Electrophoretic Forms of Citrus-Infectious-Variegation Virus

P. R. DESJARDINS, J. V. FRENCH, and C. L. NIBLETT

AT THE International Citrus Symposium held in 1968 at the University of California, Dr. J. M. Bové announced that he and his associates had found a serological relationship between citrus-infectious-variegation virus (CIVV) and cowpea-mosaic virus (CPMV). (See the addendum in reference 8.) We have confirmed this relationship in our laboratory.

In view of this demonstrated serological relationship to CPMV, we wished to investigate the possibility of electrophoretic heterogeneity in CIVV as has been demonstrated for CPMV (1, 13, 14, 15). In this paper we shall give a progress report on the studies made to date on the 2 electrophoretic forms of CIVV we have found.

Materials and Methods

The strain of CIVV used was the same as that used in earlier studies (9, 10). The strain of CPMV was originally obtained from Dr. J. S. Semancik and was the strain used by him (15) and by Niblett and Semancik (13, 14) in their studies on electrophoretic heterogeneity.

The CIVV was cultured in lemon, sour orange, and cowpea cv. Early Ramshorn. The method of virus purification was the same as that described previously (9). Virus particles purified from both citrus hosts and from cowpea were indistinguishable under the electron microscope and were found to be 30 nm in diameter, with icosahedral symmetry. The CPMV was cultured in cowpea and purified by the method of Semancik and Bancroft (16).

The CIVV antiserum used in these studies was prepared with CIVV purified out of cowpea. The CPMV antiserum was prepared by Dr. Niblett in his earlier studies (14). The titers of the antisera of both viruses were determined by the ring interface precipitin test. Serological cross reactions were made with the ring interface test and the agar gel double diffusion plate test (2). The agar gel plates were read at 2-day intervals for at least 20 days after preparation. Virus concentrations of both viruses were determined by using an extinction coefficient of $E_{260}^{0.1\%} = 8.0$ optical density units.

Density gradient centrifugation (DGC) of CIVV was carried out in preformed sucrose linear gradients. The gradients and centrifugation were prepared as described previously (9).

When CIVV was purified out of citrus the density gradient electrophoresis procedure described previously was used (9). For separation of the electrophoretic forms, the procedure was basically the same as that described by Niblett and Semancik (14) in that glycine-NaOH buffers at pH 9.0 and 9.5 were used. In the latter case the electrophoresis was carried out for 8–10 hours at 800 volts at 1 °C. The separated forms were dialyzed against glass-distilled water, 0.001 M PO_4 buffer pH 7.0, or 0.015 M ammonium acetate buffer pH 8.0 and then concentrated by high speed centrifugation.

The procedure for electrophoresis on acrylamide gels was essentially the same as that used by Semancik (15) and Niblett and Semancik (13, 14). It involved a gel column composed of a sample gel, a large pore spacer or stacking gel, and a small pore separating gel. The apparatus used was very similar to that described by Davis (7). Before the initiation of electrophoresis, the sample gel contained 50–80 μg of sample. The gels were generally stained with aniline blue black, but occasionally they were stained with coomassie blue (5). Gels stained with aniline blue black were destained in 3 or 7 per cent acetic acid by diffusion or electrophoresis and subsequently stored in 7 per cent acetic acid. Gels stained with coomassie blue were destained and stored in 10 per cent trichloracetic acid (5).

Treatment of the virus with carboxypeptidase A and B enzymes and with chymotrypsin for the experimental conversion of the slow electrophoretic form into the fast form was by the procedure described by Niblett and Semancik (14).

Results

Ring interface precipitin tests demonstrated homologous and heterologous titers of 1/32,000 for the antisera of both viruses. Agar gel double diffusion precipitin tests also demonstrated positive homologous and heterologous reactions of the 2 viruses to the 2 lots of antisera but a negative reaction with CIVV purified out of citrus (Fig. 1). The antigen suspensions in the satellite wells at 12, 3, and 6 o'clock were all adjusted to the same optical density (i.e., 0.25 OD_{260}). The center well contained a 1/64 dilution of CIVV antiserum. There is a strong positive reaction between the center well and the satellite well at 3 o'clock, which contained CIVV purified out of cowpea, and also a strong reaction with the satellite well at 6 o'clock, which contained purified CPMV. The satellite well at 9 o'clock contained saline solution while the satellite well at 12 o'clock contained CIVV purified out of citrus. Similar reactions were obtained when the same antigens were tested against the CPMV antiserum.

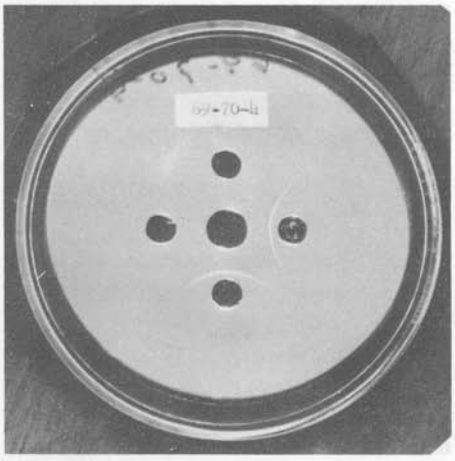


FIGURE 1. *Agar gel double diffusion plate showing the positive reaction of purified CIVV out of cowpea and CPMV to a 1/64 dilution of CIVV antiserum in the center well. Well at 12 o'clock contained CIVV purified out of citrus; well at 3 o'clock contained CIVV purified out of cowpea; well at 6 o'clock contained purified CPMV; and well at 9 o'clock contained saline solution.*

Multiple centrifugal components have been demonstrated for both CIVV (6, 9, 12, 18) and for CPMV (1, 14, 15, 17). Our method of purification of CIVV seems to eliminate the top centrifugal component of this virus. Figure 2 shows a typical density gradient centrifugation profile for purified CIVV; it includes the middle and bottom components, but the top component is missing. The single centrifugal components of CPMV are electrophoretically heterogeneous, and the single electrophoretic forms of this virus are centrifugally heterogeneous (1, 14, 15). So far our

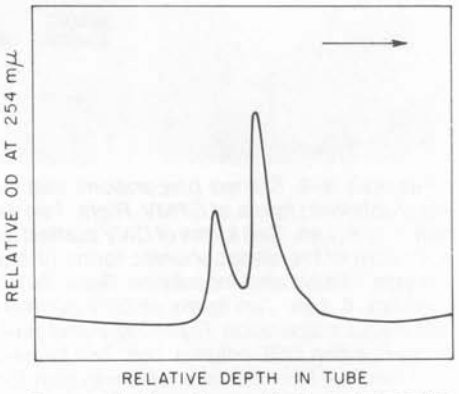
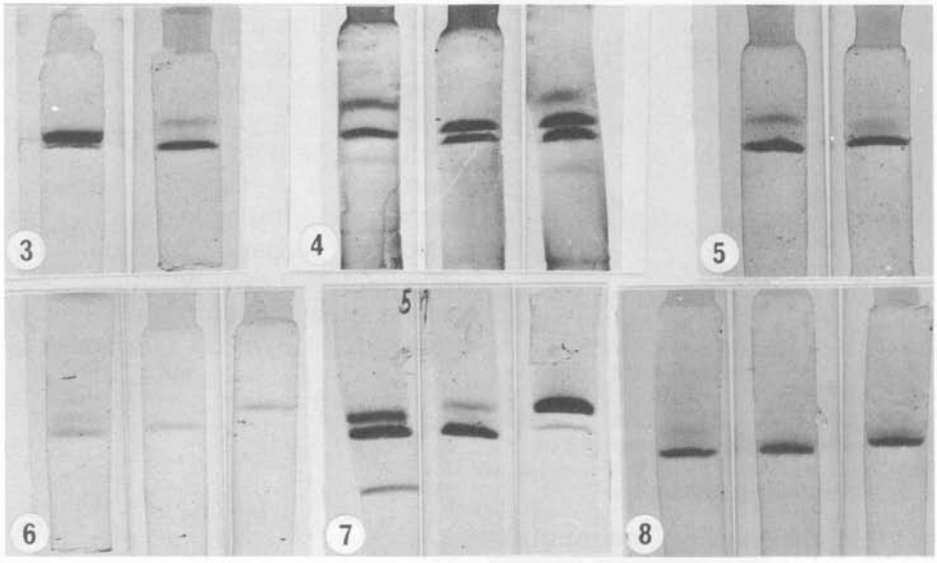


FIGURE 2. *Density gradient centrifugation scanning profile of CIVV purified out of cowpea. Bottom component is the major peak on the right. The arrow indicates the direction of sedimentation.*

studies suggest this phenomenon to be true for CIVV also, but we have not completely characterized all the viral components as yet in this regard.

Two electrophoretic forms were found for CIVV purified from both citrus and cowpea. Figure 3 illustrates a comparison of the electrophoretic forms of CPMV and CIVV; the latter in this case was purified out of cowpea. In our earlier studies, it appeared that there was a greater difference in electrophoretic mobility between the 2 forms (fast and slow) of CIVV than between the 2 forms of CPMV. Occasionally in some electrophoretic runs the reverse seemed to be true. In an attempt to resolve this apparent discrepancy, the 2 viruses were run on separate gel columns and in a mixture on the same gel column in the same electrophoretic run. Figure 4 illustrates the results obtained. The fast forms of both viruses seem to have the same electrophoretic mobility; the slow form of CPMV appears, how-



FIGURES 3–8. *Stained preparations after acrylamide gel electrophoresis.* FIG. 3. *Left. Two electrophoretic forms of CPMV. Right. Two electrophoretic forms of CIVV purified out of cowpea.* FIG. 4. *Left. Two forms of CIVV purified out of cowpea. Center. Two forms of CPMV. Right. A mixture of the electrophoretic forms of both viruses.* FIG. 5. *Left. Two forms of CIVV from cowpea 18 days after inoculation. Right. Two forms of CIVV from cowpea 22 days after inoculation.* FIG. 6. *Left. Two forms of CIVV purified out of citrus. Center. Separated fast form of the same virus preparation. Right. Separated slow form of the same virus preparation.* FIG. 7. *Effect of overloading DGE column. Left. Two forms of CIVV used as starting material for DGE. Center. Enriched fast form contaminated with slow form. Right. Enriched slow form contaminated with fast form.* FIG. 8. *Left. Untreated CIVV purified out of cowpea. Center. After treatment with carboxypeptidase A and B enzymes showing conversion of slow form to fast form. Right. After treatment with chymotrypsin indicating partial conversion of slow to fast. (In all the gel pictures the movement of the forms is from top to bottom in the gels; the cathode was below the gel as it appears in the picture.)*

ever, to have a slightly greater electrophoretic mobility than the slow form of CIVV.

It has been demonstrated that there is a change in the relative amounts of the 2 electrophoretic forms of CPMV in the host, depending upon the age of infection (14). In early stages the slow form predominates, but in the later stages the fast form predominates. This has been interpreted as natural conversion of the slow to fast form. With CIVV in cowpea the same appears to be true, but our studies so far suggest that there is a more rapid natural conversion of slow to fast in this host than is the case with CPMV. Figure 5 shows the relative amounts of the 2 forms at 18 and 22 days after inoculation of cowpea plants. At 18 days the slow form is still evident, but at 22 days it has been almost completely converted to the fast form.

The 2 electrophoretic forms of CIVV can be separated by density gradient electrophoresis (DGE) as has been shown for CPMV (14). Figure 6 illustrates the 2 forms of the virus purified out of citrus and the separated fast and slow forms from the same lot of virus after density gradient electrophoresis. As with CPMV (14) the 2 forms can be readily separated by DGE; one must, however, be careful not to overload the gradient column. The maximum amount of virus that one should place on the column is 35–40 mg of whole virus. When one overloads the column, even with careful fractionation, one obtains fractions enriched with fast or slow forms, but each contains slight amounts of the other form (Fig. 7).

Niblett and Semancik (14) have shown that the slow electrophoretic form of CPMV can be experimentally converted by carboxypeptidases A and B and by chymotrypsin to the fast form. So far in our work with CIVV, we have been trying to accumulate quantities of the 2 separated forms primarily for amino acid analysis of them. In a single preliminary experiment, the results indicate that the combined carboxypeptidase enzymes A and B do convert the slow form of CIVV to the fast and that chymotrypsin at least makes a partial conversion (Fig. 8).

Discussion

Although the failure of CIVV purified out of citrus to react to antiserum to the virus purified out of cowpea and with CPMV antiserum has not been resolved as yet, the possibility that the virus has different serological properties in the 2 different hosts is not unlikely. Bawden (4) has demonstrated this phenomenon with forms of tobacco mosaic virus from leguminous hosts and has shown it to be a reversible change. We are certain that we do not have a contaminant of CPMV in our CIVV in cowpea. The difference in mobilities of the slow electrophoretic forms of the 2 viruses indicates this. Also, recently Dr. Niblett has obtained a differential reaction to the 2 viruses in a cowpea hybrid host.

Viral protein components with different electrophoretic properties

have been demonstrated now for other plant viruses (1, 3, 11, 13, 14, 15). The results reported here demonstrate that there are 2 electrophoretic forms of CIVV. In addition, the results seem to confirm the relationship between citrus-infectious-variegation virus and cowpea-mosaic virus.

ACKNOWLEDGMENTS.—The authors gratefully acknowledge valuable discussions with Drs. J. S. Semancik and J. M. Wallace during the course of this work. Also we thank Mrs. Penelope Colbaugh, Mr. D. A. Reynolds, and Mr. R. J. Drake for valuable technical assistance.

Literature Cited

1. AGRAWAL, H. 1964. Identification of cowpea mosaic virus isolates. Mededel. Landbouwhogeschool, Wageningen 64(5): 1–53.
2. BALL, E. M. 1961. Serological Tests for the Identification of Plant Viruses. American Phytopathology Society: Ithaca, N.Y.
3. BANCROFT, J. B. 1962. Purification and properties of bean pod mottle virus and associated centrifugal and electrophoretic components. Virology 16: 419–27.
4. BAWDEN, F. C. 1958. Reversible changes in strains of tobacco mosaic virus from leguminous plants. J. Gen. Microbiol. 18: 751–66.
5. CHRAMBACH, A. et al. 1967. A procedure for rapid and sensitive staining of protein fractionated by polyacrylamide gel electrophoresis. Anal. Biochem. 20: 150–54.
6. CORBETT, M. K., and GRANT, T. J. 1967. Purification of citrus variegation virus. Phytopathology 57: 137–43.
7. DAVIS, B. J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. 121: 404–27.
8. DESJARDINS, P. R. 1969. Purification, electron microscopy and serology of citrus viruses—A review, p. 1481–88. *In* H. D. Chapman (ed.), Proc. 1st. Intern. Citrus Symp. Vol. 3. Univ. Calif., Riverside.
9. DESJARDINS, P. R., and FRENCH, J. V. 1970. Purification of the citrus infectious variegation virus by density gradient electrophoresis. Virology 40: 746–51.
10. DESJARDINS, P. R., and WALLACE, J. M. 1962. Serological investigations involving the infectious-variegation strain of psorosis virus of citrus. Virology 16: 99–100.
11. GILLASPIE, A. G., and BANCROFT, J. B. 1965. The rate of accumulation, specific infectivity, and electrophoretic characterization of bean pod mottle virus in bean and soybean. Phytopathology 55: 906–8.
12. MARTELLI, G. P., MAJORANA, G., and RUSSO, M. 1968. Investigations on the purification of citrus variegation virus, p. 267–73. *In* J. F. L. Childs (ed.), Proc. 4th Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
13. NIBLETT, C. L., and SEMANCIK, J. S. 1968. Properties of two electrophoretic forms of cowpea mosaic virus. Phytopathology 58: 1062.
14. NIBLETT, C. L., and SEMANCIK, J. S. 1969. Conversion of the electrophoretic forms of cowpea mosaic virus in vivo and in vitro. Virology 38: 685–93.
15. SEMANCIK, J. S. 1966. Studies on electrophoretic heterogeneity in isometric plant viruses. Virology 30: 698–704.
16. SEMANCIK, J. S., and BANCROFT, J. B. 1965. Stability differences between the nucleoprotein components of bean pod mottle virus. Virology 27: 476–83.
17. VAN KAMMEN, A. 1967. Purification and properties of the components of cowpea mosaic virus. Virology 31: 638–642.
18. YOT-DAUTHY, D., and BOVÉ, J. M. 1968. Purification of citrus crinkly-leaf virus, p. 255–63. *In* J. F. L. Childs (ed.), Proc. 4th Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.