Special Topics

Electrophoretic Variability Among Dianthoviruses

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ABSTRACT


Seven distinct members of the dianthovirus group were purified and their mobilities analyzed by virion electrophoresis. Each strain or strain possessed a single electrophoretic form as determined by differential staining of encapsidated RNA and coat protein by ethidium bromide and Coomassie Brilliant Blue, respectively. Two strains of sweet clover necrotic mosaic virus (SCNMV-38 and SCNMV-59) were electrophoretically distinct. Carnation ringspot virus (CRSV) strain A co-migrated with SCNMV-38, whereas CRSV-N had the slowest mobility of all the seven strains tested. Among the three red clover necrotic mosaic virus (RCNMV) strains used in this study, RCNMV-TpM 34 and RCNMV-TpM 48 had similar mobilities, whereas RCNMV-Aus was faster. The isoelectric points of all the strains were in the pH range 4.75–5.1 except that of CRSV-N, which was between 6.0–6.2. The single coat protein of all the dianthoviruses tested was estimated to have a molecular mass of about 39,000 daltons.

Additional keywords: agarose gel electrophoresis, legume viruses, strain relationships.

The dianthovirus group consists of carnation ringspot virus (CRSV), the type member, red clover necrotic mosaic virus (RCNMV), and sweet clover necrotic mosaic virus (SCNMV), and they share the following general properties: two species of single-stranded RNA molecules encapsidated in isometric particles measuring about 30–35 nm in diameter, with a single coat protein of about 39,000 daltons (8,16). Several strains of each virus have been characterized based on biological and serological properties. Serological relationships among the members and the strains revealed varying degrees of relationships as assessed by monoclonal (9,11) and polyclonal antibodies (7,11,18–20,23,26). In a previous paper, we reported the electrophoretic variability between virions of two strains of SCNMV (23). We extended this observation to several other strains of RCNMV and CRSV, and in this communication we describe the distinct differences in virion electrophoretic mobilities among dianthoviruses as determined by agarose gel electrophoresis. Preliminary results have appeared in an abstract form elsewhere (22).

MATERIALS AND METHODS

Viruses. The following virus strains were used in this study: carnation ringspot virus (CRSV), strains A and N (31); sweet clover necrotic mosaic virus (SCNMV), strains 38 and 59 (23); and red clover necrotic mosaic virus (RCNMV), strains TpM 34, TpM 48 (18), and Aus (7). Each virus was multiplied separately on Phaseolus vulgaris 'Red Kidney' and purified according to a previously published procedure (7). Final virus preparations were suspended in 50% glycerol and stored at −20 C.

Coat protein analysis. Purified virus preparations were dissociated at 100 C in the presence of sodium dodecyl sulfate (SDS) and 2-mercaptoethanol and analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) by using the discontinuous buffer system of Laemmli (14). Bands were located by staining with Coomassie Brilliant Blue R250 and destaining overnight in a destaining solution (methanol:acetic acid:water, 10:10:80).

Isoelectric focusing. Isoelectric focusing of purified virus preparations was done as previously described (23). Each virus preparation (2–5 μg) was subjected to focusing in 1% agarose gel (Isogel
agaro, FMC Corporation) containing 10% glycerol and 2% Bio-
Lyte ampholytes with pH ranges of 3-10 or 5-7 (Bio-Rad
Laboratories, Richmond, CA) using Bio-Rad’s Mini IEF cell
(model 111). Focusing and detection of proteins were done
according to the protocol supplied by the manufacturer. Focusing
was done under constant voltage conditions with voltage being
gradually increased, initially at 100 V for 15 min, 200 V for 15
min followed by 450 V for 1 hr. After focusing was complete,
the gel was fixed for 15 min in a fixative solution (30% methanol,
5% trichloroacetic acid, and 3.5% sulfosalicylic acid) followed
by a brief rinse in 95% ethanol. The gel was stained for 30 min
with 0.2% Coomassie Brilliant Blue R250 made in 28% ethanol
and 14% acetic acid followed by destaining in 28% ethanol and
14% acetic acid. The isoelectric point of each virus, calculated
from an average of three separate experiments, was determined
by a regression analysis of the pH gradient of the gel as previously
described (24).

**Agarose gel electrophoresis of virions.** Nondenaturing agarose
gel electrophoresis of purified virus preparations was done as
previously described (23). Agarose (Seakem LE, FMC Corpo-
ration) gels (0.8%) were cast in electrophoresis buffer (10 mM
Na2HPO4, NaH2PO4 buffer, pH 7.0). Samples were prepared by
mixing 20 µg of virus and 1 µl of tracking dye (0.5% Bromophenol
blue and 40% sucrose in electrophoresis buffer) and were electrophoresed at a constant voltage of 3 V/cm at
4°C with buffer recirculation (100 ml/min) to avoid the
development of a pH gradient during electrophoresis. After
electrophoresis, the gel was stained with ethidium bromide (0.5
µg/ml) in the presence of 1 mM disodium ethylenedia-
minetetraacetic acid to locate the encapsidated RNA. After a
brief rinse with distilled water, the same gel was restained with
Coomassie Brilliant Blue R250 and destained overnight in
destaining solution to locate the coat protein. Gels were photo-
graphed using Polaroid instant film (Type 57) using a red filter
(for ethidium bromide-stained gels) or an orange filter (for
Coomassie Blue-stained gels).

**RESULTS**

Coat protein profiles. A single polypeptide species of about
39,000 daltons was detected for each virus (Fig. 1). No significant
mobility differences could be seen among the viral proteins.

**Isoelectric focusing.** The isoelectric points of SCNMV-38,
SCNMV-59, CRSV-A, RCNMV-TpM 34, RCNMV-TpM 48, and
RCNMV-Aus were in the pH range of 4.75–5.1 (average of three
experiments), whereas that of CRSV-N was between 6.0 and 6.2
as determined by the regression analyses of the pH gradients of
3 and 10 or 5 and 7 following focusing (Fig. 2A and B). Each
virus preparation showed one major polypeptide band and occa-
sionally one minor band (Fig. 2B, lane 2). The differences in
the focusing pattern were consistent among the strains and the
most discernable differences were found between CRSV strains,
A and N and RCNMV strains. TpM 48 and Aus (Fig. 2A and B).

**Agarose gel electrophoresis.** When purified virus preparations
were electrophoresed in agarose gels under nonelectrophoretic
conditions, a single electrophoretic form was detected. The migrating
component is presumed to be a virion based on the observa-
tion that it was stained by ethidium bromide (viral RNA) and
Coomassie Brilliant Blue (viral protein). Significant mobility
differences were noticed among the strains of all three viruses
(Fig. 3A and B). All virions migrated from cathode to anode indicating a net negative charge for the virions under the
electrophoretic conditions used. SCNMV-38 and SCNMV-59 were
electrophoretically distinct as reported earlier (23) and CRSV-
A showed similar mobility to that of SCNMV-38. CRSV-N had
the slowest mobility of the strains tested in this study. RCNMV-
TpM 34 and RCNMV-TpM 48 migrated identically, whereas
RCNMV-Aus had a greater mobility and was similar to those of
SCNMV-38 and CRSV-A (Fig. 3A and B). The electrophoretic
profile of each strain and the relative mobility differences among
all the strains were consistent and highly reproducible when the
experiment was run several times during a period of 1 yr.

To confirm whether the mobility pattern displayed by each
strain is a characteristic feature, the electrophoretically distinct
strains were mixed in various combinations and then subjected
to nondenaturing agarose gel electrophoresis. After differential
staining as described above, each individual virus preparation in
a mixture retained its characteristic mobility pattern and
migrated as a single but distinct electrophoretic form (Fig. 4A
and B).

**DISCUSSION**

Results of the physical characterization of dianthoviruses by
agarose gel electrophoresis and isoelectric focusing provide
evidence for differences in electrophoretic mobilities among their
various strains. Similar results have been obtained for other
groups of plant viruses (2-4,15,27). The taxonomic grouping of
the dianthoviruses is based mainly on their serological relationships,
and the differences in electrophoretic mobility support this
taxonomy to some extent. The two electrophoretically distinct
strains of SCNMV were also serologically distinguishable by
immunodiffusion tests (23). Similarly, electrophoretically related strains of
CRSV, CRSV-A, and CRSV-N, which showed striking mobility
differences in this study, were serologically distinguishable (31).

The reason for conflicting data on RCNMV-TpM 34 and TpM
48 is unknown. Previous reports stated that RCNMV-TpM 34
and TpM 48 were serologically and electrophoretically distinct
(5,6,18,19); however, we found that the migration patterns of
these two strains were similar, and consistent and reproducible
among different batches of virus preparations that were made
over a period of 1 yr. Also, in our study, the isoelectric points
of RCNMV-TpM 34 and TpM 48 were very similar. A possible
explanation for this apparent discrepancy might be that one or
both of the strains used in this study could be mutants of the
RCNMV strains originally described (17), since the
symptomatology produced by our strains on selected host plants
was different from that of the original strains (25). This
discrepancy must be resolved by further investigation by using the
original strains since RCNMV-TpM 34 and TpM 48 are grouped into
two different serotypes (serotype A and serotype B respec-
tively), RCNMV-Aus, belonging to serotype D, has a different
mobility pattern and a significantly different isoelectric point from
those of RCNMV-TpM 34 and TpM 48.

There are several possible reasons for the origin of charge
heterogeneity. The host passage effect may have occurred, leading
to an adaptive mutation in a particular virus strain (32). In the case of SCNMV, the type strain (SCNMV-38) was originally isolated from sweet clover (10), whereas its new serotype was isolated from alfalfa (23). Of the two CRSV strains, CRSV-A forms stable 12-particle aggregates at acidic pH, whereas CRSV-N is a nonaggregating strain. They are also serologically distinguishable as determined by reactions of partial identity in gel diffusion tests (31). The striking differences in virion mobilities and isoelectric points of CRSV-A and CRSV-N are probably caused by differences in the amino acid composition of their respective coat proteins (30). Mobility differences have been correlated with differences in amino acid composition for strains of hibiscus chlorotic ringspot virus (HCRV) (13), thus the observed differences among the three strains of RCNMV may be explained similarly. Because the three strains were originally reported from Czechoslovakia (RCNMV-TpM 34 and TpM 48) (17) and Australia (RCNMV-Aus) (7), this geographical isolation may have allowed for some genetic drift in their coat proteins.

Virion electrophoresis has been used to detect tobacco mosaic virus in crude plant extracts (1), to study its in vitro disassembly products (12), and to characterize variants of HCRV (13), and strains of panicum mosaic virus and their associated satellites (29). It has also been used extensively in characterizing bacteriophages (28). It is easy to perform, rapid and sensitive, since only small amounts, as low as 15-30 ng/ml, of virus preparations are required (21). Furthermore, it has potential application as an aid in distinguishing electrophoretically distinct strains of a virus in mixed infections that may occur under field conditions.

Fig. 2. Isoelectric focusing of purified preparations of dianthoviruses. A, Lane 1, sweet clover necrotic mosaic virus (SCNMV)-38; lane 2, SCNMV-59; lane 3, carnation ringspot virus (CRSV)-A; lane 4, red clover necrotic mosaic virus (RCNMV)-TpM 34; lane 5, RCNMV-TpM 48; lane 6, RCNMV-Aus. The pH gradient was generated using Bio-Lyte 5-7 ampholytes. B, Lane 1, carnation ringspot virus (CRSV)-A; lane 2, CRSV-N. The pH gradient was generated using Bio-Lyte 3-10 ampholytes. Both gels were stained with Coomassie Brilliant Blue and destained overnight. Migration was from top (positive) to bottom (negative).

Fig. 3. Agarose gel (0.8%) electrophoresis of purified preparations of dianthoviruses. Lane 1, sweet clover necrotic mosaic virus (SCNMV)-38; lane 2, SCNMV-59; lane 3, carnation ringspot virus (CRSV)-A; lane 4, CRSV-N; lane 5, red clover necrotic mosaic virus (RCNMV)-TpM 34; lane 6, RCNMV-TpM 48; lane 7, RCNMV-Aus. A, Gel stained with ethidium bromide (0.5µg/ml in water containing 1 mM disodium EDTA). B, The same gel stained with Coomassie Brilliant Blue and destained overnight. Migration was from top (negative) to bottom (positive).
**Fig. 4.** Agarose gel (0.8%) electrophoresis of mixtures of purified dianthoviruses. Lane 1, sweet clover necrotic mosaic virus (SCNMV)-38; lane 2, SCNMV-38 + SCNMV-59; lane 3, SCNMV-59; lane 4, carnation ringspot virus (CRSV)-A; lane 5, CRSV-A + CRSV-N; lane 6, CRSV-N; lane 7, red clover necrotic mosaic virus (RCNMV)-TpM 34; lane 8, RCNMV-TpM 34 + RCNMV-Aus; lane 9, RCNMV-Aus; lane 10, RCNMV-TpM 48 + RCNMV-Aus; lane 11, RCNMV-TpM 48. A, Gel stained with ethidium bromide (0.5 μg/ml in water containing 1 mM disodium EDTA). B, The same gel restained with Coomassie Brilliant Blue and destained overnight. Migration was from top (negative) to bottom (positive).

**LITERATURE CITED**

virus on agarose gel electrophorograms at the nanogram level. Electrophoresis 9:299-302.