Particle Differences Related to Aphid-Transmissibility of a Plant Virus

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SUMMARY

The particles of bottom component of aphid-transmitted isolates of pea enation mosaic virus form multiple bands when electrophoresed in polyacrylamide gels; those of bottom component of non aphid-transmitted isolates form a single band. The particles in the multiple bands are separated on size, which is correlated with the presence of an extra protein. The structure of these particles is discussed as is their significance in relation to aphid transmissibility.

Pea enation mosaic virus (PEMV) is transmitted by aphids in the persistent manner and can be relatively easily sap transmitted. Isolates of the virus vary in their transmissibility. Some, e.g. the Wisconsin isolate (Izadpanah & Shepherd, 1966) and the P3 isolate (R. J. Shepherd, personal communication), do not lose aphid-transmissibility after repeated sap transmission. Others, e.g. the Californian aphid-transmitted (CAT) and New York aphid-transmitted isolates, lose aphid-transmissibility after repeated sap transmissions (French et al. 1973; Tsai & Bath, 1974; Harris et al. 1975; Clarke & Bath, 1976); the non aphid-transmissible strain derived from CAT is named CNT (Harris et al. 1975; Clarke & Bath, 1976). Many of the laboratory isolates which have been maintained by sap transmission, e.g. wild-type (Hull & Lane 1973) and the isolates of Bozarth & Chow (1966) and of Gonsalves & Shepherd (1972), are not aphid-transmissible.

Purified preparations of PEMV consist of spherical particles which sediment as two nucleoprotein components (Bozarth & Chow, 1966; Gibbs, Harrison & Woods, 1966; Izadpanah & Shepherd, 1966); the 112S and 99S components have been designated bottom and middle components respectively (Hull & Lane, 1973). Each component of the wild-type forms a discrete band on polyacrylamide gel electrophoresis, the separation being on the basis of size (Hull & Lane, 1973). This communication reports on a comparison of the gel electrophoretic patterns of the particles of aphid-transmitted and non-transmitted isolates and notes differences which might be associated with transmissibility.

On gel electrophoresis of the particles of the non aphid-transmissible isolates (New York non-transmissible, CNT, and those of Bozarth & Chow, 1966 and of Gonsalves & Shepherd, 1972) the two components are separated as reported by Hull & Lane (1973) for wild-type (Fig. 1a); in each case bottom component comprised 60 to 70 % of the total nucleoprotein and in 3-4 % polyacrylamide gels the ratio of the mobilities of middle to bottom component was 1:0.72 to 0.76.

Electrophoresis of the particles of the aphid-transmitted CAT isolate, and of the Wisconsin and P3 isolates (after sap transmission), resulted in patterns like that shown in Fig. 1(b). Sedimentation in the analytical ultracentrifuge showed that these isolates have very little middle component. When middle component of the Wisconsin isolate was isolated from sucrose gradients it formed one major band in the region of band M in Fig. 1(b) and two or three slightly slower bands (showing as M1). The material in bands M and M1 was degraded in CsCl isopycnic gradients at pH 5.0, a property of the middle component of the wild-type.
Fig. 1. Densitometer traces of the nucleoproteins of (a) the PEMV isolate of Bozarth & Chow (1966) and (b) the Wisconsin isolate, electrophoresed in 3·4 % polyacrylamide gels at 16 V/cm for 16 h using the buffer system described by Hull & Lane (1973). The two gels are from separate experiments.

Isolate (Hull & Lane, 1973); peak M also co-electrophoresed with the middle component of wild-type in 2·8 and 3·4 % gels. There was not enough of M1 to examine its properties in detail.

The bottom component of the aphid-transmitted isolates formed multiple bands (B1 to B8 in Fig. 1b). Bands B3 to B8 are regular distances apart which suggest that the major band is made up of two (B1 and B2). The ratio of the mobilities of middle component (band M) to bands B1 and B8 was 1:0-58 and 1:0-38 respectively in 3·4 % gels (pH 4·4) and 1:0-80 and 1:0-68 in 2·8 % gels. This suggests that the bands in bottom component were being separated on size rather than charge. Since the bands were regular distances apart, it appears that bottom component of these aphid-transmitted isolates is composed of particles of discrete size classes with regular increments in diameter. The bottom component of the wild-type isolate
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Fig. 2. SDS-polyacrylamide gels of PEMV proteins. (b) and (c) wild type; (a) and (d) the Wisconsin isolate. (a) and (b) were loaded with about 25 µg protein, (c) and (d) were loaded with about 5 µg. Electrophoresis conditions were as described by Bar-Joseph & Hull (1974). Arrows in (a) and (d) indicate the extra minor proteins.

Electrophoresed faster than B1, the difference in mobility corresponding to about five of the incremental distances mentioned above. The co-electrophoresis of the middle component of wild-type isolate with the middle component major band of the Wisconsin isolate suggests that all the bands of the Wisconsin isolate bottom component contain particles bigger than wild-type bottom component. An idea of the size differences can be gauged from the difference in the mobility of the wild-type bottom component and its dimer (Hull & Lane, 1973). This was about 16 incremental distances which represents a hydrated diameter difference of about 12 nm (the difference in the hydrated diam. of the monomer and dimer). Thus each incremental distance is equivalent to about 0.75 nm.

Analysis of the nucleic acids of the Wisconsin and CAT isolates of PEMV revealed the two RNA species reported for other isolates (Gonsalves & Shepherd, 1972; Hull & Lane 1972; German & de Zoeten, 1975) which were in the approximate ratio of bottom and middle components. There were no RNA components detected which could account for the range of different particle sizes found in these isolates.

The coat protein of the non aphid-transmissible isolates of PEMV forms a major band, with an apparent mol. wt. of 22 000 and often a minor band, mol. wt. 44 000, on electrophoresis in sodium dodecyl sulphate (SDS) polyacrylamide gels (Fig. 2); the minor band is considered to be formed by stable dimers of the protein in the major band. On SDS gel electrophoresis
of the proteins from Wisconsin, P3 and CAT isolates, these bands and two further minor bands are found (Fig. 2). The two extra minor bands have apparent mol. wt. of 28000 and 58000; it is thought possible that they might be monomers and dimers of the same protein. Analysis on SDS-polyacrylamide gels indicated that there was more of the extra proteins in virus extracted from bands B4 to B8 than in that extracted from bands B1 to B3. None of the protein bands stain using the periodate-Schiff's procedure and thus are unlikely to be glycoproteins. The CAT isolate has been shown to have an antigen not present in the CNT isolate (Clarke & Bath, 1976); R. G. Clarke (personal communication) associated this with a protein of mol. wt. 53000 which presumably is the same as the 58000 mol. wt. protein described here.

The gel electrophoretic behaviour of aphid-transmissible PEMV resembles that of Qβ phage which has various size classes of particles due to the replacement of coat protein molecules by the larger IIb protein molecules (Radloff & Kaesberg, 1973); the number of replacements varies from 5 to 33. If in aphid-transmissible PEMV the particles in each size category (B1 to B8) have increasing numbers of normal coat protein subunits replaced by the larger (28000) protein subunit, it can be calculated that an incremental replacement of one protein subunit per size category (i.e. B1, 5 replacements...B8, 12 replacements) would result in a mean of about 2.5% of larger protein subunits. The larger protein subunits comprise about 8.5% of the total protein in the gel (estimated from traces of stained gels and assuming each protein binds the same amount of stain per unit mass), which, when corrected for mol. wt., is about 5% of the protein subunits. This suggests that the incremental replacement number should be 2 protein subunits per size category. Thus, if this hypothesis is correct, the B1 particles would have 170 coat protein subunits and 10 larger protein subunits (being five size categories larger than the 180 coat protein subunit wild-type bottom component; Hull & Lane, 1973) and B8 particles would have 156 coat protein and 24 larger protein subunits.

The IIb protein of Qβ has been shown to be the result of failure of termination of translation of the coat protein cistron (Moore et al. 1971; Weiner & Weber, 1971). At present, the serological evidence (Clarke & Bath, 1976) suggests that the PEMV larger protein is unlikely to be a 'read through' coat protein.

There is the possibility that the PEMV larger protein molecules do not replace coat protein molecules but just adhere to the exterior of the virus particles. This is not very likely since the larger protein remains with the virion on CsCl centrifugation; furthermore, examination of the virus particles in the electron microscope does not show any adhering protein.

It would appear that the CAT isolate is a mixture of aphid-transmitted and non-transmitted strains. The non-transmitted strain (CNT) might be aphid-transmitted from the CAT culture to a small extent due to coating of its nucleic acid with the protein of the aphid-transmissible strain. Comparison of the yields in purification showed that more than 10 times as much virus could be extracted from peas infected with non aphid-transmitted isolates than from those infected with aphid-transmitted isolates. Thus, on the repeated sap transmission of the CAT isolate, the CNT strain would tend to dominate and exclude the aphid-transmitted strain.

It has been shown (Harris et al. 1975) that the particles of the CAT isolate entered the salivary glands of the vector whereas those of the CNT strain appeared to be excluded. It is interesting to speculate that the extra protein(s) found only in the aphid-transmissible isolates might be a transmission factor which enables the particles of those isolates to pass into the salivary glands.

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REFERENCES


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