Electrophoretic Heterogeneity of the Sedimenting Components of Arabis Mosaic Virus

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SUMMARY

Electrophoretic heterogeneity in preparations of arabis mosaic virus (AMV) was due to differences in net surface charge among the sedimenting components. Bottom component migrated more rapidly than top component in polyacrylamide gels and on cellulose acetate strips. When nucleic acid was removed from bottom component the nucleic acid-free protein shell showed sedimentation and electrophoretic properties similar to those of top component.

Relatively few plant viruses have been subjected to a detailed investigation of their electrophoretic properties. Most isometric viruses for which information is available exhibit one component in free-boundary electrophoresis, but some viruses appear heterogeneous and two electrophoretic forms have been recorded for cowpea mosaic virus and bean pod mottle virus (Bancroft, 1962; Agrawal, 1964). One form can be converted into the other by means of proteolytic enzymes (Niblett & Semancik, 1969), each form containing both of the nucleoprotein sedimenting components (Bancroft, 1962; Semancik, 1966). This paper reports on a phenomenon in which electrophoretic heterogeneity in preparations of arabis mosaic virus (AMV) is associated with differences in surface charge among the centrifugal components.

The investigation involved two isolates of AMV. One (AMV-S) was obtained from strawberry in Herefordshire and has subsequently been propagated in the glasshouse in Nicotiana clevelandii and cucumber. The other (AMV-W) was originally obtained from a petunia plant following infestation by nematodes (Xiphinema diversicaudatum Micol.) transferred from woodland soil collected near High Halstow, Kent. This virus was propagated in Chenopodium quinoa.

Purified preparations of AMV-S and AMV-W each separated into two distinct bands on electrophoresis in 2·6% or 3·0% polyacrylamide gels stained subsequently with Coomassie brilliant blue R. The slower band was initially attributed to virus aggregation, but only trace amounts of the slow component were detected when other, unstained gels were scanned at 254 nm using a Vitatron MPS with a densitometer attachment (Fisons Scientific Instruments Ltd.). Moreover, electrophoresis of both AMV isolates on cellulose acetate strips, to avoid differential sieving effects of the gels, produced two protein-staining bands (Fig. 1) of which only the faster band was readily detected by the u.v. scanner. These results indicated that nucleic acid was absent from the slow moving band and discounted major size differences between the components of the two bands.

AMV preparations sediment as three components (Fig. 2, inset) of which bottom (B) and middle (M) are nucleoproteins, while the top component (T) consists of protein shells devoid of nucleic acid. From the electrophoresis results it seemed possible that the slower migrating band might correspond to the T component. Accordingly, the electrophoretic components of a preparation of AMV-S were separated on a cellulose acetate strip in 0·05 M-tris-citric acid (TC) buffer, pH 8·0, for 30 min at a potential gradient of approx. 4 V/cm. While the
Fig. 1. Cellulose acetate electrophoresis of AMV-W and AMV-S in 0.05 M-TC buffer (upper strip) and 0.10 M-TC buffer (lower strip). Migration is from left to right.

strip was still wet the positions of the separated bands were observed under u.v. light. The bands were cut out, eluted into a small vol. of buffer and sprayed on to carbon-coated grids for electron microscopy using potassium phosphotungstate as a negative stain. The faster migrating band contained mainly 'full' particles whereas the slower band contained mostly 'empty' protein shells.

The observed relationship between the sedimenting and electrophoretic components was confirmed by examining B, M and T components, separated by density gradient centrifugation, in both polyacrylamide gel and cellulose acetate electrophoresis systems. In each system the B and T components behaved like the fast and slow bands, respectively, of unfractionated preparations. In gels, the M component, concentrated so as to be more readily detected by staining, migrated at an intermediate rate which for AMV-S was more similar to that of T component but for AMV-W was closer to that of B component. Similar results were obtained for the centrifugally separated components of strawberry latent ringspot virus (SLRV) which is also a member of the nepovirus group. However, the difference in migration rates of the components of this virus was considerably less than with AMV.

The mobilities of the components in unfractionated preparations of AMV were influenced by the ionic strength of the electrophoresis buffer. In 0.1 M-TC buffer the degree of separation was less than in 0.05 M-buffer (Fig. 1). A sucrose density gradient zonal electrophoresis system, similar to that described by Polsen & Cramer (1958) and as used for the purification of plant viruses (van Regenmortel, 1964) was used to measure the mobilities of the compo-
nents of AMV-S, relative to phenol red, at both ionic strengths. The positions of the various constituents were established by scanning at 280 nm using a model UA-4 light monitor (Instrumentation Specialities Co., Nebraska). The mobility of the slow component was similar in each buffer, but that of the fast component increased from $R_{\text{phenol red}} = 0.30$ in 0.1 M to $R = 0.41$ in 0.05 M-TC buffer.

The possibility was investigated that segments of virus nucleic acid might be exposed at the surface of the particles, thereby adding to the overall net charge. Pancreatic ribonuclease was added at 20 μg/ml to a preparation of AMV-S and incubated at 37 °C for 30 min. However, no difference was detected between treated and untreated samples following sucrose gradient centrifugation or electrophoresis in polyacrylamide gels. Moreover, overnight treatment of AMV preparations at 4 °C with various concentrations of formaldehyde ($\leq 2.0 \%$) or glutaraldehyde ($\leq 0.6 \%$) resulted in the loss of the charge differential between the components, presumably through interaction of the aldehyde with exposed, positively charged amino groups. Each treated preparation migrated on cellulose acetate as a single band at a rate increasing with the concentration of aldehyde used. The results of these tests were taken to indicate that the charge differential between the components was probably not due to nucleic acid being exposed at the surface of the nucleoprotein particles.

Two explanations seemed possible; either portions of the protein coat of the virus were partially de-phosphorylated or, perhaps more likely, the encapsidated nucleic acid exerted an indirect effect on the net charge on the particle, possibly by a masking or an internal neutralization of some of the normally surface-located, positive charged groups. If the second explanation is correct, retraction of the nucleic acid from the protein capsomers, or its complete removal from the virus capsid, should convert the electrophoretically faster-moving nucleocapsid into a slower moving component. Accordingly, a preparation of AMV-S was fractionated by a single cycle of density gradient centrifugation (Fig. 2, inset). Sucrose was removed and the separated B and T components concentrated by sedimentation, the pellets being resuspended in 0.05 M-TC buffer. In an attempt to release the nucleic acid from within the B component particles with minimum disruption of the protein shell, 0.5 ml samples were dialysed overnight at 18 to 20 °C against 0.1 M-glycine buffer at pH 9.0 or 10.0 in the presence of 0.2, 0.5 or 1.0 M-KCl. After a further period of dialysis against 0.05 M-TC buffer for 2 h the six samples were examined by sucrose gradient centrifugation and by electrophoresis.

Both pH treatments decreased the amount of detectable B component with a concomitant increase in the amount of a protein component which behaved centrifugally and electrophoretically like T component (Fig. 2). Additionally, there was an accumulation of low mol. wt. nucleic acid material at the top of the centrifuged gradient columns. Treatment at pH 10 was more effective than at pH 9, but varying the concentration of KCl between 0.2 and 1.0 M made little difference to the degradative process. Although a critical examination of all the possible reaction products was not made, the apparent absence of any electrophoretic intermediate in the transition from the B to the T-like component, such as an M-type of electrophoretic component, better supports the nucleic acid indirect effect explanation than the one involving protein de-phosphorylation for which a continuous range of intermediate products might be expected.

Electrophoretic heterogeneity of virus preparations has usually been attributed to differences either in particle morphology (Lister, Ghabrial & Saksena, 1972) or in the composition of the protein coat (Niblett & Semancik, 1969). However, the heterogeneity exhibited by the isolate of SLRV and by both isolates of AMV does not constitute a novel phenomenon for plant viruses. Hitchborn & Dunn (1965) observed a charge differential between the top and
Fig. 2. Centrifugal and electrophoretic examination of AMV-S components before and after treatment at pH 9 or pH 10 in the presence of 0.5 M-KCl. Inset: sucrose density gradient extinction profile at 254 nm of the virus preparation from which B and T components were obtained. Left: sucrose density gradient extinction profiles at 280 nm of untreated B and T components and of treated B component. Right: cellulose acetate electropherograms of corresponding untreated and treated components. Sedimentation and electrophoretic migration from left to right.

bottom components of wild cucumber mosaic virus during free-boundary electrophoresis in a phosphate magnesium buffer of low ionic strength. The B component exhibited a higher net negative charge than the nucleic acid-free T component. Hitchborn & Dunn were unable to detect any difference between the components by examination of the peptides released by enzymic hydrolysis. SLRV is peculiar in having two species of protein in its coat, but this could not account for its electrophoretic behaviour as both protein species occur in isolated centrifugal components which are themselves electrophoretically homogeneous (Mayo et al. 1974; M. F. Clark, unpublished results). It seems probable that the heterogeneity shown
both by SLRV and by AMV is a protein-mediated surface charge effect resulting indirectly from the presence or absence of nucleic acid in the virus capsid.

The protein surface of the virus particle is probably a major determinant of vector specificity (Harrison, 1964; Rochow, 1970; Harrison et al. 1974) and it is possible that the nature of the charge on the particle surface is one of the factors involved. Although the distribution of the RNA species among the nucleoprotein components of these two nepoviruses is such that transmission of the B component alone would ensure the passage of the entire virus genome (Murant et al. 1972), it would be of interest to investigate the relative transmissibility by nematodes of the differently charged nucleoprotein components.

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REFERENCES


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