Use of Bovine C1q to Detect Plant Viruses in an Enzyme-linked Immunosorbent-type Assay

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SUMMARY

Polystyrene microtitre plates coated with bovine C1q were used to trap antigen: antibody aggregates of three plant viruses [plum pox virus (PPV), potato virus Y and cocksfoot mild mosaic virus] and human antibody aggregates in an ELISA-type system. The aggregates were detected with alkaline phosphatase-labelled anti-rabbit or anti-human IgG. The assay was as sensitive as the double antibody sandwich method of ELISA for the detection of PPV particles; the limit of detection was 4 to 15 ng virus/ml in purified preparations or a dilution of > 1/1000 in infective Nicotiana clevelandii sap extracts. When used at low dilutions, plant sap of three of the four species tested had an inhibitory effect on the reaction.

Activation of the classical pathway of the complement system follows the binding of antigen: antibody aggregates by C1 through its subcomponent C1q (Dodds et al. 1978; Porter, 1979). C1q, mol. wt. approx. 410,000, has a complex structure consisting of a central stem with six peptide arms each ending in an immunoglobulin binding site. It binds immune complexes including aggregates of IgG molecules via the Fc portion of immunoglobulins (Reid & Porter, 1976; Roitt, 1979). Human subcomponent C1q has recently been used to detect immune complexes in human sera by various methods (Nydegger et al. 1974; Hay et al. 1976; Zubler et al. 1976). Because of the limited availability of human sera for preparation of C1q, Campbell et al. (1979) purified the molecule from bovine serum. They obtained a relatively high yield (12 to 16 mg/l serum) and showed that it not only has a similar structure and composition to human C1q, but that it can take the place of human C1q in the formation of haemolytically active C1 with human C1r and C1s (the other two subcomponents of C1). This paper reports the novel use of solid-phase bovine C1q to bind plant virus: rabbit anti-virus immunoglobulin (Ig) aggregates and the detection of the bound aggregates by alkaline phosphatase-labelled goat anti-rabbit IgG.
Table 1. Absorbance values (A_{405}) obtained in the bovine C1q immunosorbent assay with dilutions of sap from PPV-infected and healthy N. clevelandii*

<table>
<thead>
<tr>
<th>Sap dilution (reciprocal)</th>
<th>Infected sap†</th>
<th>Healthy sap†</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.11</td>
<td>0.06</td>
</tr>
<tr>
<td>100</td>
<td>0.40</td>
<td>0.07</td>
</tr>
<tr>
<td>500</td>
<td>0.49</td>
<td>0.11</td>
</tr>
<tr>
<td>1000</td>
<td>0.39</td>
<td>0.13</td>
</tr>
</tbody>
</table>

* Microtitre wells were coated with 10 µg/ml bovine C1q and enzyme-labelled anti-rabbit IgG used at a dilution of 1/1000.
† Three experiments were performed for both infected and healthy sap.

the plates were washed, 300 µl/well of 0.5 mg/ml solution p-nitrophenyl phosphate in diethanolamine buffer added and incubated for 10 to 30 min at room temperature. The reaction was stopped by adding 50 µl/well 3 M-NaOH and absorbance at 405 nm measured using a Titertek Multiskan photometer (Flow Laboratories, Irvine, Scotland).

Initial experiments showed that only C1q-coated plates trapped antibody:antigen aggregates. Thus, when plates coated with either C1q and gelatin or C1q alone were used, an A_{405} of 0.30 (average of two experiments) was obtained with a sample of plum pox virus (PPV) infective Nicotiana clevelandii sap at 1/100 dilution, and an A_{405} of 0.07 with non-infective sap of the same dilution, whereas when plates were coated with gelatin only or were not coated, an A_{405} of 0.00 was obtained with both infective and non-infective samples. All samples were mixed with 50 µl anti-PPV Ig at 1/200 dilution. Samples of PPV infective and non-infective N. clevelandii sap mixed with 50 µl anti-potato virus Y Ig at 1/200 dilution had A_{405} values similar to the background absorbance, indicating that only virus-specific Ig forms detectable aggregates.

When mixtures of purified PPV particles (prepared as described by Torrance, 1980) in serial twofold dilutions from 4 µg/ml to 1 ng/ml, assuming an A_{405} of 2.5 (200 µl), and anti-PPV Ig (50 µl of a 1/200 dilution) were tested with the C1q immunosorbent assay and these same dilutions of purified virus were tested in the double antibody sandwich method of ELISA (as described by Clark & Adams, 1977; using coating globulin at 1 µg/ml and enzyme conjugate at a dilution of 1/1000) the limits of detection were the same, between 4 and 15 ng/ml, showing that both assays were equally sensitive. Furthermore, the A_{405} values obtained in the C1q assay decreased giving a sigmoidal dilution curve similar to that obtained in the double antibody sandwich-type ELISA.

Table 1 gives the absorbance values obtained in tests to detect PPV in diluted sap from infected and healthy N. clevelandii. The end-point in these experiments was more than 1/1000 sap dilution when an absorbance value of twice the background reading was considered to be the reaction end-point. Two features shown by these results were found to be typical of the assay: (i) concentrated N. clevelandii sap had an inhibitory effect on the reaction, and (ii) there was a relatively high non-specific background absorbance at high sap dilutions.

In experiments to examine the inhibitory effect of N. clevelandii sap, purified PPV particles and N. clevelandii sap were diluted in PBS-Tween containing 2% PVP and mixed so that the final dilution of PPV was 0.25 µg/ml and that of sap was from 1/20 to 1/2000. The samples were mixed with anti-PPV Ig at 1/200 dilution. As the relative sap concentration decreased, absorbance values increased (Table 2a). Table 2(b) gives absorbance values obtained in an experiment when heated and non-heated PPV infective N. clevelandii sap were tested with the C1q assay. This, and other experiments in which extracted sap at 1/50
Table 2. Absorbance values \( (A_{405}) \) obtained in the bovine CIq immunosorbent assay in experiments to test the effect of N. clevelandii sap on PPV detection

<table>
<thead>
<tr>
<th>(a)*</th>
<th>Sample</th>
<th>( A_{405} )</th>
<th>(b)†</th>
<th>Sap dilution</th>
<th>Heated§</th>
<th>Untreated</th>
<th>Heated§</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPV</td>
<td>+ buffer</td>
<td>0.70</td>
<td></td>
<td>0.35</td>
<td>0.14</td>
<td>0.05</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>+ 1/20 sap</td>
<td>0.45</td>
<td></td>
<td>0.36</td>
<td>0.34</td>
<td>0.05</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>+ 1/200 sap</td>
<td>0.58</td>
<td></td>
<td>0.35</td>
<td>0.38</td>
<td>0.07</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>+ 1/2000 sap</td>
<td>0.64</td>
<td></td>
<td>0.35</td>
<td>0.38</td>
<td>0.07</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Buffer</td>
<td></td>
<td>0.08</td>
<td>50</td>
<td>0.14</td>
<td>0.17</td>
<td>0.09</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>1/20 sap</td>
<td></td>
<td>0.03</td>
<td>100</td>
<td>0.14</td>
<td>0.17</td>
<td>0.09</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>1/200 sap</td>
<td></td>
<td>0.05</td>
<td>1000</td>
<td>0.14</td>
<td>0.17</td>
<td>0.09</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>1/2000 sap</td>
<td></td>
<td>0.10</td>
<td>10000</td>
<td>0.14</td>
<td>0.17</td>
<td>0.09</td>
<td>0.10</td>
<td></td>
</tr>
</tbody>
</table>

* In (a), PPV particles were mixed with N. clevelandii sap at various dilutions.
† In (b), a comparison was made between heated and untreated sap from PPV-infected and healthy plants: microtitre wells were coated with 10 µg/ml CIq and enzyme-labelled anti-rabbit IgG used at a dilution of 1/1000.
‡ PPV particles (final concentration 0.25 µg/ml) were mixed with PBS-Tween containing 2% PVP or N. clevelandii sap extracted and diluted in the same buffer.
§ N. clevelandii sap at a dilution of 1/50 was heated at 40 °C for 10 min then centrifuged at 10000 g for 10 min; the supernatant was then further diluted.

Dilution was heated at 40 °C for 10 min and then centrifuged at 10000 g for 10 min before testing, showed that the inhibiting factor in sap was partly overcome by this treatment.

The non-specific background absorbance is higher in more dilute sap. Attempts to overcome this effect were by (i) using less CIq (5 µg/ml), (ii) increasing the amount of gelatin used for coating to 0.1%, (iii) addition of other proteins such as ovalbumin and bovine serum albumin to CIq, and (iv) increasing the concentration of Tween 20 (0.5%) in the extraction buffer and in the diluent for enzyme conjugate and anti-virus Ig; however, none of these procedures influenced the non-specific absorbance. The high background is probably caused by the presence of antibody aggregates in the anti-virus Ig and the enzyme conjugates which are bound by CIq not occupied by virus:antibody aggregates. Use of enzyme-labelled Fab fragments of anti-rabbit IgG or removal of the aggregates by column chromatography of the Ig preparations before use might help to eliminate the non-specific absorbance. However, the background absorbance is very low at sap dilution of up to 1/500; therefore, sap extracts made at ≤ 1/500 dilution would not be affected.

The CIq assay was applied to detect potato virus Y ordinary strain in diluted Nicotiana tabacum cv. White Burley sap and cocksfoot mild mosaic virus strains in diluted saps of Setaria italica and barley. The test seems very sensitive; the limits of detection in sap were > 1/1000 for both viruses. There was an inhibitory effect by saps of N. tabacum and S. italica at dilutions ≤ 1/50 but not by barley sap. The assay was also used successfully in tests to detect heat-aggregated human IgG (L. Torrance, unpublished observations).

The bovine CIq binding assay was applied successfully to detect three plant viruses, and can also detect human antibody aggregates. It was as sensitive as the double antibody sandwich-type ELISA system in the detection of PPV. The main advantages of this assay are that it requires only preparation of immunoglobulin to the specific virus under test and expensive antibody:enzyme conjugates do not have to be prepared. Moreover, a single sample can be tested for the presence of a range of different viruses side by side in one plate. Bovine CIq can be obtained in greater amounts than human CIq so it would be readily available for use in the detection of immune complexes in human sera. The inhibition effect with low dilutions of sap of certain plant species may prove to be a limitation on its use in detecting plant viruses, but heating sap helps to overcome this problem.
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REFERENCES


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