Disc Electrophoretic Separation of Elongated Plant Viruses in Polyacrylamide-agarose Gels

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Disc electrophoresis in polyacrylamide gels has often been used for investigation of virus proteins (McCarthy, 1968; Sarkar & Schilde-Rentschler, 1968; Semancik, 1966), but has seldom been used to separate whole viruses (Semancik, 1966; Tiselius, Hjerten & Jerstedt, 1965). Tiselius et al. (1965) were the first to fractionate a plant virus, turnip yellow mosaic virus (TYMV) by electrophoresis in polyacrylamide gels. Semancik (1966) and Niblett & Semancik (1969, 1970) used this method for fractionation and characterization of the components of purified isometric plant viruses, but separation in polyacrylamide gel was not possible for viruses with elongated particles, such as tobacco mosaic virus (TMV) or tobacco rattle virus.

In this paper we report the electrophoresis of viruses with elongated particles in polyacrylamide-agarose gels of low polyacrylamide concentration. This method has been used before to fractionate nucleic acids and ribosomes (Peacock & Dingman, 1968; Dahlberg, Dingman & Peacock, 1969). Mixtures of purified TYMV, TMV and potato virus X (PVX) could be separated from each other and from plant proteins. Also, the viruses could be separated from plant proteins in clarified sap. This made it possible to use polyacrylamide disc electrophoresis with advantage for the fractionation of plant viruses with elongated particles.

TMV and PVX were purified from systemically infected leaves of Nicotiana tabacum L. cv. Samsun by the ether-carbon tetrachloride method (Wetter, 1960). TYMV was isolated from systemically infected leaves of Brassica chinensis with butanol and chloroform (Steere, 1959).

Leaves were ground in a mortar with an equal weight of tris + HCl buffer, 0.05 M, pH 7.8, containing 0.1% cysteine. The slurry was incubated at 50°C for 10 min. followed by centrifugation at 17,000 g for 10 min.

Disc electrophoresis was set up in tubes (0.6 cm. x 10 cm.) with gels of 2%, 2.5%, or 3% polyacrylamide, 0.5% agarose and 0.09 M-tris + boric acid buffer solution, pH 8.3, containing 0.0025 M-Na2-EDTA (Peacock & Dingman, 1968). In other experiments 0.07 M-tris + HCl, pH 7.5, was used for gels with a reservoir solution of 1 g./l. tris and 5.52 g./l. diethylbarbituric acid, pH 7.0 (Williams & Reisfeld, 1964). Also, a buffer system modified from Davis (1964) has been used with 0.1 M-tris + HCl buffer, pH 8.3, for the gels and a solution of 0.6 g./l. tris and 2.88 g./l. glycine, pH 8.3, for the reservoirs. The running gels were not covered by spacer gels.

Volumes of 40 µl. of virus suspension or 50 µl. of sap were used for electrophoresis; the TYMV suspension contained 2 mg. virus/ml. and the TMV suspension 4 mg./ml. The samples, mixed with a trace of bromophenol blue, were layered above the gels and a current of 1 mA/tube applied until the virus had penetrated the gel. The current was then increased to 2 mA/tube for 3 to 5 hr, depending on the buffer system.

The gels were stained after electrophoresis in 0.1% Amido Black solution in 7% acetic acid or in an 0.25% solution of Coomassie Blue in methanol, glacial acetic acid and water (5:1:5, v/v) and destained in 7% acetic acid or methanol, acetic acid and water (5:1:5, v/v).

Samples of each virus suspension were run in several gels of which one was incubated in
7% trichloroacetic acid to detect the virus bands. Slices were cut from the other gels in the band positions and were homogenized in 0.5 to 1 ml. phosphate buffer, 0.05 M, pH 7, using a Potter–Elvehjem homogenizer. After centrifugation the supernatant fluids were tested serologically by the microprecipitin test on slides and were checked for intact virus particles in the electron microscope. TYMV and TMV were also identified by the agar diffusion test. For this each PAA gel was embedded in an agar gel so that virus diffusing from the PAA gel precipitated with antibodies diffusing through the agar from a parallel trough filled with antiserum. Infectivity was tested by rubbing homogenized gel slices on carborundum dusted leaves of host plants.

As shown in Fig. 1 (a, b, c, and g), TMV migrated into gels of 2% PAA + 0.5% agarose and separated into two distinct bands, whereas we found, like Tiselius et al. (1965), that it did not migrate into gels of 3% PAA. However, TMV has a normal length of only 300 nm. and it was therefore of interest to determine whether a longer and more flexuous virus was

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**Fig. 1.** Electrophoresis under various conditions of TMV, TYMV and PVX in polyacrylamide-agarose gels. Anode, cathode and direction of migration are shown at the left.

(a) Separation of a mixture of purified TMV and TYMV in 2% polyacrylamide + 0.5% agarose gel. Buffer solution: tris + boric acid + EDTA, pH 8.3 (Peacock & Dingman, 1968). Upper bands: TMV; lower bands: TYMV.

(b) As (a), but tris + HCl buffer, pH 7.5 (Williams & Reisfeld, 1964).

(c) As (a), but tris + HCl buffer, pH 8.3, modified from Davis (1964). TYMV ran with the front (arrow).

(d) As (a), with addition of 0.1% sodium dodecyl sulphate to the virus suspension 15 min. before electrophoresis. TYMV ran with the front (arrow).

(e) Clarified sap of Chinese cabbage infected with TYMV. Conditions as (a). Upper two bands, TMV; lower zone, cell particles (ribosomes).

(f) Clarified sap of healthy Chinese cabbage.

(g) Clarified sap of TMV infected leaves of *Nicotiana tabacum* L. cv. Samsun. Conditions as (a). Upper two bands, TMV; lower zone, cell particles (ribosomes) and proteins.

(h) Clarified sap of healthy *N. tabacum* L. cv. Samsun leaves.

(i) Purified PVX in 2.0% polyacrylamide + 0.5% agarose gel. Buffer solution as (c). One band of PVX in the upper part of the gel, host plant protein and degraded virus protein ran with the front (arrow).

(j) Clarified sap of PVX-infected leaves of *N. tabacum* L. cv. Samsun. Conditions as (i).

(k) Clarified sap of healthy *N. tabacum* leaves. Conditions as (i).
able to penetrate such a gel. Fig. 1 i shows the electrophoresis of purified PVX, of normal length about 515 nm., in a 2 % PAA + 0.5 % agarose gel. In this experiment PVX migrated as one band in the upper part of the gel with a lower electrophoretic mobility than that of TMV under comparable conditions (Fig. 1c).

Fig. 2. Double-diffusion test showing light zones of antigen-antibody precipitation. Gel of Fig. 1(a) (middle) embedded in 0.7 % agar, TYMV → antiserum trough on left, TMV → antiserum trough on right side. Zones of antigen-antibody precipitation appear light.

As it is often difficult to separate a mixture of two or more viruses, we tried to fractionate a mixture of purified TYMV and TMV and found that this was easily done (Fig. 1a, b, c). It was also possible to separate the virus of crude saps from host components. The virus bands (Fig. 1e, g, j), whose intensity depends on the virus concentration in the host plant, clearly separated from the ribosome and protein bands derived from the hosts. Virus particles extracted from the gel were morphologically intact, serologically active, and infectious to host plants. Purified preparations of mixtures of TYMV and TMV and saps of plants infected with either of these viruses always gave two bands (Fig. 1a, b, e, g). When 0.1 % sodium dodecylsulphate (SDS) was added to a mixture of purified TYMV and TMV before
electrophoresis each virus formed only one band (Fig. 1d); virus particles did not degrade in this concentration of SDS. After adding SDS, particles separated in electrophoresis only according to particle size (Shapiro, Vinuela & Maizel, 1967), so it is likely that the two bands obtained in the absence of SDS occurred because of different charges on the virus particles.

No investigation has been made of the two electrophoretic forms of the individual viruses. Fig. 2 shows the same gel as that of Fig. 1a embedded in agar for the double-diffusion test. From the serum trough on the left, TYMV antibodies have diffused and have formed the curved precipitation line with the TYMV from the electrophoresis gel. The TMV antiserum, diffusing from trough on the left, formed the double curved line very close to the gel. The elongated TMV particles have a very low diffusion velocity in agar compared with that for the isometric TYMV. Particles of the two electrophoretic forms of TMV therefore precipitated very close to the gel to form a precipitation line of slight double curvature. This could mean that the two electrophoretic forms of the TMV particles have identical serological properties.

The appearance of two electrophoretic forms in disc electrophoresis has been reported for bean pod mottle virus (Bancroft, 1962) and cowpea mosaic virus (Agrawal, 1964). Although we do not know the cause of the electrophoretic heterogeneity of the TMV, PVX and TYMV that we used, it is unlikely that this is the result of mixed virus populations. One of the two components may be a slightly degraded form of the other as reported by Niblett & Semancik (1969, 1970) for cowpea mosaic virus and bean pod mottle virus. This hypothesis requires confirmation.

Clarified sap from diseased plants (Fig. 1e and g) often formed in disc electrophoresis a remarkably thicker band of cell particles (ribosomes) than did sap from healthy plants (Fig. 1f and h). Following the reports by Reid & Matthews (1966) and McCarthy, Jarvis & Thomas (1970) that there is an increase of ribosomes in virus infected plant tissue, the thicker band noted here for sap from diseased plants may be caused by a higher concentration of ribosomes.

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REFERENCES


Short communications


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