Demonstration of Serological Relationships among Isolates of Barley Yellow Dwarf Virus by Using Polyclonal and Monoclonal Antibodies

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SUMMARY

Hybridomas secreting monoclonal antibodies against three isolates of barley yellow dwarf virus (BYDV) were established. Two monoclonal antibody preparations were generated against the MAV isolate, one against RPV and six against P-PAV. None of the monoclonal antibody preparations reacted with healthy host components. Reactions of monoclonal antibody preparations or unlabelled polyclonal antisera, in an indirect enzyme-linked immunosorbent assay (ELISA) indicated that all three virus isolates share a common epitope. BYDV particles dissociated when incubated in carbonate-bicarbonate coating buffer at pH 9.6 but could be stabilized by prior dialysis against 2% formaldehyde or 2% glutaraldehyde. In indirect ELISA, unlabelled polyclonal antisera bound to both stabilized and dissociated particles of homologous and heterologous BYDV isolates. However, conjugated polyclonal antisera were incapable of binding to dissociated particles or to stabilized particles of heterologous isolates. Experiments with monoclonal antibodies in a competition ELISA indicated the presence of at least two epitopes on the coat protein of P-PAV.

INTRODUCTION

Barley yellow dwarf virus (BYDV), the type member of the luteovirus group (Rochow & Israel, 1977), comprises a number of obligately aphid-transmitted, phloem-limited viruses of Gramineae that occur worldwide in wheat, barley, oats and many perennial grasses (Rochow, 1970). Several distinct isolates have been given acronyms based upon their specificity of aphid transmission (Rochow, 1969; Johnson & Rochow, 1972). Of these, 'RPV' is specifically transmitted by Rhopalosiphum padi, 'MAV' is specifically transmitted by Macrosiphum (= Sitobion) avenae, and 'PAV' is transmitted by either aphid species. These three isolates also differ immunogenically such that, in tests with polyclonal rabbit antisera, MAV and PAV are distinct but related, whereas RPV is unrelated to either (Lister & Rochow, 1979; Rochow & Carmichael, 1979). In this paper, we describe the production of monoclonal antibodies (Köhler & Milstein, 1975) against these three isolates of BYDV and the use of these antibodies in studies of relationship by enzyme-linked immunosorbent assays (ELISA). We report the presence of a common epitope on all three virus isolates and the presence of at least two specific epitopes on the P-PAV isolate. This is the first reported evidence for serological relationship among all three virus isolates. A preliminary report has appeared (Diaco et al., 1983).

METHODS

Cells and virus. The mouse myeloma cell line SP2/O-Ag-14, obtained from R. A. Van Deusen, National Veterinary Services Laboratories, Ames, Iowa, U.S.A. was maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS; Hazelton-Dutchland Laboratories, Denver, Pa., U.S.A.), 25 mM HEPES, 6 g/l glucose, 0.5 g/l sodium bicarbonate and 1.2 g/l L-glutamine.

The RPV, MAV, PAV (RC-PAV) isolates were kindly supplied by W. F. Rochow, and the P-PAV isolate was that previously described (Hammond et al., 1983). All BYDV was purified at Purdue University as previously described (Hammond et al., 1983) and shipped frozen to Iowa State University.
Establishment of hybridoma clones. Four-week-old BALB/c mice (Charles River Breeding Laboratories, Wilmington, Ma., U.S.A.), two for each isolate, were injected intraperitoneally with 0.3-0.4 ml containing 50 μg of purified virus (based on $A_{260,1cm} = 5.6$; Hammond et al., 1983) emulsified in Freund's complete adjuvant, followed by an additional 25 μg of virus in 0.1 ml-sodium phosphate pH 6.0, 4 to 6 weeks later. Four days after hyperimmunization, the mice were exsanguinated, and the serum was retained as a positive control for future testing. Spleen cells were fused by a modification of previously described methods (Van Deusen & Whetstone, 1981).

Cells were collected by centrifugation at 225 g for 10 min and resuspended at a density of $2.5 \times 10^6$ myeloma cells/ml in a medium consisting of equal volumes of conditioned medium (CM; DMEM that had supported growth of SP2/O-Ag-14 cells for 3 to 4 days) and DMEM supplemented with $10^{-4} \mu$M-hypoxanthine, $4 \times 10^{-7} \mu$M-aminopterin and $1 \times 10^{-5} \mu$M-thymidine (DMEM-CM-HAT, Hazelton-Dutchland Laboratories). They were plated at 0.2 ml/well into 96-well tissue culture plates (Costar) and the cultures were incubated at 37°C in humidified 5 to 7% CO$_2$ in air.

Wells containing microscopically visible hybrid clusters were tested for relevant antibody production by ELISA (see below). Cultures expressing positive reactions were slowly weaned by replacement of the HAT medium with HT medium (i.e. HAT with no aminopterin, Hazelton-Dutchland Laboratories). Specific antibody-producing hybridomas were cloned three times by limiting dilution (Galfr6 & Milstein, 1981) into DMEM-CM supplemented with an additional 10% FBS. Positive cell lines were used for in vitro propagation of monoclonal antibody. Once established, cell lines were frozen at $-70°C$ in Nunc cryogenic vials (Vanguard International, Neptune, N.J., U.S.A.) at a cell density of $10^6$ to $2 \times 10^6$ cells/ml in FBS containing 10% dimethyl sulfoxide and then stored in liquid nitrogen. Ascites fluid was obtained by intraperitoneal injection of BALB/c mice, primed 3 to 4 weeks earlier by intraperitoneal injection of 0.5 ml pristane (2,6,10,14-tetramethylpentadecane), with $0.5 \times 10^7$ cells/ml in a medium consisting of equal volumes of conditioned medium (CM; DMEM that had supported growth of SP2/O-Ag-14 cells for 3 to 4 days) and DMEM supplemented with $10^{-4} \mu$M-hypoxanthine, $4 \times 10^{-7} \mu$M-aminopterin and $1 \times 10^{-5} \mu$M-thymidine (DMEM-CM-HAT, Hazelton-Dutchland Laboratories). They were plated at 0.2 ml/well into 96-well tissue culture plates (Costar) and the cultures were incubated at 37°C in humidified 5 to 7% CO$_2$ in air.

ELISA. All ELISA procedures with monoclonal antibodies utilized optimized conditions, found by calculation of P/N ratios (Hill et al., 1981), to yield the highest response (P) over background (N). This was necessary to eliminate false positive reactions frequently observed when suboptimal conditions were used (R. Diaco, unpublished results). ELISA procedures with polyclonal antisera were as described by Lister & Rochow (1979).

Purification and biotinvlation of IgG. Monoclonal antibodies were purified by affinity chromatography of ascites fluid with Protein A-Sepharose CL-4B (Pharmacia). IgG was bound from solution in 0.1 M-sodium phosphate pH 8.0, and eluted with 5.0% acetic acid in saline pH 3.0. Concentrations of IgG were determined by $A_{290,1cm} = 1.4$. Samples were adjusted to pH 7.0 to 8.0 with NaOH, diluted 1:1 with glycerol, and stored as described for ascites fluid. The purified monoclonal antibody preparations were biotinylated by the method of Bayer et al. (1979). Biotinyl-N-hydroxysuccinimide ester (Sigma) dissolved in dimethyl formamide was added to Protein A-purified IgG in a 1:50 (v/v) and 10:1 (mol/mol) ratio. The mixture was rotated for 4 h at room temperature, stored at 4°C overnight, and then extensively dialysed against 0.01 M-sodium phosphate pH 8.0, at 4°C. The biotinylated antibodies were stored as described for ascitic fluid.

Immunoglobulin subclass determination. The antibody subclass and light chain component of each of the monoclonal antibody preparations was determined by ELISA using each of the following class- and subclass-specific rabbit anti-mouse immunoglobulins: rabbit anti-mouse IgA, IgG1, IgG2a, IgG2b, IgG3, IgM, kappa light chain and lambda light chain (Zymed Laboratories, Burlingame, Ca., U.S.A.).
A410 of the sample was greater than the mean A410 of the controls plus three standard deviations; samples with A410 values below this level were considered negative. Background reactions were consistently less than or equal to 0.08. Experiments performed to optimize this ELISA showed that the formulation of the blocking buffer had significant effects on the values obtained. This was measured by the P/N (Hill et al., 1981) or response ratio, calculated as the A410 values of samples containing virus divided by the A410 values of corresponding control samples without virus. The response ratio, which takes into account well-to-well and day-to-day variations, is necessary because it is not possible to ensure equivalent binding of virus particles in each individual well, especially when polyclonal capture sera or plant extracts are used. Also, because non-specific reactions tend to increase with higher concentrations of reactants (Clark & Adams, 1977; Koenig, 1978) the response ratio helps to normalize the data. The optimal blocking buffer was PBS containing 0.05% Tween 20 and 1% bovine serum albumin and the optimal dilution of the enzyme conjugate was 1:3200. The P/N optimum was independent of coating antigen concentration (50 or 250 ng antigen/well), and the same reactions were obtained when either monoclonal anti-BYDV IgG or polyclonal mouse anti-BYDV serum was used in the assay. No background reactions were obtained in the optimized ELISA when preimmune mouse sera or blocking buffer were used as negative controls (data not shown).

**Double sandwich ELISA using rabbit polyclonal capture antibody.** Plates were coated at 37 °C for 4 h with 1:400 dilutions of polyclonal anti-BYDV antisera (100 μl/well) in carbonate–bicarbonate coating buffer. After one wash, and incubation at room temperature for 90 min with blocking buffer, plates were washed twice more, and filled with 100 μl/well of test samples. Samples of plant extracts consisted of extracts [ground 1:6 (w/v) in 0.1 M-sodium phosphate pH 6.0] from either healthy plants or plants infected with the homologous isolate. Tests with purified virus utilized virus diluted to 2 μg/ml in 0.1 M-sodium phosphate pH 7.0 (storage buffer) or storage buffer alone. After incubation of the test samples overnight at 4 °C, plates were washed three times and incubated for 2 h at 37 °C with 50 μl/well of purified monoclonal antibodies diluted to 10 μg/ml in wash buffer. Plates were then washed three times, incubated for 90 min at 37 °C with alkaline phosphatase-conjugated rabbit anti-mouse IgG, washed again, and incubated with substrate solution.

**Stability of barley yellow dwarf virus in coating buffer.** To assess the stability of BYDV in carbonate–bicarbonate coating buffer, purified virus (MAV isolate) was mixed with 9 vol. coating buffer or storage buffer (control), washed again, and incubated with substrate solution.

**Dissociation of barley yellow dwarf virus for use in ELISA.** To ensure complete dissociation of the BYDV particles, purified virus was diluted 1:16 with coating buffer and incubated for 4 h at room temperature followed by overnight incubation at 4 °C. The dissociated virus was then mixed for 30 s on a vortex mixer and diluted in an appropriate buffer. For coating, dissociated virus was diluted in coating buffer to 2 μg/ml and used at 50 μl/well.

**Indirect ELISA with polyclonal antibodies.** The indirect ELISA was performed by using unlabelled and alkaline phosphatase-conjugated rabbit polyclonal antisera at the dilutions used in the standardized polyclonal ELISA of Lister & Rochow (1979). In these assays, 100 μl samples of formalin-stabilized or dissociated virus were used to coat plates at 2 μg/ml. After blocking and washing, 100 μl/well of unlabelled polyclonal anti-BYDV serum diluted 1:400 in wash buffer or conjugated polyclonal anti-BYDV serum diluted 1:200 in wash buffer were reacted for 90 min at 37 °C. Plates containing conjugated anti-BYDV serum were then reacted with substrate solution for 1 h at 37 °C; plates incubated with unlabelled polyclonal anti-BYDV serum were reacted for 90 min with alkaline phosphatase-conjugated goat anti-rabbit IgG (diluted 1:750 in wash buffer) and then with substrate.

**Polyclonal double sandwich ELISA with intact and dissociated BYDV.** The cross-reactivities of intact and dissociated BYDV isolates were analysed by the standardized polyclonal antibody-based double sandwich ELISA of Lister & Rochow (1979). In this assay, particles of purified and intact or dissociated virus were dialysed for 2 h at room temperature against wash buffer. The viral samples were diluted to 10 μg/ml in wash buffer followed by twofold serial dilution in healthy plant extract from 2.0 to 0.0625 μg virus/ml. These samples were added (50 μl/well) to plates coated with 1:400 dilutions (100 μl/well) of unlabelled polyclonal anti-BYDV sera. After 4 h at 37 °C, the plates were washed, reacted overnight with 1:200 dilutions of conjugated anti-BYDV sera, and subsequently reacted with substrate solution.

**Competition ELISA for epitope analysis.** In this assay, 50 ng of virus in 50 μl of coating buffer was bound to each well of a 96-well microtitre plate. After unbound protein-binding sites were blocked with blocking buffer, an excess of individual unlabelled antibody (determined empirically for each preparation) was added to each well and allowed to react with the virus and bind to its specific epitope. Unbound antibody was washed off after 2 h incubation at 37 °C. As a control to ensure that all available sites had been bound, a biotinylated form of the same antibody preparation was reacted with the virus–antibody complex. Alkaline phosphatase-labelled avidin was added, followed by addition of the substrate. Total inhibition of binding of labelled antibody indicated that a sufficient quantity of unlabelled antibody had been added to bind to all available epitopes. In the
competition assay, a different unlabelled antibody preparation was added to immobilized virus, followed by
addition of the biotinylated antibody. When the biotinylated antibody was specific for all or part of the same
epitope as the unlabelled antibody, the binding of the biotinylated antibody was inhibited. The amount of
immobilized biotinylated antibody was determined by reaction with avidin-labelled alkaline phosphatase and
substrate.

RESULTS

Production and characterization of monoclonal antibodies

Hybrids were consistently obtained in more than 90% of the wells plated by using the
fusion protocol described in this study. Two weeks after hybridization, 20 to 38% of the
primary hybrids tested produced relevant antibody. Although a large number of the cultures
examined initially produced relevant antibodies, only a few of the hybrids survived the
expansion process. Most cultures either stopped producing immunoglobulins or stopped
growing during the first month after hybridization. Once cloned, the cell lines were more stable.

The monoclonal antibodies did not react in indirect ELISA with healthy host proteins (*Avena
sativa* L. cv. 'Clintland 64') extracted by the method of Lister & Rochow (1979), at a
concentration of 2500 ng per well.

Nine stable cell lines producing monoclonal antibody preparations were generated. Six, two
and one of these were produced against the PAV, MAV and RPV isolates, respectively (Table
1). All antibodies contained kappa light chains and were of the IgG1 subclass. No reactions were
observed with anti-IgA or anti-IgM. Only antibody purified by affinity chromatography from
ascites fluid was used in subsequent assays.

Reactivity of antiviral antibodies

Indirect ELISA with mouse monoclonal antibodies showed that most, but not all,
preparations reacted with both the homologous and heterologous isolates of BYDV (Table 1).
P-PAV 3A10 was the only isolate-specific monoclonal antibody reacting exclusively with the
PAV isolates of BYDV. P-PAV 3A11 reacted with both PAV isolates and with MAV, but not
with RPV. Some of the preparations showed responses with the heterologous isolates that were
equal to those obtained with the homologous isolates. None, however, reacted with healthy host
tissue or with purified preparations of the unrelated cowpea mosaic, lettuce mosaic, soybean
mosaic or Newcastle disease viruses (data not shown).

Monoclonal antibodies were also incorporated into a double sandwich ELISA using rabbit
polyclonal capture antibody. To allow a direct comparison between the different polyclonal
capture systems, reactions are reported as a response ratio. When the monoclonal antibodies
were reacted with virus captured by polyclonal capture sera, the cross-reactivities of the

Table 1. *Cross-reactivity of monoclonal antibodies against barley yellow dwarf virus isolates as
determined by indirect ELISA*

<table>
<thead>
<tr>
<th>Monoclonal antibody*</th>
<th>Minimum concentration (ng/well) of antibody required for detection of BYDV isolates†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RC-PAV</td>
</tr>
<tr>
<td>P-PAV 2A5</td>
<td>0.5</td>
</tr>
<tr>
<td>P-PAV 1D7</td>
<td>50</td>
</tr>
<tr>
<td>P-PAV 3B9</td>
<td>5</td>
</tr>
<tr>
<td>P-PAV 3A10</td>
<td>500</td>
</tr>
<tr>
<td>P-PAV 3B10</td>
<td>50</td>
</tr>
<tr>
<td>P-PAV 3A11</td>
<td>50</td>
</tr>
<tr>
<td>MAV 2B12</td>
<td>500</td>
</tr>
<tr>
<td>MAV 4F7</td>
<td>50</td>
</tr>
<tr>
<td>RPV 3F10</td>
<td>50</td>
</tr>
</tbody>
</table>

* Designation indicates the BYDV isolates used to generate the hybridoma cell line.
† Data are mean values of three determinations.
‡ No reaction at the levels tested.
Table 2. Cross-reactivity of monoclonal antibodies against barley yellow dwarf virus isolates as determined by double sandwich ELISA utilizing rabbit polyclonal antisera

<table>
<thead>
<tr>
<th>Monoclonal antibody*</th>
<th>P-PAV Pure</th>
<th>P-PAV Extract</th>
<th>MAV Pure</th>
<th>MAV Extract</th>
<th>RPV Pure</th>
<th>RPV Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-PAV 2A5</td>
<td>8.2</td>
<td>6.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P-PAV 1D7</td>
<td>10.2</td>
<td>7.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P-PAV 3A10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.3</td>
<td>2.0</td>
</tr>
<tr>
<td>P-PAV 3B10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P-PAV 3A11</td>
<td>10.0</td>
<td>5.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MAV 2B12</td>
<td>-</td>
<td>-</td>
<td>5.0</td>
<td>7.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MAV 4F7</td>
<td>-</td>
<td>-</td>
<td>2.4</td>
<td>2.3</td>
<td>2.0</td>
<td>1.9</td>
</tr>
<tr>
<td>RPV 3F10</td>
<td>-</td>
<td>-</td>
<td>1.3</td>
<td>2.1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Designation indicates the BYDV isolate used to generate the hybridoma cell line.
† Response ratio less than 1.2.

preparations were significantly different from those obtained by indirect ELISA (Table 2). In this assay, only MAV 4F7 was cross-reactive; it was able to bind MAV and RPV, but not the PAV isolate. P-PAV 2A5, P-PAV 1D7, P-PAV 3A11 and MAV 2B12 reacted only with the homologous isolates against which they were produced. P-PAV 3A10 did not react at all, whereas P-PAV 3B10 and RPV 3F10 could bind only to the heterologous isolates RPV and MAV, respectively.

Effect of BYDV dissociation

Density gradient profiles indicated that particles of the MAV isolate were essentially fully dissociated after 2 h incubation at 37 °C in carbonate buffer pH 9.6 (Fig. 1 f) but unaffected by treatment in storage buffer (Fig. 1 c). Reduction of the virus peak was accompanied by an increase in u.v. light-absorbing material at the meniscus. The dissociation was only slightly less when particles were incubated at 4 °C or room temperature (Fig. 1 d, e), but was completely prevented when particles were dialysed at room temperature for 1 h with either 2% (v/v) glutaraldehyde (Fig. 1 g, h, i) or 2% (v/v) formaldehyde.

When monoclonal antibodies were used in indirect ELISA with BYDV isolates dissociated by prolonged incubation in coating buffer, antibody specificity was similar to that found without the dissociating pretreatment (Table 3). The only significant difference was that monoclonal antibody preparation P-PAV 3A10 no longer reacted with the P-PAV isolate. The others all displayed the same degree of cross-reactivity and similar levels of sensitivity, corresponding to plus or minus one dilution.

Reactivity of polyclonal antisera

Intact BYDV particles reacted with polyclonal antibody in the double-sandwich ELISA system of Lister & Rochow (1979) with characteristic homologous responses and slight cross-reactivity between the MAV and P-PAV isolates. Dissociated BYDV was not reactive (data not shown).

Indirect ELISA using polyclonal anti-BYDV antisera and either dissociated or stabilized BYDV particles gave contrasting results. Unconjugated polyclonal anti-BYDV antisera recognized the homologous and heterologous isolates of BYDV in both the dissociated and stabilized forms (Table 4). Conjugated polyclonal anti-BYDV antisera, however, reacted only with stabilized particles and only with those of the homologous isolates (Table 4).

Competition ELISA for epitope mapping

Competition assays using biotinylated antibody P-PAV 2A5 and unlabelled antibodies P-PAV 1D7, P-PAV 3A11 and P-PAV 2A5 showed that P-PAV 1D7 and P-PAV 2A5 totally inhibited binding of the biotinylated antibody. Antibody P-PAV 3A11 had virtually no effect on
Fig. 1. Stability of BYDV in coating buffer. All panels represent $A_{254}$ absorbance profiles of the MAV isolate of BYDV subjected to ultracentrifugation on sucrose gradients after treatment as follows: (a to e) 2 h incubation in 9 vol. storage buffer at 4 °C, room temperature or 37 °C, respectively; (d to f) 2 h incubation in 9 vol. coating buffer at 4 °C, room temperature or 37 °C, respectively; (g) predialysis with 2% glutaraldehyde for 1 h preceding room temperature incubation in coating buffer; (h, i) predialysis with 2% formaldehyde for 1 h before room temperature incubation in coating buffer or storage buffer, respectively.

Table 3. Reaction of monoclonal antibodies against dissociated barley yellow dwarf virus isolates as determined by indirect ELISA

<table>
<thead>
<tr>
<th>Monoclonal antibody*</th>
<th>P-PAV</th>
<th>MAV</th>
<th>RPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-PAV 2A5</td>
<td>5</td>
<td>50</td>
<td>500</td>
</tr>
<tr>
<td>P-PAV 1D7</td>
<td>5</td>
<td>50</td>
<td>500</td>
</tr>
<tr>
<td>P-PAV 3A10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P-PAV 3B10</td>
<td>500</td>
<td>50</td>
<td>500</td>
</tr>
<tr>
<td>P-PAV 3A11</td>
<td>50</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>MAV 2B12</td>
<td>500</td>
<td>50</td>
<td>500</td>
</tr>
<tr>
<td>RPV 3F10</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

* Designation indicates the BYDV isolate used to generate the hybridoma cell line.
† These values represent the minimum concentration (ng/well) of antibody required for a positive response as determined by serial tenfold dilution in the indirect ELISA. Average of four determinations.
‡ No reaction at the levels tested.
Table 4. Reaction of unlabelled and alkaline phosphatase-conjugated polyclonal antibodies in indirect ELISA with dissociated (D) and formalin-stabilized (F-S) barley yellow dwarf virus isolates

<table>
<thead>
<tr>
<th>Polyclonal antibody*</th>
<th>F-S</th>
<th>D</th>
<th>F-S</th>
<th>D</th>
<th>F-S</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-P-PAV</td>
<td>11.5</td>
<td>9.5</td>
<td>4.0</td>
<td>4.0</td>
<td>2.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Anti-MAV</td>
<td>7.5</td>
<td>3.5</td>
<td>8.5</td>
<td>5.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Anti-PAV</td>
<td>5.0</td>
<td>5.0</td>
<td>4.5</td>
<td>4.5</td>
<td>7.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Conjugated anti-P-PAV</td>
<td>4.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2.0</td>
<td>–</td>
</tr>
<tr>
<td>Conjugated anti-MAV</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Conjugated anti-RPV</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2.0</td>
<td>–</td>
</tr>
</tbody>
</table>

* Polyclonal antisera were produced and conjugated as described previously (Lister & Rochow, 1979).
† Response ratio less than 1.2.

DISCUSSION

Indirect ELISA experiments with the monoclonal antibodies made to these BYDV isolates showed a high degree of cross-reactivity and therefore indicate the presence of a common epitope on the three BYDV isolates examined. The only isolate-specific monoclonal antibody was preparation P-PAV 3A10; all other monoclonal antibody preparations reacted with at least two different isolates. This cross-reactivity is in contrast to previous reports using polyclonal antisera (Lister & Rochow, 1979; Rochow & Carmichael, 1979). These results, which have been recently confirmed using serologically specific electron microscopy (Diaco et al., 1984, 1986), were not due to non-specific reactions in our ELISA. No antibody preparations reacted with healthy host protein at concentrations 25-fold greater than the amount of virus antigen used in ELISA tests. Furthermore, this ELISA was optimized with respect to several important parameters, including the formulation of the blocking buffer and P/N ratio (Hill et al., 1981). Also, all the monoclonal antibodies are of the IgG1 subclass, and therefore, because of their common isotypic nature, they differ only in their idiotypic markers. Because the rabbit anti-mouse enzyme-conjugated antibody was raised against whole-mouse IgG, it would be expected to recognize isotypic and not idiotypic markers and should be equally proficient at detecting any of the monoclonal antibodies.

Some antibody preparations were equally reactive in ELISA against heterologous or homologous BYDV isolates but others, although of equivalent titre were more reactive (higher endpoint A410 response) with heterologous than with homologous isolates. For example, the endpoint absorbance for MAV 4F7 was 0.71 with P-PAV, 0.70 for RC-PAV, 0.42 with RPV and 0.45 with MAV. Similarly, endpoint values for RPV 3F10 were 0.17, 0.18, 0.13 and 0.26 for P-PAV, R-PAV, RPV and MAV, respectively. These preparations were thus heteroclitic, or heterospecific (Al Moudallal et al., 1982).

When the monoclonal antibodies were tested in double sandwich ELISA with rabbit polyclonal capture antisera, only limited cross-reactivity was seen. This is consistent with the work of Hsu et al. (1984). Several potential problems are inherent in this approach, however. Binding of virus-specific rabbit polyclonal antisera to viral polypeptides has been shown to interfere with subsequent binding of monoclonal antibodies to the same virus (Hill et al., 1984). If only a limited number of common epitopes are present in a virus and these are bound by the polyclonal capture antisera, detection of these epitopes would not be expected by using a monoclonal antibody specific for them. This may account for the altered reactivity of some of the anti-BYDV monoclonal antibodies in this type of ELISA. Additionally, binding of one antibody to a viral protein has been shown to enhance or reduce binding of a second antibody to
that protein (Howard et al., 1978, 1979); this suggests that substantial changes can occur in the three-dimensional conformation of a protein when coupled with an antibody molecule. Capturing of BYDV particles by polyclonal anti-BYDV antisera may have altered the antigenic configuration of the virus enough to mask existing epitopes. Steric hindrance by the prebound polyclonal antisera may have also caused reduced binding of the monoclonal antibodies. Furthermore, it is very difficult to bind equivalent amounts of each virus isolate by using specific polyclonal antiserum preparations of potentially different avidity; use of this type of assay, therefore, makes direct comparison of the results of cross-reactivity tests virtually impossible. This is especially significant when establishing taxonomic relationships. Recent results obtained using mixed polyclonal–monoclonal systems for serotyping of viral groups (Briand et al., 1982; Gugerli & Fries, 1983; Hsu et al., 1983, 1984) must therefore be viewed with caution. Indirect ELISA testing of monoclonal antibodies with virus bound directly to the solid phase is likely to be more reliable for such analysis.

Our experiments indicate that BYDV dissociates in the presence of carbonate–bicarbonate coating buffer. Also, our selection of monoclonal antibody-producing cell lines was based on an indirect ELISA with antigen that was presumably dissociated in the coating process. When our monoclonal antibodies were used in indirect ELISA with fully dissociated BYDV, only one had significantly altered reactivity. This suggests that the virus need not remain intact for reactivity with most of our monoclonal antibodies. For these reasons, we thought the monoclonal antibodies recognized determinants internally oriented in the intact particle (i.e. 'cryptotopes' sensu van Regenmortel, 1966). This could explain the cross-reactivity observed in our experiments in contrast to the results of previous attempts to examine serological relationships among these isolates, which used virus-specific capture antibody to sequester the virus from solution (Lister & Rochow, 1979; Rochow & Carmichael, 1979). Such assays may have recognized only intact virus and hence may have failed to detect common internal epitopes.

Unlabelled polyclonal antisera also reacted in indirect ELISA with the homologous and heterologous isolates of BYDV in both the stabilized and fully dissociated forms. Cross-reactivity, therefore, was not a phenomenon peculiar to mouse monoclonal antibodies. Rabbit polyclonal antisera were fully capable of detecting serological relationships between the BYDV isolates when the antisera reacted, in an unlabelled form, in indirect ELISA. Furthermore, the common epitopes recognized probably are not exclusively internally oriented because the polyclonal antisera are still capable of cross-reacting with stabilized particles in indirect ELISA. Conjugated polyclonal antisera would react only with the homologous isolates and then only after the virus had been stabilized before coating. Evidently, conjugation made the polyclonal antisera unable to react with the common BYDV epitopes. It has been reported that conjugation of antibodies with enzyme decreases their binding ability (Koenig, 1978) presumably due to spatial impairments or conformational changes in the combining site. This work also suggested that homologous, or heterologous, antibodies with low avidity in the native configuration may become non-reactive after conjugation (Koenig, 1978).

The double sandwich ELISA assays of Lister & Rochow (1979) were incapable of reacting with dissociated virus or showing cross-reactivity among RPV and the other isolates. This can be explained by the results obtained in the indirect ELISA; the Lister & Rochow (1979) assay used conjugated polyclonal anti-BYDV antisera, which were unable to bind to dissociated or heterologous isolates of BYDV. These factors clearly demonstrate limitations inherent in using double sandwich immunoassays for serological differentiation and establishment of serotypes and may be important in the design of other plant virus immunoassays.

Results of competition ELISA showed the presence of at least two epitopes on the coat protein of the P-PAV isolate of BYDV and suggest that monoclonal antibodies P-PAV 2A5 and P-PAV 1D7 are specific for the same or overlapping epitopes and that antibody P-PAV 3A11 is specific for a different epitope on the P-PAV isolate. Further experiments of this nature may yield important information about the basic structure and function of BYDV. Such information may be useful in understanding the mechanisms underlying virus–vector specificity and enhancing breeding programmes for resistance to BYDV strains.

Our results clearly define some fundamental properties of BYDV capsid proteins. Monoclonal
antibody preparation P-PAV 3A10 delineates the presence of a unique epitope on the PAV isolates of BYDV, antibody 3A11 delineates the presence of group-specific epitopes on the PAV and MAV isolates of BYDV, and the other seven monoclonal antibodies delineate the presence of common epitopes on all the BYDV isolates examined. The apparent existence of conserved-common and group-specific epitopes suggests an evolutionary continuum among BYDV isolates. Because our monoclonal antibodies react with dissociated virus, the epitopes they recognize may be dependent on the primary structure of capsid protein rather than secondary or tertiary structure. Assays designed to detect these common BYDV epitopes must present the antigen to the antibody in a form appropriate for recognition.

Monoclonal antibodies are highly individual reagents capable of reacting with a small portion of an otherwise complex antigen. However, proper interpretation of such reactions requires consideration of the form of the antigen being analysed, and the ability of the antibody to react under conditions of the assay.

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