Nucleic Acids in the Potato Virus X Group and in some Other Plant Viruses: Comparison of the Molecular Weights by Electrophoresis in Acrylamide-agarose Composite Gels

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The molecular weight of the nucleic acid is a basic property of a virus, and has been shown to be of great value for classifying animal viruses (Bellett, 1967). However, this property is known for few plant viruses. In the potato virus X group, for example, only the RNA of potato virus X (PVX) itself has a known molecular weight. The reported value \(2.1 \times 10^6\) has been calculated from the total particle weight of the virus and its phosphorus content (Knight, 1963).

Recently electrophoresis in acrylamide gels of low concentration has been successfully used to estimate the molecular weights of large RNA molecules (Loening, 1968; 1969). Studies including the RNAs of five plant viruses have been reported by Bishop, Claybrook & Spiegelman (1967), and the RNA of tobacco mosaic virus (TMV) has been used as a marker in the re-examination of the molecular weight of poliovirus RNA (Tannock, Gibbs & Cooper, 1970). Gels prepared with less than 3.5% acrylamide are difficult to handle as vertical slabs, but acrylamide-agarose composite gels are more stable and have increased the usefulness of the technique (Peacock & Dingman, 1968). With slight modifications this technique was used in the work reported here to compare the RNA molecular weights of viruses in the PVX group, namely PVX, cactus virus X (CaVX), white clover mosaic (WCMV), clover yellow mosaic (CYMV) and pawpaw mosaic (PMV) viruses, as well as of some other plant viruses, namely TMV, sowbane mosaic (SMV), belladonna mottle (BMV) and brome mosaic (BrMV) viruses.

The method used to extract infectious RNAs from purified viruses or ribosomal marker RNAs from plant, animal or microbial cells was similar to that described by Bockstahler & Kaesberg (1965). It was essential to free all equipment from nucleases using the treatments suggested by these authors, and deionized water had to be autoclaved. With most viruses more RNA was obtained when 0.02 M-tris-borate buffer, pH 8.8 containing 0.089 M-EDTA, 1% sodium dodecylsulphate (SDS) and 1% Bentonite was used instead of the acetate buffer used by Bockstahler & Kaesberg. In some experiments 1% Macaloid or 5% diethylpyrocarbonate (DEPC) were used instead of Bentonite. After phenol extraction the RNAs were precipitated with ethanol at \(-20^\circ\) overnight, the precipitates were washed with 70% ethanol and dissolved in an electrophoresis buffer of tris-EDTA-boric acid, pH 8.3 (Peacock & Dingman, 1968). To remove any intact virus particles which might have interfered in infectivity tests, the samples were ultracentrifuged for 1 hr at 130,000 g. Most virus RNAs were unstable in solution and had to be used as soon as possible. The infectivity of the various RNAs was tested on the following host plants: *Gomphrena globosa* for PVX, CaVX, CYMV and PMV, *Chenopodium quinoa* for CYMV, *Phaseolus vulgaris* cv. Saxa for WCMV and *Nicotiana glutinosa* for BMV. The infectivity of all the virus nucleic acids was destroyed completely when they were incubated for 30 min. at 37° with 1 µg./ml. pancreatic ribonuclease.

Electrophoresis experiments were done in the vertical apparatus of Stegemann (1970) using either the standard buffer of Loening (1969) or that of Peacock & Dingman (1968).
with 0.2% SDS with gels containing 1.25% agarose and acrylamide at concentrations ranging between 1.5% and 2.5%. Current was applied for 1.5 to 3 hr depending on the acrylamide concentration and the voltage used (between 200 and 400 v). After electrophoresis the gels were placed in 1 M-acetic acid for 20 min., rinsed in running tap water for 30 min., stained with 0.02% methylene blue in 0.4 M-sodium acetate buffer, pH 4.7, for 3 to 4 hr and destained with running tap water overnight. The molecular weights estimated by this method seemed to be independent of buffer, acrylamide concentration and voltage used.

![Image]

**Fig. 1.** Electrophoresis at 200 v and 160 mA for 3 hr of RNAs of the PVX group and of marker RNAs in a 1.25% agarose-2% acrylamide gel with 36 mM-tris, 30 mM-NaH$_2$PO$_4$, 1 mM-EDTA and 0.2% sodium dodecylsulphate at pH 7.9. Column a from top: RNA from TMV, 25 s and 18 s RNA from carrot; columns b, c, d, e, f and g: RNAs from WCMV, PVX, CaVX strain K11, CaVX strain B1, CYMV and PMV, respectively; column h from top: RNA from SMV, RNAs from BrMV. The fourth band from top in column h is due to an unidentified component in the BrMV preparation.

**Fig. 2.** Relationship between molecular weight and electrophoretic migration of marker RNAs. The conditions of electrophoresis were the same as in Fig. 1. ⊖, PVX RNA; ▲, TMV RNA; ○, 28 s and 18 s ribosomal RNA from rabbit; ● and ■, 25 s and 18 s ribosomal RNA from radish and carrot, respectively; ⊙, SMV RNA, △, BrMV RNAs; ▼, ribosomal RNA from *Euglena*.

The following RNAs served as markers (mol. wts. and references in parentheses): ribosomal RNAs from carrot and radish (0.7 x 10$^6$ and 1.29 x 10$^6$; Loening, 1968), from *Euglena gracilis* (0.85 x 10$^6$; Loening, 1968) and from rabbit liver (0.7 x 10$^6$ and 1.72 x 10$^6$; Loening, 1968), RNA from TMV (2.0 x 10$^6$; Boedtker, 1960), from PVX (2.1 x 10$^6$; Knight, 1963), from SMV (1.27 x 10$^6$; Kado & Black, 1968) and from BrMV (1.0 x 10$^6$, 0.7 x 10$^6$ and 0.3 x 10$^6$; Bockstahler & Kaesberg, 1965).
Fig. 1 shows an electropherogram of nucleic acids of viruses of the PVX group together with virus and ribosomal marker RNAs. With all plant viruses studied with the exception of BrMV (Bockstahler & Kaesberg, 1965) only one strong RNA band was formed. The faint smear of stain in front of the RNA bands (Fig. 1, WCMV) indicates a degradation of the RNAs which was probably due to traces of nucleases.

Plant nucleases have been found to be strongly inhibited by DEPC (Solymosy et al. 1968). In our experiments with some viruses (PVX, WCMV) a stronger band was obtained with DEPC in the extraction medium, whereas with others the band was not affected (BMV) or was almost completely absent (PMV, 3 strains of CaVX, SMV). The position of the bands of the RNAs of these viruses was not changed by DEPC. The infectivity of all RNAs, however, was lost completely or almost completely (PVX, CaVX) with DEPC in the extraction medium. Similar observations have recently been made by Öberg (1970) with poliovirus RNA.

The distances that the marker RNAs migrated were plotted against the log values of their reported molecular weights (Fig. 2). The molecular weights of the PVX and SMV RNAs, which so far have not been studied by acrylamide electrophoresis, fit well into this straight line relationship. The size of the RNA of BMV was estimated to be $2 \times 10^6$ on the basis of this relationship. This estimate also agrees well with a value of $1.8$ to $2.0 \times 10^6$ obtained from the total particle weight and its phosphorus content (Paul et al. 1968).

For the RNAs of the other viruses in the PVX group the following sizes were estimated (number of observations in parentheses): CaVX strain B'l $2.1 \times 10^6$ daltons (12), CaVX strain K 11 $2.1 - 10^6$ (14), CYMV $2.4 \times 10^6$ (17), PMV $2.2 \times 10^6$ (15), WCMV $2.4 \times 10^6$ (13). The standard errors of each mean were less than $1\%$.

The size of the nucleic acid of a virus is one of the properties encoded in the virus cryptograms suggested by Gibbs et al. (1966). The work reported here shows that the cryptograms of viruses in the PVX group are, at present:

- CaVX: R/I: 2.1/* : E/E: S/*
- CYMV: R/I: 2.4/* : E/E: S/*
- PMV: R/I: 2.2/* : E/E: S/*
- PVX: R/I: 2.1/6: E/E: S/O (Fu)

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