Comparison of the Effects of Sodium Dodecyl Sulfate on Some Isometric Viruses

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ABSTRACT

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Twenty-two viruses or virus strains were used in a comparison of the effects of sodium dodecyl sulfate (SDS), ethylenediaminetetraacetic acid (EDTA), and $MgCl_2$ on spherical virus particles. At pH 7, brome mosaic (BMV), broad bean mottle (BBMV), turnip crinkle (TCV), cucumber mosaic (CMV), tomato aspermy viruses (TAV), and four strains of cowpea chlorotic mottle virus (CCMV) were completely dissociated into protein and RNA by 0.2% SDS or less. At pH 5, all but TAV were less sensitive to SDS and two strains of CCMV were not dissociated in 7.5% SDS. With certain SDS concentrations, BMV, BBMV, TCV, CCMV, TAV, CMV, and carnation ringspot virus (CRSV) formed components which were probably SDS-swollen virus particles. At pH 7, $MgCl_2$ decreased the SDS sensitivity of BMV, BBMV, TCV, CCMV strains, saguaro virus, and strains of CRSV. Particles of SDS-sensitive strains of CCMV, and CRSV that had been reacted with formaldehyde at pH 5 were totally resistant to SDS at pH 5; at pH 7, however, they became increasingly swollen with increasing SDS concentrations. At pH 7, some particles in cucumber necrosis virus, tobacco necrosis virus, and carnation mottle virus preparations were dissociated by low concentrations of SDS, but the remaining particles were not dissociated in 15% SDS. Turnip yellow mosaic virus and two comoviruses (echtes Ackerböhnemosaik and radish mosaic viruses) were resistant to SDS in the presence of EDTA. Southern bean mosaic, tomato bushy stunt, cocksfoot mottle, and sowbane mosaic viruses were resistant to SDS alone but were dissociated in SDS-EDTA solutions at pH 7.

Boatman and Kaper (3, 4) added increasing concentrations of SDS to suspensions of 13 plant viruses at room temperature and after brief exposure observed the effect of this treatment in the analytical ultracentrifuge. They found that alfalfa mosaic, brome mosaic (BMV), peanut stunt, cucumber mosaic (CMV), broad bean mottle (BBMV), cowpea chlorotic mottle (CCMV), and turnip crinkle (TCV) viruses were dissociated completely into RNA and protein by low concentrations of SDS. Some of the virions in turnip yellow mosaic (TYMV), southern bean mosaic (SBMV), and carnation mottle (CarMV) virus preparations were dissociated at moderate SDS concentrations, but all of the virions did not dissociate at any SDS concentration. Tomato bushy stunt (TBSV), tobacco ringspot, and bean pod mottle viruses did not dissociate at any SDS concentration. The SDS-sensitive viruses predominantly are stabilized by protein-RNA interactions, but most of the SDS-resistant viruses have strong protein-protein interactions (9, 10). Boatman and Kaper (3) proposed that SDS binding specifically interferes with protein-RNA interactions. Later, Boatman and Kaper (4) used radioactive SDS to determine the amount of SDS bound to CMV, BMV, and TYMV. Because TYMV did not bind SDS under the conditions of their experiment, they concluded that resistance to SDS was not necessarily correlated with the strength of protein-protein interactions.

Nelson and Tremaine (14) observed that a virus from saguaro cactus (SV) was dissociated by 0.05% SDS at pH 7 and 0.1% SDS at pH 5. On the basis of these results and of its stability at pH 7 and 5, they concluded that SV was stabilized by protein-RNA interactions and pH-dependent protein-protein interactions. Tremaine et al. (24) isolated three strains of carnation ringspot virus which differed greatly in sensitivity to SDS (23).

We now report the effect of SDS on 22 viruses or strains at both pH 5 and pH 7 using the density-gradient technique described previously (14, 23). The effect of Mg^{++}, ethylenediaminetetraacetic acid (EDTA), and formaldehyde treatment on the SDS sensitivities of certain viruses also are reported.

MATERIALS AND METHODS

The viruses.—A list of viruses and their sources is given in Table 1. Most of the viruses were purified in 0.1 M sodium acetate buffer (pH 5.0) by polyethylene glycol precipitation and differential centrifugation (24). The viruses CarMV, CMV, and TAV, which precipitate at pH 5, were purified as described previously (18, 20) and suspended in 0.1 M sodium phosphate buffer, pH 7.0. The absence of nonviral material in preparations was

demonstrated by analytical and density-gradient centrifugation. These preparations were used in SDS tests shortly after their purification but almost identical results were obtained with the same preparations that had been stored for 2 years.

Treatment of virus with sodium dodecyl sulfate.—The effect of SDS was tested in 0.1 M sodium acetate buffer, pH 5.0 and in 0.1 M sodium phosphate buffer, pH 7.0. Concentrated virus preparations were diluted in either buffer, then mixed with SDS in the same buffer to yield virus at approximately 700 μg/ml in the required SDS concentrations. After standing at 22-26 C for 20 minutes, 0.25 ml of the mixture was layered on a 50 to 350 mg/ml sucrose gradient in the same buffer (23), and centrifuged for 1 hour at 39,000 rpm in a Beckman SW 41 rotor at 20 C. Sodium dodecyl sulfate was not included in the gradients because it interfered with ultraviolet scans, and contributed to the density of the gradient. Centrifugation was started 30 minutes after the samples were mixed with SDS. The gradients were scanned at 254 nm in an ISCO Model UA-4 ultraviolet monitor with a Model 612 recorder, Model D density-gradient fractionator, and a Model 184 tube-piercing device. The percentage dissociation of some viruses by SDS was estimated from the areas under the nucleic acid and virus component peaks in absorbance scans of density gradients. In some experiments the viruses were added to SDS solutions containing ethylenediaminetetraacetic acid (EDTA) or $MgCl_2$ to yield desired concentrations. Appropriate control experiments also were done with SDS, EDTA, and $MgCl_2$.

Determination of virus concentration.—Virus concentration was determined spectrophotometrically using the extinction coefficient at 260 nm appropriate for each virus.

Electron microscopy.—To examine virus particles in SDS a drop was allowed to stand on a grid for a few minutes and the grid was washed by dripping gently with 20-40 drops of either 2% sodium phosphotungstate, pH 7.0 (PTA) or 2% uranyl acetate, pH 5.0 (UA). The grid was drained by touching with filter paper, leaving a thin layer of stain, and then allowed to dry. The grids were examined immediately in a Philips EM 200 or EM 300 electron microscope.

RESULTS

Viruses dissociated by low concentrations of sodium dodecyl sulfate.—*Bromoviruses.*–1) Cowpea chlorotic mottle virus strain T (CCMV-T).—At pH 5, CCMV-T sedimented about half way down the sucrose density-gradient tube as a single component (Fig. 1). Amounts of virus decreased and amounts of dissociation products (protein and RNA components) increased with increasing SDS concentrations between 0.025% and 0.2%, but undissociated virus was still present at 0.2% SDS. At pH 7, the virus sedimented more slowly, probably because of pH-induced swelling (2). Two nucleoproteins were found upon reaction of CCMV-T with 0.005% or 0.01% SDS at pH 7: one sedimented to the same depth as unreacted virus; the other sedimented more slowly and was called intermediate sedimenting material (IS). The quantities of IS at 0.005% and 0.01% SDS were similar but the virus peak was greater at 0.005% SDS; the RNA peak was greater at 0.01% SDS. These results suggest that IS is an intermediate in the dissociation of virus into RNA and protein. The width of the IS peak demonstrated heterogeneity and $A_{280/260}$ measurements indicated that this component contained a slightly lower RNA content than the native virus. Electron microscopy of CCMV-T at pH 7 in 0.005% SDS, stained with PTA, showed spherical particles with diameters 28-32 nm, as well as 25-nm

TABLE 1. Viruses and virus strains tested for dissociation in sodium dodecyl sulfate solutions

Virus name	Abbreviated designation	Source or reference
Broad bean mottle virus	BBMV	Agrawal and Tremaine (1)
Brome mosaic virus	BMV	Agrawal and Tremaine (1)
Carnation mottle virus	CarMV	Tremaine (20)
Carnation ringspot virus		
strain aggregating at high temperature	CRSV-N	Tremaine et al. (24)
strain aggregating at low temperature	CRSV-R	Tremaine et al. (24)
Cocksfoot mottle virus	CfMV	A. Brunt, Littlehampton, England
Cowpea chlorotic mottle virus		
type strain	CCMV-B	A. Thomas, Seattle, Washington
mild strain	CCMV-T	Agrawal and Tremaine (1)
bean strain	CCMV-A	Fulton et al. (7)
bean yellow stipple strain	BYSV	Fulton et al. (7)
Cucumber mosaic virus	CMV	Stace-Smith and Tremaine (18)
Cucumber necrosis virus	CNV	Tremaine (21)
Echtes Ackerböhnemosaik virus	EAMV	R. Stace-Smith, Vancouver, Canada
Radish mosaic virus	RMV	R. Stace-Smith, Vancouver, Canada
Saguaro cactus virus	SV	Nelson and Tremaine (14)
Southern bean mosaic virus (bean)	SBMV	Tremaine and Wright (25)
Sowbane mosaic virus	SoMV	R. Stace-Smith, Vancouver, Canada
Tobacco necrosis virus	TNV	R. Stace-Smith, Vancouver, Canada
Tomato aspermy virus	TAV	Stace-Smith and Tremaine (18)
Tomato bushy stunt virus (prunus)	TBSV	Tremaine (19)
Turnip crinkle virus	TCV	Tremaine and Chidlow (22)
Turnip yellow mosaic virus	TYMV	R. Stace-Smith, Vancouver, Canada

diameter spherical particles. The smaller resembled particles seen in untreated virus preparations. The larger are probably either SDS-swollen virus or IS component. Particles with similar properties have been obtained upon reaction of CRSV and BMV with SDS (4, 23).

The effect of length of exposure and virus concentration on the reaction of CCMV-T with 0.005% SDS at pH 7 was investigated. Virus exposed to SDS for periods of 0.25-4.0 hours gave almost identical absorbance profiles. Apparently the reaction with SDS occurs within 15 minutes and does not proceed further during 4 hours. The reaction is more dependent on SDS concentration than on virus concentration. Dissociation was slightly greater with virus at 700 μg/ml than at 1,800 μg/ml and increased further at 140 μg/ml. However, dissociation was increased more by doubling the SDS concentration from 0.005% to 0.01% (Fig. 1) than by the fivefold decrease in virus concentration.

2) Cowpea chlorotic mottle virus strain B (CCMV-B).—This strain induced a yellow chlorotic mottle on cowpea plants, whereas CCMV-T produced mild mosaic symptoms, but they gave reactions of identity in gel diffusion serological tests with CCMV-B antiserum. Although the pattern of degradation was similar, CCMV-B was more resistant to SDS than CCMV-T. The concentrations of SDS required for 50% dissociation of CCMV-B were twice as great at pH 7, and ten times as great at pH 5, than that required for 50% dissociation of CCMV-T.

3) Cowpea chlorotic mottle virus serotypes.—At pH 5, almost all CCMV-A particles were resistant to SDS concentrations up to 15% (Fig. 2) but the virus peaks became broader as the SDS concentration increased. Nonideal sedimentation (5) caused by interaction of virus with high concentrations of SDS may be responsible for these broad peaks. The small quantity of nucleic acid released at 0.1% was probably from denatured virions and it did not increase with increasing SDS concentration.

At pH 7, CCMV-A sedimented as two components (Fig. 2); the faster component sedimented as far as the virus did at pH 5. The slower component is probably a pH-induced swollen virus particle. Bancroft et al. (2) observed both swollen and nonswollen virus particles in CCMV preparations dialyzed to pH 7.05; the proportions of the two particle types depended on the age of infected tissue used for virus preparation. At 0.005% SDS all the virus sedimented at the rate of the slower-sedimenting component (Fig. 2). This low concentration of SDS apparently converted the faster-sedimenting form to the slower form. With increased SDS concentrations of 0.01%, 0.025%, and 0.05%, increasing amounts of IS,

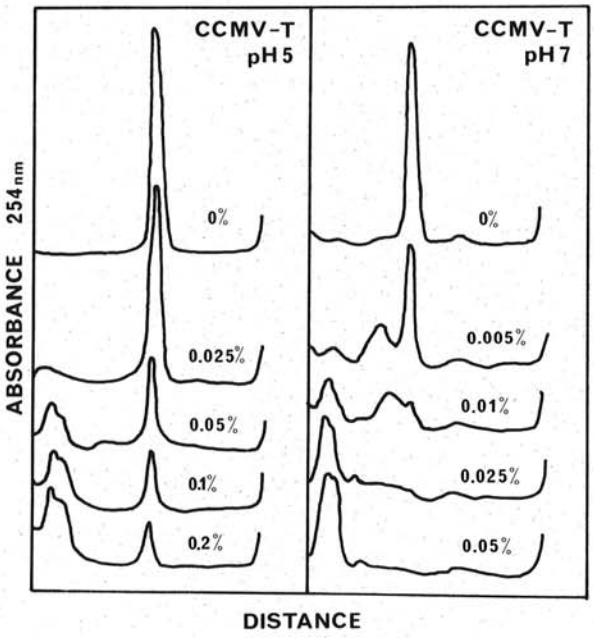

Fig. 1. The effect of concentration of sodium dodecyl sulfate (SDS) on cowpea chlorotic mottle virus strain T (CCMV-T) at pH 5 and pH 7. Tracings are of 254-nm absorbance scans of sucrose density gradients after centrifugation at 20 C for 1 hour in a Beckman SW 41 rotor at 39,000 rpm. Sedimentation was from left to right. The virus was exposed to the concentration of SDS indicated for 30 minutes before centrifugation.

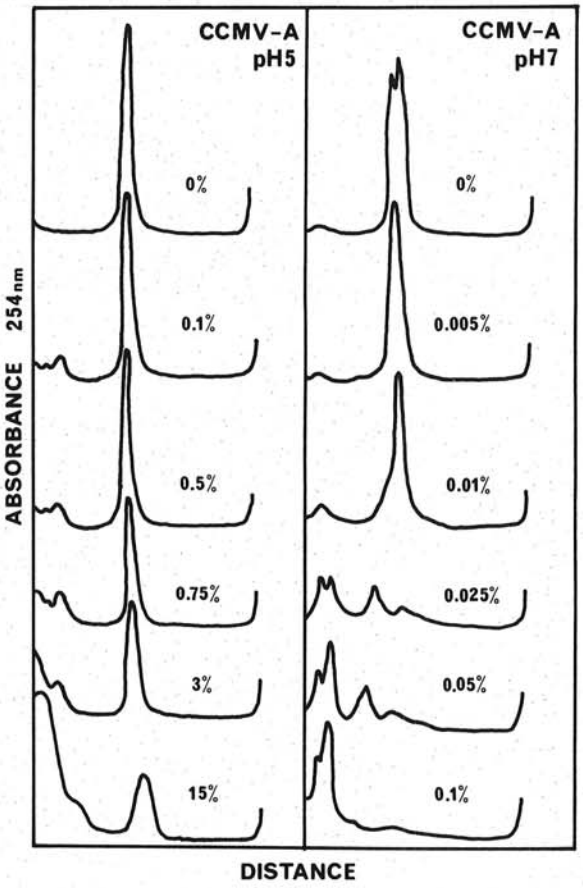

Fig. 2. The effect of concentration of sodium dodecyl sulfate (SDS) on cowpea chlorotic mottle virus strain A (CCMV-A) at pH 5 and pH 7. Tracings are of 254-nm absorbance scans of sucrose density gradients after centrifugation at 20 C for 1 hour in a Beckman SW 41 rotor at 39,000 rpm. Sedimentation was from left to right. The virus was exposed to the concentration of SDS indicated for 30 minutes before centrifugation.

protein, and nucleic acid were observed; then at 0.1% SDS the IS component dissociated into RNA and protein.

At 0.01% and 0.025% SDS (Fig. 2, pH 7) most of the RNA molecules released were small but at higher SDS concentrations larger RNA molecules also were released. Some CCMV particles contain a molecule of each of the smaller RNA-3 and RNA-4; others contain a single molecule of the larger RNA-1 or RNA-2 (11). Our results at pH 7 demonstrated that particles containing RNA-3 and RNA-4 are more sensitive to SDS than particles containing the larger RNA-1 or RNA-2. Comparable differences in SDS sensitivity were not observed at pH 5.

Two preparations of serotype BYSV were tested with SDS. One preparation, purified without DIECA, had a dark-brown color which persisted after two cycles of differential centrifugation and passage through an agarose 4-B column. This pigment probably was composed of oxidized polyphenols which were not present in the preparation purified with DIECA. The absorbance patterns obtained with both preparations in SDS experiments were identical, and similar to those obtained with CCMV-A (Fig. 2) with the exception of 7.5% and 15% SDS at pH 5. Both preparations of BYSV were almost completely degraded in 15% SDS and an IS component was detected in 7.5% SDS.

The large differences in sensitivity to SDS at pH 5 among CCMV strains are similar to those reported among CRSV strains. (23). When the SDS-resistant BYSV was exposed to 2.5% SDS at pH 5 for 2 and 3 days, dissociation was 73% after 2 days, and 85% after 3 days. With the SDS-resistant CRSV-R, dissociation was 22% after 2 days and 30% after 3 days. Therefore, these strains reacted with SDS more slowly than did the sensitive strains.

4) Brome mosaic virus (BMV).—At pH 5, BMV sedimented half way down the gradient tube as a single large peak preceded by a small peak which was probably dimer virus (Fig. 3). Sodium dodecyl sulfate at 0.01% had no effect but at 0.025% BMV was precipitated. At 0.05% and 0.1% SDS, levels of RNA components increased but undissociated virus was not detected. The absorbance profile of BMV treated with 0.01% SDS at pH 7, showed heterogeneous material preceding and following the virus peak. With the same SDS concentrations there was more dissociation of BMV at pH 7 than at pH 5.

The nature of the precipitate formed by BMV in 0.025% SDS at pH 5 was investigated by electron microscopy. The reaction mixture, stained with UA, showed aggregated intact spheres 28-30 nm in diameter, but untreated virus showed spheres 25 nm in diameter. The swollen particles of BMV were insoluble but their size and appearance was similar to the soluble IS component of CCMV-T (Fig. 1).

5) Broad bean mosaic virus (BBMV).—At pH 5, BBMV dissociated to the same extent as BMV in the same concentrations of SDS, but BBMV did not precipitate. At pH 7, BBMV in 0.01% SDS formed a minor component

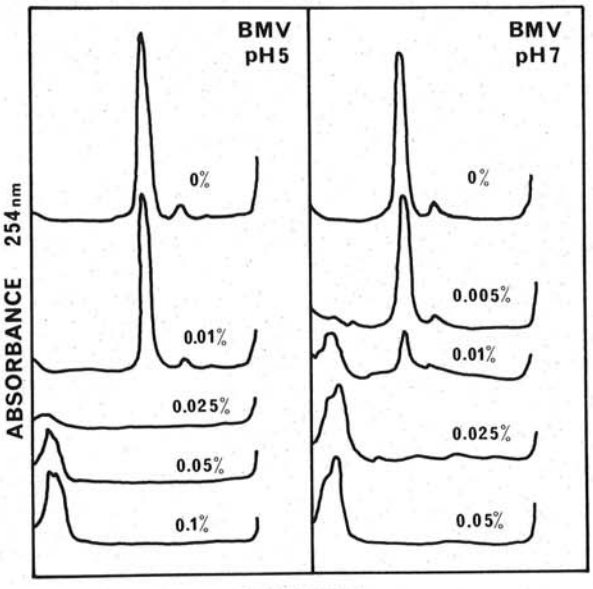

Fig. 3. The effect of concentration of sodium dodecyl sulfate (SDS) on brome mosaic virus (BMV) at pH 5 and pH 7. Tracings are of 254-nm absorbance scans of sucrose density gradients after centrifugation at 20 C for 1 hour in a Beckman SW 41 rotor at 39,000 rpm. Sedimentation was from left to right. The virus was exposed to the concentration of SDS indicated for 30 minutes before centrifugation.

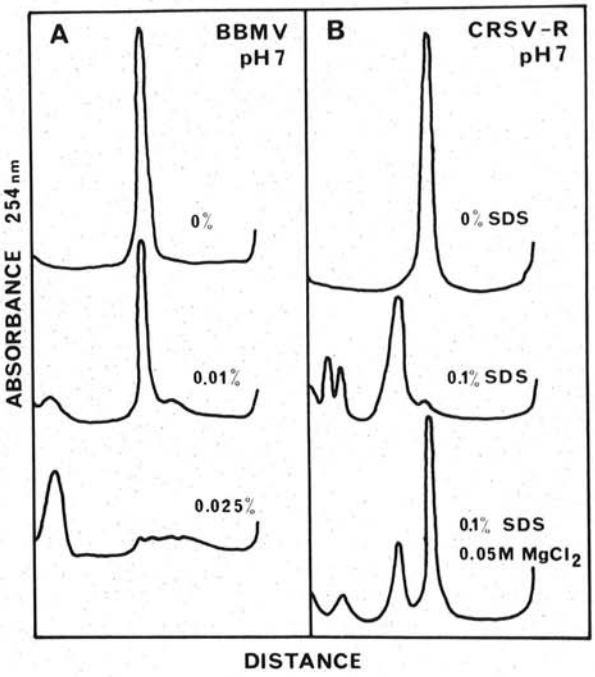

Fig. 4-(A, B). **A)** The effect of concentration of sodium dodecyl sulfate (SDS) on broad bean mottle virus (BBMV) at pH 7; **B)** The protective effect of 0.05 M $MgCl_2$ on SDS-treated carnation ringspot virus strain R (CRSV-R). Tracings are of 254-nm absorbance scans of sucrose density gradients after centrifugation at 20 C for 1 hour in a Beckman SW 41 rotor at 39,000 rpm. Sedimentation was from left to right. The virus was exposed to the concentration of SDS indicated for 30 minutes before centrifugation.

that sedimented faster than the virus (Fig. 4-A). Most of the virus dissociated in 0.025% SDS, leaving a small amount of residual virus and heterogeneous components which sedimented faster than the virus. Broad bean mosaic virus was more sensitive to SDS at pH 7 than at pH 5.

Electron microscopy of BBMV in 0.025% SDS at pH 7 showed swollen particles with diameters up to 35 nm and aggregated particles of 25-35 nm (Fig. 5-A).

Turnip crinkle virus (TCV).—At pH 5, absorbance profiles of TCV were similar to those of BMV at the same SDS concentrations. However, 0.005% and 0.01% SDS induced the formation of dimers of TCV. Electron micrographs of TCV in 0.025% SDS showed large aggregates of irregularly shaped particles, and spheres 32-36 nm in diameter. Most of the spheres were no larger than particles in untreated virus preparations. At pH 7, TCV was less sensitive to SDS than BMV. In 0.01% SDS, TCV formed an IS component. Turnip crinkle virus was more sensitive to SDS at pH 7 than at pH 5.

Cucumoviruses.—Dissociation of CMV and TAV in 0.005%-0.02% SDS was shown by analytical ultracentrifugation (4) and by density-gradient centrifugation (8). In our tests, CMV and TAV were dissociated over a similarly narrow range (0.005%-0.05%). We found that both viruses were precipitated by SDS at pH 7, and that lower concentrations of SDS were required for release of the smaller RNA components than for release of the larger RNA components. Both viruses precipitated at pH 5, but dissociated on addition of SDS. Tomato aspermy virus was more sensitive to SDS at pH 5 than at pH 7, but CMV showed the opposite relationship.

Effect of magnesium ion and ethylenediaminetetraacetic acid.—Bromoviruses and CRSV swell when the pH

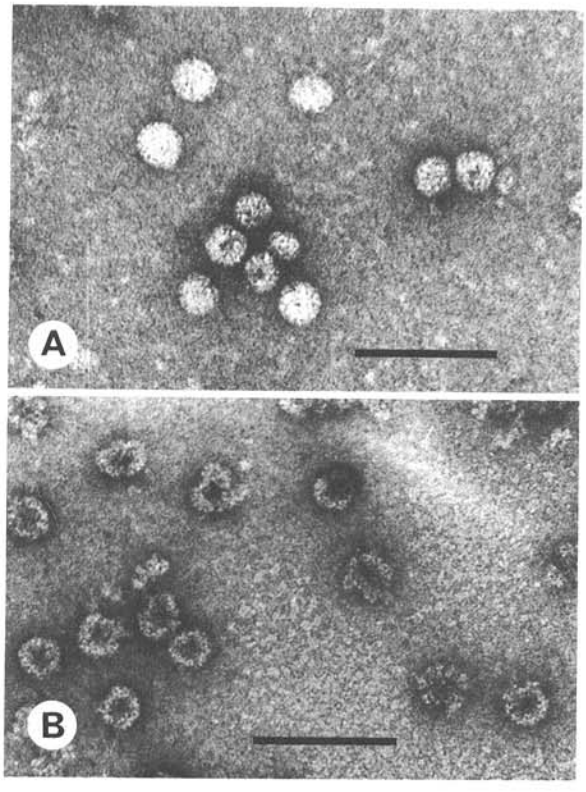

Fig. 5-(A, B). **A)** Electron micrograph of broad bean mottle virus (BBMV) treated with 0.025% sodium dodecyl sulfate (SDS) at pH 7. **B)** Electron micrograph of pH 7 formaldehyde-reacted cowpea chlorotic mottle virus strain T (CCMV-T-F7) treated with 15% SDS at pH 7. Viruses were negatively stained with 2% uranyl acetate. Bars indicate 100 nm.

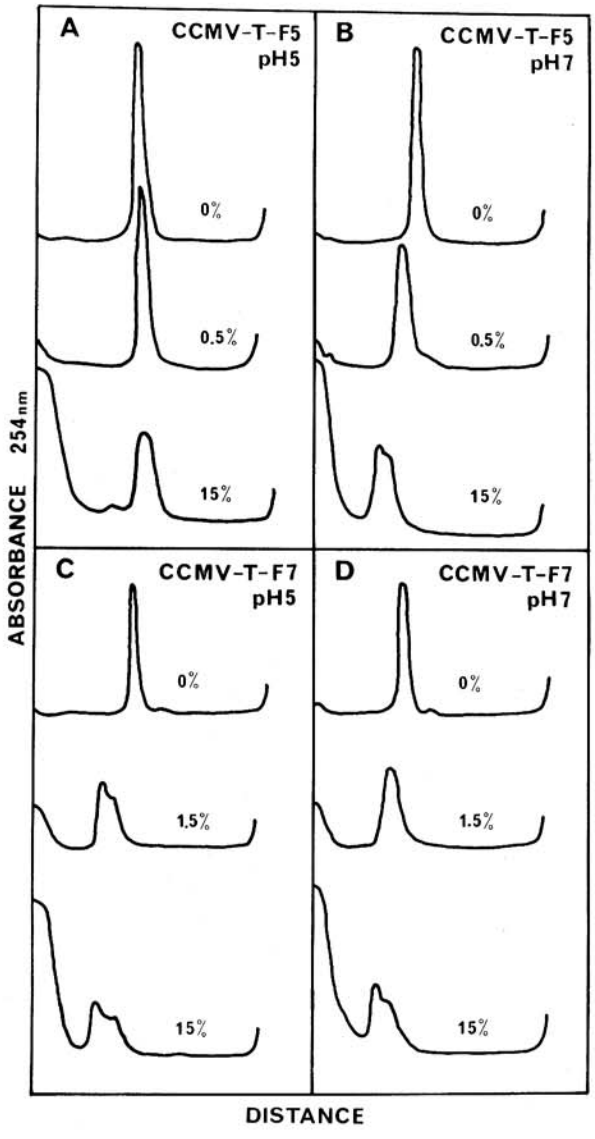

Fig. 6-(A to D). The effect of concentration of sodium dodecyl sulfate (SDS) on pH 5 formaldehyde-reacted cowpea chlorotic mottle virus strain T (CCMV-T-F5); **A)** at pH 5 and **B)** at pH 7. The effect of concentration of SDS on pH 7 formaldehyde-reacted cowpea chlorotic mottle virus strain T (CCMV-T-F7); **C)** at pH 5 and **D)** at pH 7. Tracings are of 254-nm absorbance scans of sucrose density gradients after centrifugation at 20 C for 1 hour in a Beckman SW 41 rotor at 39,000 rpm. Sedimentation was from left to right. The virus was exposed to the concentration of SDS indicated for 30 minutes before centrifugation.

is changed from 5 to 7 but swelling can be reduced by the addition of Mg^{++} (11, 23). Both of those viruses, TCV, and SV were more sensitive to SDS at pH 7 than at pH 5. The effect of 0.05 M $MgCl_2$ and 0.01 M EDTA (a chelator of divalent ions) on the reaction of these viruses with SDS was assessed at pH 7.0. Each virus was tested with and without EDTA in a concentration of SDS chosen to dissociate 30% of the virus. Bromoviruses, TCV, and CRSV-N were affected similarly in EDTA-SDS solutions as in SDS alone, but EDTA enhanced the dissociation of SV by SDS. Each virus was also tested with and without $MgCl_2$ in a concentration of SDS chosen to dissociate 60% of the virus. In the presence of Mg^{++}, dissociation of bromoviruses, TCV, SV, and CRSV-N was reduced to 40%-50%.

The effect of Mg^{++} and EDTA on the SDS reaction was much greater with CRSV-R than with other viruses. In 0.1% SDS, almost all CRSV-R was dissociated into protein and two RNA components or converted into IS (Fig. 4-B). In the presence of $MgCl_2$ much of the virus was unaffected by SDS and the RNA sedimented as a single heterogeneous component (Fig. 4-B); $MgCl_2$ probably induced aggregation of the two RNA components of CRSV-R. In 0.025% SDS (not shown) less dissociation of CRSV-R occurred and less IS formed than in 0.1% SDS shown in Fig. 4-B. Addition of EDTA to CRSV-R in 0.025% SDS caused all of the virus and much of the IS to become dissociated.

The amount of unaffected CRSV-R in tests with 0.1% SDS (Fig. 4-B) was much less than that obtained with another preparation of CRSV-R (23). Our experiments with $MgCl_2$ and EDTA may explain this disparity; the divalent ion content may vary from one preparation to another.

Effect of formaldehyde treatment.—Reaction of a virus with formaldehyde can result in the formation of covalent intersubunit bonding (8) and these bonds should be stable in SDS. Cowpea chlorotic mottle virus strain T was reacted with formaldehyde at pH 5 (CCMV-T-F5), and at pH 7 (CCMV-T-F7). Treatment of these products with SDS at pH 5 or 7 caused little dissociation (Fig. 6). At pH 5, SDS had little effect on CCMV-T-F5 (Fig. 6-A). At pH 7, the sedimentation rate of CCMV-T-F5 decreased as the SDS concentration increased, and at 15% SDS two components were observed (Fig. 6-B). The behavior of CCMV-T-F7 in SDS at both pH 5 and 7 (Fig. 6-C, D) was similar to that of CCMV-T-F5 at pH 7.

The slower-sedimenting components in Fig. 6-B, C, and D are probably SDS-swollen virus particles. Electron microscopy of CCMV-T-F7 in 15% SDS at pH 7 showed irregular particles with diameters up to 35 nm (Fig. 5-B).

Tests with SDS on CRSV-N previously reacted with formaldehyde at pH 5 gave results similar to those obtained with CCMV-T-F5. However, this formaldehyde-reacted CRSV was not soluble at pH 7 in the absence of SDS.

Viruses partially resistant to sodium dodecyl sulfate.—The effect of increasing concentrations of SDS on CNV was different at pH 5 than at pH 7. At pH 5 (Fig. 7-A), CNV dissociation increased with increasing SDS concentration but dissociation of all the virions did not occur, even in 15% SDS. At pH 7, CNV dissociation was progressive with SDS concentrations up to 0.05%, but further increases in SDS concentration did not induce further dissociation. At pH 5, TNV was totally resistant to SDS, but at pH 7 its behavior (Fig. 7-B) was similar to that of CNV.

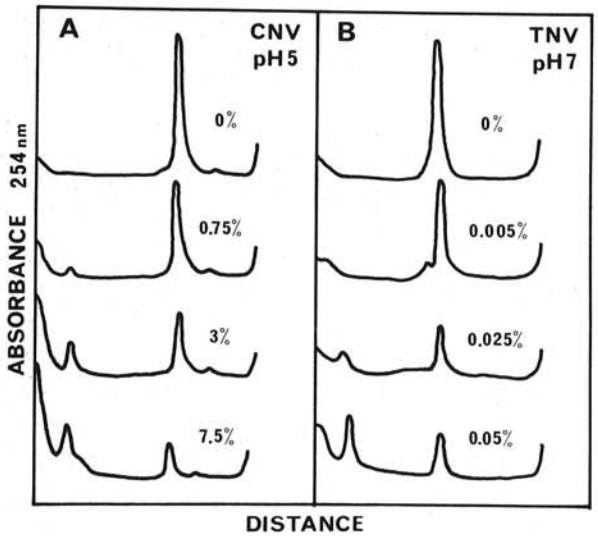

Fig. 7-(A, B). **A)** The effect of concentration of sodium dodecyl sulfate (SDS) on cucumber necrosis virus (CNV) at pH 5. **B)** The effect of concentration of SDS on tobacco necrosis virus (TNV) at pH 7. Tracings are of 254-nm absorbance scans of sucrose density gradients after centrifugation at 20 C for 1 hour in a Beckman SW 41 rotor at 39,000 rpm. Sedimentation was from left to right. The virus was exposed to the concentration of SDS indicated for 30 minutes before centrifugation.

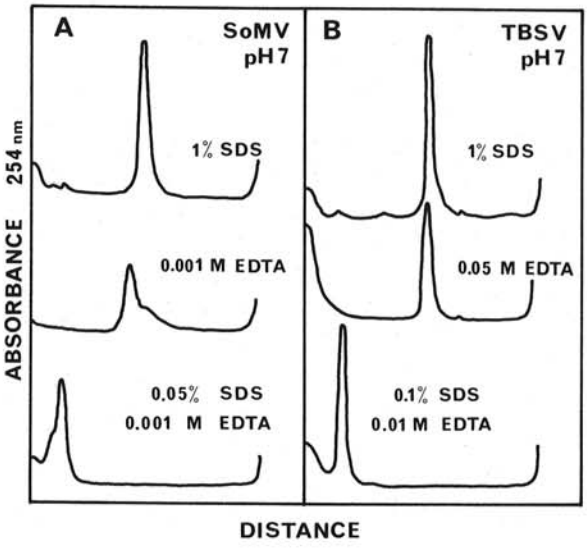

Fig. 8-(A, B). **A)** The effect of concentration of sodium dodecyl sulfate (SDS) and ethylenediaminetetraacetic acid (EDTA) at pH 7 on sowbane mosaic virus (SoMV) and **B)** on tomato bushy stunt virus (TBSV). Tracings are of 254-nm absorbance scans of sucrose density gradients after centrifugation at 20 C for 1 hour in a Beckman SW 41 rotor at 39,000 rpm. Sedimentation was from left to right. The virus was exposed to the concentration of SDS indicated for 30 minutes before centrifugation.

The effect of SDS on the dissociation of CarMV at pH 5 and 7 was similar to that of CNV. However, CarMV was only partly soluble at pH 5 and formed large quantities of an IS component in SDS at both pH 5 and 7.

Dissociation of CNV in 1% SDS at pH 7 increased with length of exposure. Dissociation after 30 minutes, 2 hours, 1, 2, and 3 days was 24%, 38%, 81%, 91%, and 95%, respectively. Dissociation of CarMV also increased with length of exposure to 1% SDS. These results indicate that the partial resistance of these viruses to SDS is due to their slower reaction rate with SDS.

Addition of 0.01 M EDTA to CNV or CarMV in 0.05% SDS at pH 7, induced complete dissociation. The addition of 0.05 M $MgCl_2$ caused complete dissociation of CNV in 0.05% SDS at pH 7, but it reduced the dissociation of CarMV. The addition of 0.1 M or 0.2 M NaCl to CNV in 0.05% SDS did not increase dissociation. Therefore, the enhanced dissociation of CNV in EDTA or $MgCl_2$ was not necessarily a result of chelation, or higher salt molarities.

Sodium dodecyl sulfate-resistant viruses.—Little dissociation of TYMV, EAMV, RMV, TBSV, SBMV, CfMV, and SoMV occurred in 3%, 7.5%, or 15% SDS at either pH 5 or 7. The peaks obtained with these viruses in 7.5% and 15% SDS were broad, probably because of nonideal sedimentation.

The effect of longer periods of exposure of these viruses to 1% SDS at pH 7 was studied. The SoMV was greatly affected by long exposure: 23%, 69%, and 80% of the virus was dissociated after 1, 2, and 3 days, respectively. Dissociation of the other viruses also increased with exposure period. Dissociation after 3 days was 3% for RMV, 6% for EAMV, 7% for TBSV, 10% for SBMV, and 16% for TYMV.

The effect of EDTA on the SDS-resistant viruses was investigated at pH 5 and 7. At pH 5, none of these viruses was affected by 0.01 M EDTA, either with or without 1% SDS. At pH 7, TYMV, EAMV, and RaMV were not affected by 0.01 M EDTA and 1% SDS, but SBMV, SoMV, CfMV, and TBSV were dissociated. Unlike CNV, none of these viruses was dissociated in 0.05 M $MgCl_2$ and 1% SDS at pH 7. Therefore, the effect of EDTA on the reaction of SDS with some of the viruses was probably the result of chelation of divalent ions.

The effect of length of exposure of SBMV to 0.02 M EDTA at pH 7.5 was investigated by Sehgal and Sinha (17). During the first 30 minutes all the virus particles disorganized into a transient 92 Svedberg (S) entity that was then progressively converted into a 50 S "subviral entity" by the loss of some protein subunits (17). In our experiments with SBMV in 0.01 M EDTA at pH 7, 60% of the virus particles were converted in the first 30 minutes to an entity that sedimented 10% slower than the virus, and the remainder were converted over a 2-hour period. We did not detect "subviral entity" even after 20 hours. We found the addition of 1% SDS had no effect on unaltered virus particles but it dissociated the slower-sedimenting entity. The dissociation of SBMV in 0.1% SDS increased with the concentration of EDTA. In 0.01 mM EDTA the virus was unaffected, but in 0.1 mM EDTA there was 40% dissociation. In 1 mM, 10 mM, or 50 mM EDTA there was a constant level of 60% dissociation.

Some SoMV (Fig. 8-A) and CfMV particles formed a slower-sedimenting component in 0.001 M EDTA alone. Both SoMV (Fig. 8-A) and CfMV were completely dissociated in 0.05% SDS and 0.001 M EDTA. Tomato bushy stunt virus was unaffected by 0.05 M EDTA but was completely dissociated in 0.1% SDS and 0.01 M EDTA (Fig. 8-B).

DISCUSSION

The viruses in this study fit into four groups based on their reaction with SDS for 30 minutes at pH 7: group 1–viruses with all particles dissociated, or swollen, by low concentrations of SDS; group 2–viruses with some particles dissociated by low concentrations of SDS, but the remainder not dissociated at any SDS concentrations; group 3–viruses with particles affected by SDS, only in the presence of EDTA; and group 4–viruses with particles unaffected by SDS. Our groups differ from those proposed by Boatman and Kaper (4) because of the differences in SDS sensitivity we found among strains of CCMV and CRSV. In addition, we studied more viruses and the effect of $MgCl_2$ and EDTA on the SDS-induced viral dissociation.

Nonviral proteins lacking RNA also differ in their reaction with SDS (13). Most proteins readily bind large amounts of SDS and this binding is accompanied by gross denaturation (16). Some proteins bind SDS slowly; others are resistant or do not bind SDS. Precise explanations for this variation in reactivity are not known (13).

The SDS-sensitive viruses of group 1 have common physical and chemical properties, listed by Kaper (9, 10), which indicate their virions are stabilized by protein-RNA interactions. Most of the viruses in this group are dissociated by lower concentrations of SDS in 0.1 M sodium phosphate pH 7 than in 0.1 M sodium acetate pH 5. Some strains of CCMV and CRSV are resistant to SDS at pH 5 for 30 minutes, but dissociate on prolonged exposure. They may bind SDS slowly at pH 5 or binding may slowly induce conformational changes which expose more sites for SDS binding. The swelling of bromoviruses and CRSV at pH 7 (11, 23) requires conformational changes in their proteins. These changes probably expose new sites for SDS binding resulting in greater sensitivity to SDS. Since Mg^{++} reduces swelling at pH 7 (11, 23), this may explain the decrease in SDS sensitivity when Mg^{++} was added. An alternative explanation may be the removal of SDS by Mg^{++}. This is unlikely because Mg^{++} increased the SDS dissociation of CNV.

The formation of SDS-swollen virus particles (IS) with most of group 1 viruses may elucidate the mechanism of SDS action. Swelling of the virus particle may be due to conformation changes on binding of SDS or to a repulsion between negative charges of the bound SDS. The swelling probably increases with further SDS binding, intermolecular bonding is destroyed, and the particles dissociate. The progressive swelling of CCMV-T-F7 with increasing concentration of SDS was probably a result of increasing SDS binding. However, the strong covalent formaldehyde bonding prevented dissociation of the particle. The failure of CCMV-T-F5 to swell when treated with SDS at pH 5 may indicate that no SDS binding occurred. The site of formaldehyde reaction is probably on surface lysine side chains (6) and these

groups are highly reactive SDS-binding sites in bovine serum albumin (12, 15). However, CCMV-T-F5 swells in the presence of SDS at pH 7. Possibly a pH-induced conformational change exposes SDS-binding sites in these particles.

The SDS-resistant viruses of groups 3 and 4 react with SDS slowly under the conditions tested. The viruses, SBMV, SoMV, CfMV, and TBSV are probably stabilized by divalent ions. The removal of these ions by EDTA at pH 7 caused SBMV, CfMV, and SoMV to swell and this swelling probably exposed SDS-binding sites. At pH 5, EDTA is not an effective chelator and therefore did not predispose SBMV, CfMV, SoMV, or TBSV to dissociation in SDS.

The major difference between the viruses of group 2 and those of groups 3 and 4 is probably the initial rate of binding of SDS. Within 30 minutes in low SDS concentrations at pH 7, 30-60% of CNV, TNV, and CarMV particles are dissociated. The remaining CNV and CarMV particles are dissociated in a few days. The greater sensitivity of CNV, TNV, and CarMV to SDS at pH 7 than at pH 5 is surprising. Unlike bromoviruses, CRSV, and SV, these viruses are stable at pH 7. The SDS sensitivity of CNV and CarMV at pH 7 is increased by EDTA. Although $MgCl_2$ stabilizes CarMV in SDS, it enhances dissociation of CNV by SDS. Similar anomalies were reported by Habili and Francki (8) who found that $MgCl_2$ stabilizes TAV but precipitates CMV; and EDTA stabilizes CMV but degrades TAV.

The mechanisms proposed here for the action of SDS on viruses are speculative. We have observed that most of the SDS-sensitive viruses are degraded by proteolytic enzymes at pH values above 7 (Agrawal and Tremaine, *unpublished*). This indicates a loose surface structure accessible to the large molecule of a proteolytic enzyme. Similar studies have not been made with SDS-resistant viruses. These studies, and determination of the SDS sensitivities of other plant viruses, may provide information of great value in virus classification.

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