STUDIES ON FIJI DISEASE VIRUS WITH SPECIAL REFERENCE TO THE VIRAL NUCLEIC ACID

by

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SUMMARY

1. Spherical particles 55-60 nm in diameter have been purified from leaf galls of sugarcane infected with Fiji disease virus (FDV) by differential centrifugation, treatment with the nonionic detergent Nonidet P40 and sucrose density-gradient centrifugation; they are subviral particles.

2. Immunization of mice with preparations of FDV subviral particles resulted in the production of antibodies to both viral protein and viral RNA. These antibodies were present in both the blood serum and in ascitic fluid produced in response to injection with Krebs 2 Ascites tumour cells.

3. Positive serological reactions were observed in immunodiffusion tests between FDV and antisera to rice dwarf and maize rough dwarf viruses, and between maize wallaby ear virus and antisera to FDV. However, the reactions were shown to result not from the precipitation of viral proteins but of double-stranded (ds) RNA.

4. The specificities of antibodies to ds-polyribonucleotides in sera from animals immunized with three plant viral antigens and one synthetic ds-RNA were studied in immunodiffusion tests with four distinct ds-RNA antigens. Although antibodies in all four antisera reacted with all four antigens tested, the antibodies showed some specificity.

5. Nucleic acid isolated from subviral particles of FDV was identified as ds-RNA by the following properties: (1) Positive orcinol reaction; (2) Negative diphenylamine reaction; (3) Resistance to ribonuclease (RNase) in 1 x SSC (0.15M-sodium chloride and 0.015M-sodium citrate buffer, pH 7) but not in 0.1 x SSC; (4) Susceptibility to RNase in 1 x SSC after thermal denaturation; (5) Sharp thermal denaturation curve with a melting temperature of 76°C in 0.01 x SSC; (6) Buoyant density of 1.60 g/cm^3 in Cs_2SO_4; and (7) No increase in ultraviolet absorption on treatment with formaldehyde at 37°C. On electrophoresis in polyacrylamide gel, FDV-RNA separated into nine RNA segments with a total molecular weight of 15.3×10^6.

6. RNA-dependent RNA polymerase activity was detected in concentrated extracts of gall tissue from FDV-infected leaves but not in similar extracts from healthy leaf tissue. The polymerase activity was correlated with FDV antigen and some polymerase activity was also detected in preparations of FDV subviral particles. Optimal polymerase activity occurred at 35°C, at pH between 8.5 and 9.0, and in the presence of 8 mM $MgCl_2$ and 200 mM NH_4Cl. The polymerase product was single-stranded RNA which was apparently transcribed from FDV ds-RNA.

7. On the basis of data presented in this thesis FDV can be included in the family Reoviridae.