>Ii2-BmlI</td>
<td>1 to 10</td>
<td>1 to 10</td>
<td>1 to 10</td>
</tr>
<tr>
<td>Ii2-Dsl</td>
<td>1 to 10</td>
<td>1 to 10</td>
<td>1 to 10</td>
</tr>
<tr>
<td>Ii2-Agl2</td>
<td>1 to 10</td>
<td>1 to 10</td>
<td>1 to 10</td>
</tr>
<tr>
<td>Op5-BmlI</td>
<td>1 to 10</td>
<td>All except 3</td>
<td>1 to 10</td>
</tr>
<tr>
<td>Op5-BmlI</td>
<td>1 to 10</td>
<td>All except 3</td>
<td>1 to 10</td>
</tr>
<tr>
<td>Op5-Dsl</td>
<td>1 to 10</td>
<td>All except 3</td>
<td>1 to 10</td>
</tr>
<tr>
<td>Op5-Agl2</td>
<td>1 to 10</td>
<td>All except 3</td>
<td>All except 4</td>
</tr>
<tr>
<td>Op5-Ii2</td>
<td>1 to 10</td>
<td>All except 4</td>
<td>1 to 10</td>
</tr>
<tr>
<td>Op5-Ha5</td>
<td>ND‡</td>
<td>ND‡</td>
<td>All except 4</td>
</tr>
</tbody>
</table>

* In addition to migrational differences in comparable genome segments, submolar RNA bands, or the resolution of a genome segment into two or more submolar bands, were observed in the 3% PAGE system with the genomic RNA of Op5 CPV, in the 10% PAGE system with the RNA of BmlI, Dsl, Op5 and (as a result of a deletion in some copies of genome segment 2) of Ha5 CPV. No submolar bands were detected after AGE.

‡ ND, Not done. Comparison of Ha5 and Op5 CPV genomic dsRNAs by 0-75% AGE and 4-5% PAGE (Galinski et al., 1983) have shown no significant migrational differences, except as the result of a deletion of RNA sequence in genome segment 2 of Ha5 CPV, resulting in the appearance of an additional smallest genome segment (Rubinstein & Harley, 1978).
Still more differences were detected by 10% PAGE between the genome segments of Ag12 and either Bm1C or Bm11 CPV than were detected by either 3% PAGE or AGE (Fig. 1a; Fig. 3, lanes 1, 2 and co-run in lane 5), resulting in the failure of any of the comparable genome segments to comigrate exactly (summarized in Table 2).

Collectively, these results demonstrate that the AGE system is most effective for detecting similarities between those CPV isolates analysed which are presently grouped within a single electropherotype (type 1), while still retaining the ability to distinguish different electropherotypes. The 10% PAGE system appeared to be most effective for the analysis of variations within a single electropherotype, particularly for the detection of minor bands and hence mixed virus populations within a single isolate. However, because of migrational variations, which are dependent on the concentration of polyacrylamide used during PAGE, not all of the differences between genome segments detected using the 3% polyacrylamide gel system were also detected by PAGE in the 10% gel system and vice versa.

**Antigenic properties**

The antigenic properties of both the polyhedrin and virus particle fractions were examined to analyse the inter- and intra-electropherotype serological relationships between CPV structural proteins.
Polyhedrin

In agarose gel immunodiffusion tests, using alkali-dissolved polyhedra and antisera prepared against the polyhedrin fraction, no cross-reactions were observed between Bm1C, Ii2 and Ha5 CPV antigens (Fig. 4). In contrast, Bm1C and Dsl CPV antigens cross-reacted, giving a line of apparent identity with Bm1C polyhedrin antiserum. Ha5 and Op5 polyhedrins were also indistinguishable by their reactions with homologous and heterologous antisera (Fig. 4). These results indicate that CPV polyhedrin antigens of distinct CPV electropherotypes do not cross-react, while cross-reactions are strong between viruses classed within the same electropherotype.

Antigenic relationships were evaluated in more detail using the indirect ELISA method. The results obtained (Fig. 5) show that there were no significant cross-reactions, at a range of antigen concentrations, between the polyhedrin antigens of the distinct CPV electropherotypes Bm1C, Ii2 and Ha5. In contrast the two electropherotype 5 polyhedrin antigens cross-reacted strongly. Polyhedrins of Bm1C and Dsl both reacted with antibodies to Bm1C polyhedrin, as did that of Ag12, the order of the intensity of reaction being Bm1C > Dsl > Ag12 (Fig. 5). These results indicate, as suggested by the overall similarity between their genome profiles (Fig. 1), the existence of some antigenic similarity between the polyhedrin antigens of electropherotype 1 and 12 viruses, although at a much lower level than observed between the different isolates of electropherotype 1 CPV.

Virus particle antigens

The indirect ELISA was also used to assess the antigenic relationships between structural proteins of CPV virus particles, using IgG raised against purified virions (Fig. 6). The results obtained showed considerable similarity to those produced with polyhedrin IgG. No significant serological cross-reactions were detected between virions of CPV electropherotypes 1, 2 or 5, the virion proteins reacting only with homologous antisera. Antigenic relationships between Bm1C, Dsl and Ag12 virion proteins were clearly demonstrated, with particularly strong cross-reactions between Bm1C and Dsl antigens (Fig. 6). Virus particle structural proteins of Op5 and Ha5 also cross-reacted at a high level (data not shown). When IgG raised against the artificial dsRNA poly(rI–rC) (Payne & Churchill, 1977) was used in these assays in place of anti-virus antibody, no positive reactions were detected. This confirms that none of the cross-reactions observed was due to antibodies to viral dsRNA.
Fig. 6. Serological comparisons of virus particles of Bm1C, Ds1, li2, Ha5 and Agl2 CPVs as measured by indirect ELISA. Virus particles were used as antigen at concentrations of 0.1 to 3.0 μg/ml as indicated. (●) Bm1C, (○) li2, (■) Ha5, (△) Ds1, (◇) Agl2. Specific antibodies to the virus particles of the different viruses were used separately to obtain the results presented as follows: (a, b) anti-Bm1C (V) [antibody to virus particles of electropherotype 1 CPV (cuboidal polyhedrin) from B. mori]; (c) anti-Agl2 (V); (d) anti-Ha5 (V); (e) anti-li2 (V).

DISCUSSION

Within the limited number of virus isolates assessed in this study the virus particle and polyhedrin proteins of CPV both show variations of their antigenic properties between different virus isolates (as detected by ELISA and agar gel immunodiffusion tests), which correlate well with the virus RNA electropherotype. High levels of cross-reaction were detected in both polyhedrin and virus structural proteins between members of a single electropherotype, and relatively lower or no cross-reaction was detected between any of these proteins from different electropherotypes. Collectively, these results support the validity of the existing CPV classification by electropherotype, indicating that with the exception of type 1 and 12 viruses, each electropherotype is serologically unrelated to the others. The CPV electropherotypes 1 and 12 are clearly related antigenically and show some similarity in their overall genome profiles. However, the low level of cross-reaction observed suggests that these viruses should still be classed as distinct although related virus types.

These results contrast with those from reovirus, rotaviruses and orbiviruses (Both et al., 1984; Grubman et al., 1983; Appleton & Letchworth, 1983; Huismans & Erasmus, 1981; reviewed by Holmes, 1983; Joklik, 1983; Gorman et al., 1983), where those outer capsid proteins directly involved in serum neutralization show high levels of serotype-specific variability, while at least some of the core structural proteins and non-structural proteins show serological cross-reactions and include serogroup-specific antigens. These differences may well result from the absence of neutralizing antibodies in members of the Arthropoda and a consequent absence of antibody selective pressure during the replication of CPV. Such pressure could be expected to cause a relatively increased rate of antigenic variation in those outer capsid proteins of other members of the Reoviridae which bind neutralizing antibodies during infection of mammalian hosts. The CPV structural and polyhedrin proteins may therefore be more directly comparable in their antigenic properties to the core structural and non-structural proteins of other members of the Reoviridae.

Cross-hybridization and sequencing studies with these other members of the Reoviridae have also demonstrated a higher level of RNA sequence variation which correlates with virus serotype, in those genome segments which code for the outer capsid proteins, while high levels of serogroup-specific sequence conservation are detected in the remainder of the virus genome (Bodkin & Knudson, 1986; Mertens et al., 1987; Gonzalez & Knudson, 1987, 1988; Gould, 1987; Purdy et al., 1985, 1986; Huismans & Cloete, 1987; Both et al., 1984; Kowalik & Li, 1987;
In contrast, cross-hybridization studies show relatively uniform sequence variation in all 10 CPV genome segments (P. P. C. Mertens, S. Pedley & C. C. Payne, unpublished results; Galinski et al., 1983). The antigenic variation of the CPV structural proteins and the RNA sequence variation detected correlate well with the classification of CPV isolates by electropherotype.

The results of serum neutralization tests for those members of the Reoviridae which replicate in mammalian hosts are clearly important, particularly in view of the significance of such interactions with the hosts' immune system, in the replication, spread and control of these viruses. However, classification based solely on the results of these tests inevitably ignores similarities or variations which exist in those proteins (and in the portions of the genome from which they are translated) that are not involved in the reaction with neutralizing antibodies. Since CPVs do not possess outer capsid proteins, and members of the Arthropoda do not produce antibodies, the whole concept of classification by serotype is arguably not relevant to these viruses. However, electropherotype analysis not only analyses the whole of the CPV genome, but also produces results which correlate well with the antigenic properties of the viral proteins and the results of cross-hybridization studies.

Since AGE appears to separate dsRNA molecules primarily on the basis of $M_r$ (Pedley et al., 1988; Mertens et al., 1987), variations in RNA sequence and base composition which appear to cause additional variations in their migration rates during PAGE analysis are not detected. The close similarity of genome profiles from viruses within single orbivirus serogroups when analysed by this system (Gonzalez & Knudson, 1988; Knudson, 1986; Pedley et al., 1988; Kowalik & Li, 1987) suggest that the large differences in genome profiles observed between distinct CPV electropherotypes are indicative of differences that are as great or greater than those between orbivirus serogroups. This conclusion is supported by both the low levels of serological cross-reactivity between polyhedrin and virus structural proteins from different CPV electropherotypes and by RNA cross-hybridization studies (P. P. C. Mertens, S. Pedley & C. C. Payne, unpublished results; Galinski et al., 1983).

It remains to be seen whether the genome profile variations observed between rotavirus isolates when analysed by PAGE are also detected by AGE.

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REFERENCES


CPV classification


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