Heterologous Reactivity of Tobacco Mosaic Virus Strains in Enzyme-linked Immunosorbent Assays

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SUMMARY

Enzyme-linked immunosorbent assay (ELISA) differentiated between the antigens of the type strains of tobacco mosaic virus (TMV) and those of the avocado isolate (TMV-A). The ELISA specificity in the heterologous antibody systems was affected mainly by the behaviour of the free phase (conjugate antibodies) whereas antibodies used for coating cross-reacted as in double-diffusion tests. Binding of bovine serum albumin to the TMV and TMV-A antibodies used for coating impaired their activity when tested in homologous reactions but did not make them completely specific in the heterologous system. The complexes of both TMV and TMV-A antigens bound to their homologous and heterologous coated antibodies were stable to acid dissociation. Some possible explanations of ELISA specificity are discussed.

Recently we reported that the tobacco mosaic virus (TMV) strain obtained from avocado seedlings TMV-A cross-reacted in agar double-diffusion tests with TMV antiserum (Alper et al. 1978). Spur formation between the precipitation lines of the two strains, however, indicated the existence of certain differences between the antigenic determinants of these two strains. This paper describes the application of enzyme-linked immunosorbent assay (ELISA) to the serological differentiation between these two strains, and discusses some possible explanations of ELISA specificity.

The common TMV and TMV-A strains, kindly provided by M. Alper, were propagated in Nicotiana tabacum L. cv. Turkish Samsum. Viruses were purified by polyethylene glycol-6000 precipitation (Gooding & Hebert, 1967) followed by sucrose gradient centrifugation.

Rabbits were immunized by two intravenous injections of 2 mg (in 1 ml) antigen at 1-week intervals, followed by a single intramuscular injection on the 10th day after the first injection with 2 mg of antigen emulsified in Freund's complete adjuvant. The rabbits were bled 8 to 10 weeks after the first injection. The partially purified γ-globulin fractions were obtained from TMV and TMV-A antiserum by using DE23 diethylaminoethyl cellulose (Whatman) for column chromatography (Clark & Adams, 1977). The γ-globulin purified from TMV antiserum cross-reacted with TMV-A and gave spur formation similar to that reported by Alper et al. (1978) for unfractionated antiserum. Alkaline phosphatase (type VII, Sigma P-4502, Sigma Chemical Co., St Louis, Mo., U.S.A.) was conjugated to partly purified γ-globulin using glutaraldehyde (Avrameas, 1969). BSA (A-4378, Sigma, 1-6 mg/ml) was conjugated to purified γ-globulin (0-7 mg/ml) of TMV and TMV-A antiserum using 0-08% glutaraldehyde for 4 h at room temperature. Enzyme-linked immunosorbent (ELISA) tests were conducted in polystyrene microplates (Dynatech M129A) essentially as described by Clark & Adams (1977). Microplates were coated with partially purified γ-globulin at concentrations of 3 μg/ml. Similar concentrations of γ-globulin were also used with BSA conjugates. The plates were incubated at 33 to 35 °C for 3 h, washed three times with phosphate-buffered saline containing 0-5 ml Tween 20 (PBS-Tween); 0-2 ml of each virus suspension at the appropriate dilutions in PBS+2% polyvinylpyrrolidone 40000 (PBS-PVP 40) was then added to each well. The plates were incubated at 6 °C for 16 to 18 h,
Fig. 1. ELISA values at $A_{405}$ obtained with different concentrations of virus antigens using homologous and heterologous combinations of coating and conjugated antibodies to (a) type strain tobacco mosaic virus (TMV) and (b) the avocado isolate (TMV-A). T and A refer to TMV and TMV-A. 1, 2 and 3 refer to coating antibodies, antigen (purified virus) and conjugated antibodies, respectively. Details on ELISA and amounts of coating and conjugate antibodies are described in the text.

The results of ELISA tests using homologous and heterologous combinations of coating antibodies to TMV and TMV-A, respectively, are depicted in Fig. 1(a, b). In homologous reactions ($T_1$, $T_2$, $T_3$ — where $T$ refers to TMV and 1, 2 and 3 refer to coating, antigen and conjugate components of the ELISA system, respectively), 1 $\mu$g/ml of TMV antigen gave an ELISA value of 0.53 $A_{405}$, whereas in $T_1$, $A_2$, $T_3$ (A refers to TMV-A), a concentration at a magnitude one hundred times higher could not be specifically detected. In heterologous antibody reactions it can be seen that the replacement of the homologous coating antibodies with the heterologous TMV-A antibodies ($A_1$, $T_2$, $A_3$) reduced the ELISA values by 63 to 86%, depending on antigen concentration. Replacement of the homologous conjugated antibodies by the heterologous conjugate ($A_1$, $A_2$, $A_3$) almost completely prevented the ELISA reaction even at the higher antigen concentrations. TMV-A antiserum gave a markedly weaker homologous reaction and was less efficient in differentiating between TMV-A and TMV antigens; 100 $\mu$g/ml of TMV solution ($A_1$, $T_2$, $A_3$) gave ELISA values (Fig. 1 b) comparable to those obtained with 1 $\mu$g/ml of the homologous antigen ($A_1$, $A_2$, $A_3$). In heterologous antibody reactions, replacement of homologous conjugated antibodies ($A_1$, $A_2$, $A_3$) gave consistently lower ELISA values than those obtained from the coating replacements ($T_1$, $A_2$, $A_3$). The TMV-A antigen, like TMV (Bar Joseph et al. 1979), was washed as before and 0.2 ml of $\gamma$-globulin alkaline phosphatase conjugate, at 2.5 $\mu$l/ml, was added to the wells and the plates were incubated at 33 to 35 °C for 3 h. The plates were again washed, shaken dry and 0.2 ml of enzyme substrate (0.6 mg/ml $p$-nitrophenyl phosphate in 10% diethanolamine) was added to each well. Incubation was carried out at room temperature. The enzyme-substrate reaction was stopped after 30 min by adding about 0.05 ml 3 M-NaOH. The dissociation reactions of homologous and heterologous coated antibody-antigen complexes were tested by placing 0.3 ml of 0.2 M-glycine-HCl, pH 2.2, or 0.05 M-sodium carbonate-NaOH, pH 12.1, buffers into the microtitre wells for 30 min (Bar-Joseph et al. 1979). The results (ELISA values) are expressed as the absorbance ($A$) at 405 nm determined in a Varian Techtron 635 spectrophotometer, using standard silica cuvettes with a 1 cm light path.
Fig. 2. ELISA values (A405) obtained with 100 μg/ml TMV and TMV-A antigens in homologous and heterologous systems using BSA conjugated γ-globulin of TMV (T) and TMV-A (A) for coating. Other details as in Fig. 1.

...dissociated from its homologous coated antibodies by 0.05 M-sodium carbonate/NaOH, pH 12.1 (ELISA value 0.08), but not by treatment with 0.2 M-glycine/HCl, pH 2.2, for 30 min (ELISA value 0.50). Similar dissociation reactions were obtained with the heterologous combination A1, T2 (ELISA values 0.11 and 0.60 for the high and low pH treatments, respectively).

With BSA conjugated TMV and TMV-A γ-globulin for coating in homologous reactions (T1, T2, T3; A1, A2, A3) the ELISA values were 69.5 and 21.6% of those obtained with coating of non-bound antibodies (T1, T2, T3; A1, A2, A3) (Fig. 2). With the same method in heterologous reactions (A1, T2, T3; T1, A2, A3) ELISA values were 25.4 and 61.3%, respectively, of those obtained with A1, T2, T3; T1, A2, A3. However, ELISA values of A1, T2, T3 were still higher than those obtained in the alternative heterologous combinations of T1, T2, A3 (Fig. 2).

The ELISA technique was a sensitive test for the differentiation of the closely related TMV and TMV-A antigens. This differentiation was more specific when TMV antibodies, rather than those of TMV-A, were used. This might have been caused by differences in the immunological response of the rabbits or might indicate the possibility of a common antigenic determinant in TMV-A that was more active in antibody production than that in type strain TMV particles. In experiments on the strain selectivity of ELISA, Koenig (1978) showed that conjugates prepared to one strain of Andean potato latent virus failed to detect other serologically related strains. However, Koenig (1978) was comparing the specificity of purified γ-globulin in the ELISA test with that of unfractionated antibodies in the microprecipitin test. In the present study, similar fractions of γ-globulins were used both in double-diffusion and ELISA tests, thus eliminating the possibility that loss of IgMs during γ-globulin purification caused the differences. The ELISA specificity in heterologous antibody systems is mainly affected by the behaviour of the conjugated antibodies, whereas the antibodies used for coating seem to cross-react as in double-diffusion tests. The reasons for the selectivity of the conjugate antibodies are not fully understood. The dissociation
reaction experiments showed similar resistance to glycine elution both for the homologous (T₁, T₂; A₁, A₂) and the heterologous combination (A₁, T₂), whereas complexes of several other viruses including citrus tristeza virus, carnation mottle virus (Bar-Joseph et al. 1979) and a non-bound TMV-antibody complex (Langenberg & Schlegel, 1967) were reported to be dissociable by acidification. This indicates a similarity in the nature of bonds that are formed between coated antibody and the homologous and heterologous antigen, and that this type of bond is different from that formed by TMV antigen and the non-bound antibody. It has been proposed that the conjugation process causes spatial impairments or conformational changes in the combining sites and, as a result, the avidity or the binding ability of the antibodies decreases (Koenig, 1978). The results of the present work indicate that binding of BSA to TMV and TMV-A antibodies used for coating impaired their activity when tested in homologous and heterologous reactions; however, it did not make them become specific in the heterologous systems. This may be due to conformational differences caused by the two different proteins forming the conjugates. However, the dissociation experiments favour an assumption that the specificity is less pronounced in antibodies coated on the plate because they form strong (irreversible) bonds with the heterologous antigen whereas in free phase the conjugated antibodies form a weak (reversible) bond with the same antigen. The specificity of ELISA in differentiating closely related TMV strains limits its use for field detection of TMV, a virus with many differing strains, but shows that it might be useful for in vivo competition experiments among strains that can be serologically differentiated.

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