VARIATION IN ALFALFA MOSAIC VIRUS WITH SPECIAL REFERENCE TO ITS IMMUNOCHEMICAL PROPERTIES

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Thesis submitted to the University of Adelaide in fulfilment of the requirement for the degree of Doctor of Philosophy

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August, 1989

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Summary

1 - Alfalfa mosaic virus (AMV) was isolated from lucerne (Medicago sativa) plants with a variety of disease symptoms in each of 13 sites in South Australia indicating that the virus is widespread in the state. Host range and symptomatology of the collected field isolates were determined on a limited number of host plant species and shown to be extremely variable. Pathogenicity of twelve single local lesion AMV isolates was studied in detail by mechanical inoculation to 22 different plant host species under two different environmental conditions. These experiments showed that each isolate was biologically distinct and that the host range and symptomatology of each isolate was affected by the environmental condition. Differences were observed in host range and symptomatology between some of the field isolates with their corresponding single local lesion isolates indicating that there was heterogeneity in the viral populations of the AMV field isolates. The host range and variability of the twelve single local lesion AMV isolates precluded their grouping into strains of the virus.

2 - Five biologically most distinct single local lesion isolates of AMV (H4, N20, S30, S40 and W1) were selected for further study and their biochemical and biophysical properties were compared. It was shown that the isolates have different physical stabilities and particle length ratios. Their coat proteins had similar molecular weights but different numbers of disulphide bonds as revealed by polyacrylamide gel-electrophoresis under reduced and non-reduced conditions. The analysis of total RNA of each isolate by agarose gel electrophoresis showed that each has at least four RNA components designated RNAs 1-4 in order of decreasing molecular weight. The size of each segment was indistinguishable between the isolates, but their relative amounts varied. Additional RNA segments found in preparations of some AMV isolates were shown to have AMV sequences by northern blot analysis. Nucleotide sequence analysis of all RNA segments of these isolates by northern and dot-blot hybridization showed that despite their biological and physicochemical differences all five isolates have a high degree of homology.

3 - Despite the high degree of sequence homology between the coat protein genes of the five AMV isolates, their capsids had various requirements for stability as it was not

possible to prepare soluble coat protein preparations of all the isolates by any of the previously published methods. Consequently, a method for the preparation of soluble coat protein of all AMV isolates was developed (Appendix 1). However, protein solubility was retained only in the presence of 0.1 M $CaCl_2$. If the salt concentration was reduced below 0.1 M, protein from some of the AMV isolates precipitated. Proteins prepared by this method were shown to be immunoreactive and to activate the infectivity of the AMV genome. However, during prolonged exposure to buffer containing 0.1 M $CaCl_2$, AMV coat protein undergoes slow proteolysis thereby loosing its ability to activate the AMV genome but not its immunoreactivity.

4 - Polyclonal antisera were raised in rabbits against preparations of coat proteins from two of the AMV isolates and against native as well as glutaraldehyde-fixed virus particles of all five isolates. Antisera were also raised in chickens against native and glutaraldehyde-fixed virus particles of two of the AMV isolates. All antisera raised in rabbits were titrated against different antigenic forms of AMV (native and glutaraldehyde-fixed as well as coat protein subunits) in gel-immunodiffusion tests. Antibodies recognised isolated coat protein in some of the antisera raised against native or glutaraldehyde-fixed AMV and in all antisera raised against isolated coat proteins by gel-immunodiffusion, indirect ELISA and western immunoblotting.

Comparisons of immunogenicity of native and glutaraldehyde-fixed preparations of all five AMV isolates showed that glutaraldehyde-fixation enhanced its immunogenicity. It was also found that glutaraldehyde-fixation renders AMV more efficient as a test antigen in gel-immunodiffusion tests when compared to native virus or isolated coat protein preparations, irrespective of the type of immunogen used for antiserum production. The binding of glutaraldehyde-fixed virus to antibodies was shown to be specific as antibodies to non-related viruses were not able to recognise glutaraldehyde-fixed AMV particles. The better reactivity of glutaraldehyde-fixed AMV in this test was demonstrated to be correlated with its enhanced stability. In contrast to gel-immunodiffusion tests, the fixed AMV was the least reactive test antigen in indirect ELISA compared to native or isolated coat protein preparations, irrespective of the type of antibodies used.

5 - The poor antigenic reactivity of glutaraldehyde-fixed AMV in indirect ELISA was investigated using ^{35}S-labelled AMV (Appendix 2). It was observed that glutaraldehyde-fixed virus bound very poorly to microtitre plates in 10 mM phosphate buffer, pH 7.0, when compared to that of native virus. However, the glutaraldehyde-fixed virus bound better than native virus to microtitre plates precoated with anti-AMV sera, irrespective of the type of immunogen used for raising the antisera. Furthermore, the adsorption of glutaraldehyde-fixed AMV to microtitre wells could be enhanced by raising pH or increasing ionic strength of coating buffer.

The adsorption properties of proteins from native virus preparations of the different AMV isolates to the microtitre well was studied using ^{35}S-labelled virus. When 10 mM phosphate buffer, pH 7.0, (in which all AMV particles remained intact) was used as coating buffer, different isolates had different ability to be adsorbed to the plates. The serological comparison between AMV isolates using this buffer gave indications of wide differences between AMV isolates. When virus particles were applied in conventional coating buffer, (carbonate buffer, pH 9.6), all isolates had similar binding abilities. However, in this buffer, no intact AMV particles were detected and all isolates were serologically similar. These data demonstrate that the results of indirect ELISA were directly correlated with the binding ability of the isolates and this test is not a reliable method for studying serological relationships among AMV.

6 - The serological comparison of biologically distinct AMV isolates was investigated by three different tests; gel-immunodiffusion, two formats of ELISA and western immunoblotting. The comparison was made at the level of isolated coat protein, native and glutaraldehyde-fixed particles using their corresponding polyclonal antisera. It was shown that the biologically distinct isolates of AMV were serologically very closely related but not identical. However, the degree of relationships depended on the type of antisera and test antigens used. The presence of isolate-specific epitopes were best revealed in gel-immunodiffusion tests when using antisera against isolated coat protein or native virus particles and glutaraldehyde-fixed or native virus particles as test antigens. Under these experimental conditions it was possible to obtain isolate-specific or group specific

polyclonal antisera to some of the isolates. Western immunoblotting was not able to differentiate any of the AMV isolates.

7 - Mouse monoclonal antibodies (McAb) were produced to a mixture of native virus preparations of five AMV isolates and screened by three formats of indirect ELISA to select a maximum diversity of McAbs. Seven McAbs were able to differentiate between biologically diverse AMV isolates. They reacted with unique isolate-specific epitopes on the coat protein subunits, native virus or glutaraldehyde-fixed virus particles in indirect ELISA. A number of McAbs were also obtained which had been directed against common antigenic determinants of all the AMV isolates. Two McAbs were selected which precipitated either native or glutaraldehyde-fixed virus, respectively, in gel-immunodiffusion tests. A heterospecific McAb was obtained which precipitated native virus preparations of cucumber mosaic virus (CMV) but not AMV. This McAb differentiated glutaraldehyde-fixed virus preparation of CMV from its corresponding native virus particle by the formation of a pronounced spur in immunodiffusion tests.

8 - Both polyclonal and McAbs revealed the presence of three types of antigenic determinants (**cryptotopes, metatopes** and **neotopes**) on different antigenic conformations of AMV. Evidence is presented to show that isolate-specific epitopes are located on the surfaces of intact AMV particles, and are those antigenic determinants which are exposed on the surface of the isolated coat protein.