Effect of Sodium Dextran Sulfate on Some Isometric Plant Viruses

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ABSTRACT

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Nine isometric plant viruses were treated with sodium dextran sulfate (NDS) at levels up to 20 μg NDS per 100 μg virus (20% NDS/virus ratio) at pH 5.0 and at pH 7.5 with and without ethylenediaminetetraacetic acid (EDTA). None of the viruses were affected by NDS at pH 5.0. Turnip yellow mosaic and cowpea mosaic viruses were unaffected by NDS under any of the conditions. Brome mosaic (BMV), carnation ringspot (CRSV), and turnip crinkle viruses (TCV) were affected by NDS at pH 7.5; however, tomato bushy stunt (TBSV), southern bean mosaic (SBMV), sowbane mosaic (SoMV), and turnip rosette viruses (TRosV) were affected at pH 7.5 only after treatment with EDTA. As increasing levels of NDS were used with BMV and CRSV, increasing amounts of the RNAs were observed in sucrose density gradients until at a 20% NDS/virus ratio nearly all virus was dissociated. Insoluble isometric particles, formed of protein and NDS, and with diameters characteristic of T = 1 and T = 3 particles were observed in these preparations with the electron microscope. Only 50% of the TCV or TBSV particles were dissociated at a 20% NDS/virus ratio and insoluble T = 1 particles were formed with TCV. The three sobemoviruses (SBMV, SoMV, and TRosV) were dissociated into RNA by a 20% NDS/virus ratio, but at lower ratios soluble components were formed that sedimented at rates between those of swollen virus particles and RNA. Electron micrographs of these preparations showed T = 1, T = 3 and deformed particles as well as individual amorphous masses with highly irregular outlines. Swollen BMV was dissociated and its RNA and protein were separated by affinity chromatography on NDS-agarose columns by retention of the protein.

Sodium dextran sulfate (NDS) is an anhydroglucose polymer with introduced sulfate ester groups. It is a polyanion resembling single-stranded nucleic acid, and has been used as a nucleating agent for the assembly of viruslike particles with proteins of bromoviruses (1) or southern bean mosaic virus (SBMV) and sowbane mosiac virus (SoMV) (9).

After the loss of Ca^{++}-dependent and/or pH-dependent protein-protein interactions certain isometric virus particles are stabilized predominantly by interactions between the anionic nucleic acid and the cationic side chains of the coat protein (4,5,7). We investigated the effect of NDS on the interaction between the RNA and the protein coat of some of these isometric viruses. Turnip yellow mosaic virus (TYMV) and cowpea mosaic virus (CPMV) were also tested because their particles are resistant to certain reagents that degrade other viruses and are presumed to be stabilized by very strong protein-protein interactions (5,7).

MATERIALS AND METHODS

Tests of viruses with sodium dextran sulfate. The sources of the viruses brome mosaic virus (BMV), bean strain of SBMV, SoMV, prunus strain of tomato bushy stunt virus (TBSV), high-temperature aggregating strain of carnation ringspot virus (CRSV), and TYMV, were stated previously (7). Turnip crinkle (TCV) and turnip rosette viruses (TRosV) and Sb strain of CPMV were obtained from R. Hull and G. Bruening, respectively. All of the viruses except CPMV were purified by clarification at pH 5.0, polyethylene glycol precipitation and differential centrifugation. CPMV was purified by the method of van Kammen and de Jager (14) and it and SBMV were dissolved in 0.01 M sodium phosphate buffer, pH 7.0, containing 0.1 M NaCl. The other viruses were dissolved in 0.01 M sodium acetate buffer, pH 5.0, containing 0.1 M NaCl (NAN 5). Virus concentration was determined spectrophotometrically by measuring the extinction coefficient appropriate for each virus. Concentrations of all virus preparations were at 10–50 mg/ml and sodium azide (0.02%) was added as a preservative.

Tests with all viruses were conducted at pH 7.5 with and without EDTA. Virus preparations were diluted to 4 mg/ml with 0.01 M tris acetate buffer, pH 7.5, containing 0.1 M NaCl (TAN 7.5), then diluted with an equal volume of 0.2 M tris-HCl buffer, pH 7.5, with or without 30 mM EDTA. After 1 hr in an ice bath, aliquots (200 μl) of these dilutions were added to 100 μl of solutions of various concentrations of sodium dextran sulfate (NDS) in TAN 7.5 and left for 30 min. Samples (200 μl) of each solution were placed on 5–35% sucrose gradients in TAN 7.5 and centrifuged at 38,000 rpm in an SW 41 Beckman rotor for 2 hr at 5 C. Viruses were also tested at pH 5 without EDTA by a similar procedure. Tests were done at least once with two preparations of each virus.

Sodium dextran sulfate 2000 was obtained from Pharmacia, Uppsala, Sweden, and also was prepared from dextran with an average molecular weight of 2×10^6. The sedimentation coefficient of the NDS at 3 mg/ml was determined to be 9S in TAN 7.5 (*unpublished*). This result and the assembly of a broad size range of small spherical particles with this NDS and SBMV or SoMV protein (9) indicates that the NDS is smaller than 2×10^6 and may be heterodisperse.

Gradient analysis and electron microscopy. Density gradients were scanned at 254 nm in an ISCO model UA-4 ultraviolet monitor with a model 612 recorder and model 184 tube-holding device. In some experiments, appropriate 0.25- or 1-ml fractions of the gradient were collected in an ISCO model 270 fraction collector. The ultraviolet absorbance spectra of selected 1-ml fractions was determined in a Gilford model 250 recording spectrophotometer and corrected for light scattering (13). The protein content was determined on three 0.15-ml aliquots from these fractions by the dye-binding method of Bradford (2) using the appropriate virus as a calibration standard. The nucleic acid content of the fraction was calculated from the absorbance at 260 nm after subtraction of the contribution of the protein to this absorbance (13).

A drop of each virus preparation was placed on a carbon-collodion-coated grid and the grid was washed gently with 20–40 drops of 2% uranyl acetate. The grid was drained by touching with filter paper and dried. The grids were examined immediately in a Philips EM 300 or Hitachi H-600 electron microscope. The outline of virus particles after reaction with NDS often deviated

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considerably from a circle. Therefore, the diameters given in the text are estimates, but are sufficiently accurate to distinguish T = 1 and T = 3 particle sizes.

Analytical ultracentrifugation. Components in virus preparations containing NDS were examined in a Beckman model E analytical ultracentrifuge by schlieren optics at 20 C. Sedimentation coefficients were calculated by the graphical method of Markham (6).

Affinity chromatography. Dextran sulfate immobilized on 4% beaded agarose was obtained from Pierce Chemical Co., Rockford, IL 61105 and contained 0.5–0.6 mg of NDS per milliliter of gel. Nine milliliters of the gel in a 1.6 × 20-cm column with flow adaptors was equilibrated in TAN 7.5 or NAN 5. The column flow rate was 7.1 ml/hr and 0.5-ml fractions were collected. The A_{260} and A_{280} of each fraction was measured in a Gilford model 250 spectrophotometer and protein was determined on 0.25-ml aliquots by the dye binding method (2).

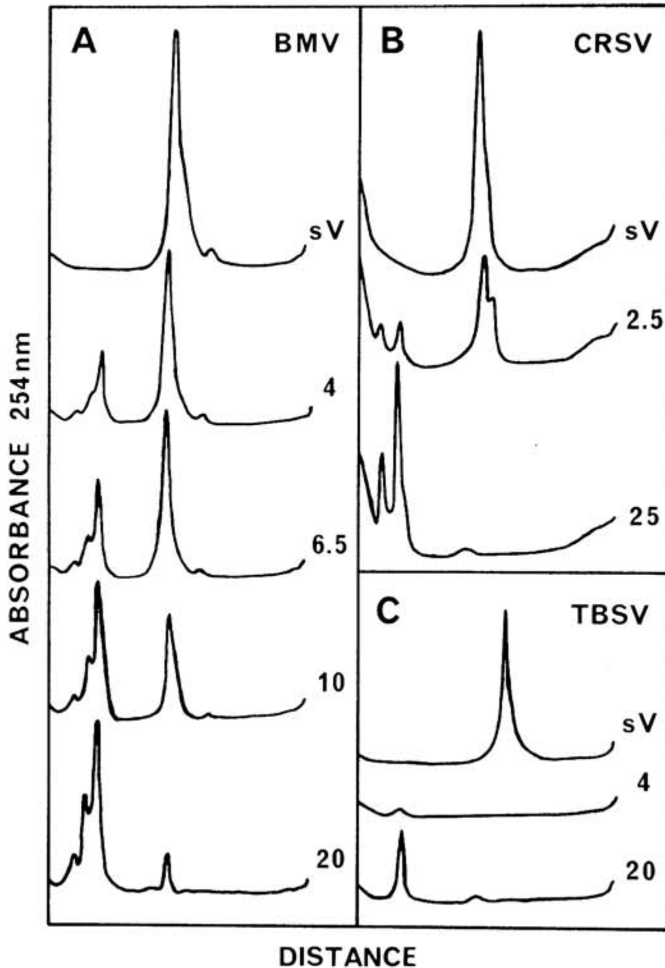


Fig. 1. Effect of sodium dextran sulfate (NDS) concentration on **A,** brome mosaic virus (BMV); **B,** carnation ringspot virus (CRSV); and **C,** tomato bushy stunt virus (TBSV). The viruses were exposed to 15 mM EDTA at pH 7.5 for 1 hr, then to NDS at the indicated percentage NDS/virus ratio for 30 min. sV denotes virus treated with EDTA only. Tracings are of 254 nm absorbance scans of sucrose density gradients after centrifugation at 5 C for 2 hr in a Beckman SW 41 rotor at 39,000 rpm. Sedimentation is from left to right.

RESULTS

Resistance to sodium dextran sulfate by viruses with protein-protein interactions. TYMV and CPMV, viruses with strong protein-protein interactions (5), were unaffected by NDS at 20 μg of NDS per 100 μg virus (a 20% NDS/virus ratio) at pH 5.0 or at pH 7.5 with EDTA. The viruses with Ca^{++}-dependent protein-protein interactions (TBSV, SBMV, TRosV, and SoMV) were also unaffected by the 20% NDS/virus ratio at pH 5.0 or at pH 7.5 in the absence of EDTA. Viruses with pH-dependent protein-protein interactions (BMV, CRSV, and TCV) were resistant to NDS only at pH 5.0.

Dissociation of brome mosaic, carnation ringspot, and turnip crinkle viruses. At pH 7.5 in EDTA, BMV sedimented about halfway down the sucrose gradient as a single large component preceded by a small amount of dimer (Fig. 1A). Amounts of virus decreased and amounts of dissociation products (presumably RNAs) increased with increasing NDS/virus ratios until at a 20% NDS/virus ratio most of the virus was dissociated. Fractions from the gradient were analyzed for protein (not shown in Fig. 1A) and protein was detected only in the RNA or virus peaks. The amount of protein relative to the A_{254} of the peak was very low for RNA but very high for virus. The level of protein in the RNA peaks was similar to that found in RNA prepared from SBMV or cowpea chlorotic mottle virus by the 1 M NaCl method (13,15).

Examination in the electron microscope of preparations of BMV treated at a 20% NDS/virus ratio showed particles with diameters of 17 or 26 nm (Fig. 2A). Many of these particles were clumped together and occasionally unusual double-shelled structures were observed (inset Fig. 2A). These structures resemble reassembled cowpea chlorotic mottle virus protein (1). The particles in Fig. 2A are probably T = 1 and T = 3 structures with NDS as the nucleating agent.

The experiment with a 20% NDS/virus ratio was repeated and 200 μl of the NDS-treated BMV was layered on 1.2 ml of a 5% sucrose cushion (TAN 7.5 buffer) in a 1.9-ml centrifuge tube. This was centrifuged in an Eppendorf model 5412 centrifuge at 12,800 *g* for 5 min, drained, and the pellet resuspended in TAN 7.5 buffer. The pellet contained clumps of isometric particles that were readily detected in the electron microscope (Fig. 2B). In one experiment, the pellet was dissolved in 0.2 ml of a 0.1% solution of sodium dodecyl sulfate. The ultraviolet absorption spectrum of this

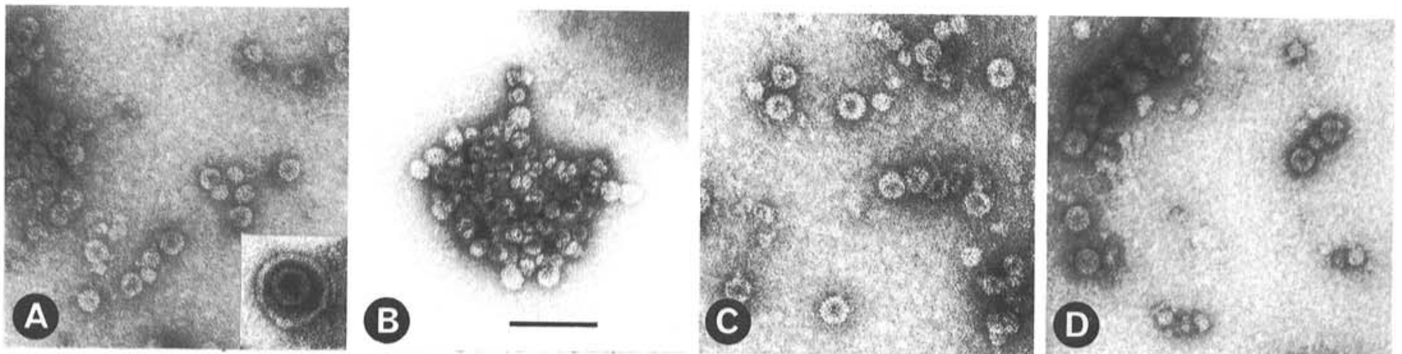


Fig. 2. Electron micrographs of various viruses treated with a 20% sodium dextran sulfate/virus ratio. **A and A inset,** brome mosaic virus; **B,** brome mosaic virus sedimented through a sucrose cushion by low-speed centrifugation; **C,** carnation ringspot virus; and **D,** turnip crinkle virus. Particles were negatively stained with 2% uranyl acetate. All micrographs including inset are at the same magnification. Bar indicates 100 nm.

solution indicated the absence of RNA. In tests with BMV not treated with NDS few, if any, isometric particles were detected in drained centrifuge tubes by electron microscopy.

CRSV was almost completely dissociated into RNAs at a 25% NDS/virus ratio, but at a 2.5% NDS/virus ratio, the sum of absorbancies of virus and RNA indicated some of the virus particles had precipitated (Fig. 1B). An electron micrograph of CRSV treated at a 25% NDS/virus ratio showed isometric particles with diameters of 22 or 33 nm (Fig. 2C) and these were probably T = 1 and T = 3 structures assembled around NDS. These particles were pelleted by low-speed centrifugation as described for BMV and clumps of isometric particles were found.

The sedimentation profiles obtained with TCV were similar to those of BMV and CRSV except TCV has only one RNA component and at a 20% NDS/virus ratio only 50% of the virions were dissociated. Sixty percent of the virions were dissociated at a 100% NDS/virus ratio, but almost complete dissociation occurred with a 20% NDS/virus ratio at pH 8.5. Protein analyses showed that virus protein dissociated from the RNA was pelleted. Electron microscopy of preparations at a 20% NDS/virus ratio showed particles with 22 or 33 nm diameter (Fig. 2D). These types of particles were pelleted by low-speed centrifugation. Untreated virus contained only 33-nm-diameter particles and these were not pelleted by low-speed centrifugation.

The effect of NDS on BMV, TCV, and CRSV at pH 7.5 in the absence of EDTA was similar to that described above with EDTA. However, at each NDS/virus ratio there was more dissociation in the presence of EDTA.

Dissociation of tomato bushy stunt virus. TBSV treated with EDTA was almost completely precipitated at a 4% NDS/virus ratio and very little dissociation occurred (Fig. 1C). Approximately 30% of EDTA-treated TBSV was dissociated into RNA at a 20% NDS/virus ratio; most of the remaining 70% was precipitated. Electron micrographs of this precipitate showed only 33-nm-diameter particles, which were indistinguishable from particles in EDTA-treated TBSV preparations.

Dissociation of SBMV. SBMV sediments as a sharp peak (not shown) but EDTA-treated swollen virus sediments as a broad peak (Fig. 3). At increasing NDS/virus ratios, amounts of material sedimenting at the rate of swollen virus decreased and components sedimenting slower than swollen virus increased. The distance of sedimentation and the protein content of these components decreased as the NDS/virus ratio increased (Fig. 3, Table 1). At a 20% NDS/virus ratio, the single sharp peak containing little protein was very similar in shape and area to the control virus peak. Aliquots of 0.25-ml fractions taken at the peaks were examined in the electron microscope. Electron micrographs of the swollen virus peak at 4% NDS/virus ratio showed many stain-penetrated particles with a diameter of 30 nm, a few with a diameter of 20 nm, and some deformed particles (Fig. 4A). The peak at 8% NDS/virus ratio contained irregularly shaped particles with a diameter of 20 nm, some deformed particles, and some individual amorphous masses of material with highly irregular outlines (Fig. 4B). The types of particles found in the fractions from the remainder of the experiment are tabulated in Table 1. No particles were found in the

TABLE 1. Effect of sodium dextran sulfate (NDS) on southern bean mosaic virus

Material	Gradient distance[a] (%)	Particle type[b]	Protein RNA[c]
Virus	100	L	180
Swollen virus	86	L	176
NDS/virus ratio[d]			
1%	62; 84	S, A; L	145; 150
2%	59; 85	S, A; L	153; 201
4%	56; 86	S, A; L	113; 141
8%	40	S	37
20%	32	–	8

[a] Sedimentation distance of each component as a percentage of virus sedimentation.
[b] Particle type observed in peak fraction(s) in the electron microscope. Abbreviations: S = small particle, ~20 nm in diameter; A = amorphous mass with highly irregular outline; L = large particle, ~30 nm in diameter; and – = no particle.
[c] Moles of southern bean mosaic virus protein per mole of RNA.
[d] Weight of NDS as a percentage of virus weight.

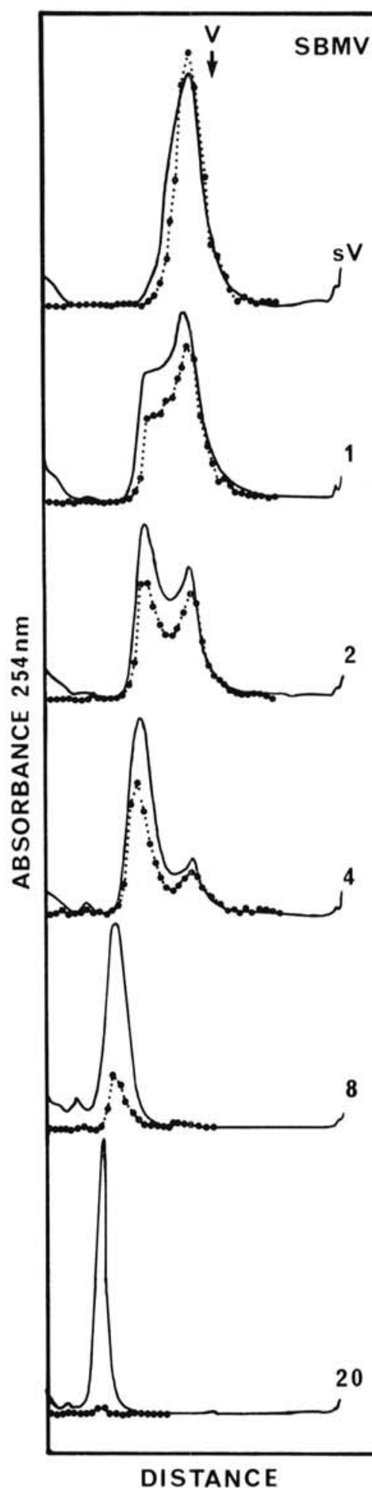


Fig. 3. Effect of sodium dextran sulfate (NDS) concentration on southern bean mosaic virus (SBMV). SBMV was exposed to 15 mM EDTA at pH 7.5 for 1 hr, then to NDS at the indicated percentage NDS/virus ratio for 30 min. sV denotes virus treated with EDTA only. These samples were centrifuged on density gradients at 5 C in a Beckman SW 41 rotor at 39,000 rpm for 2 hr. The gradients were scanned at 254 nm and fractionated into 0.25-ml fractions. —— = tracing of A_{254} scan; ●·······● = protein content of fractions. Sedimentation is from left to right.

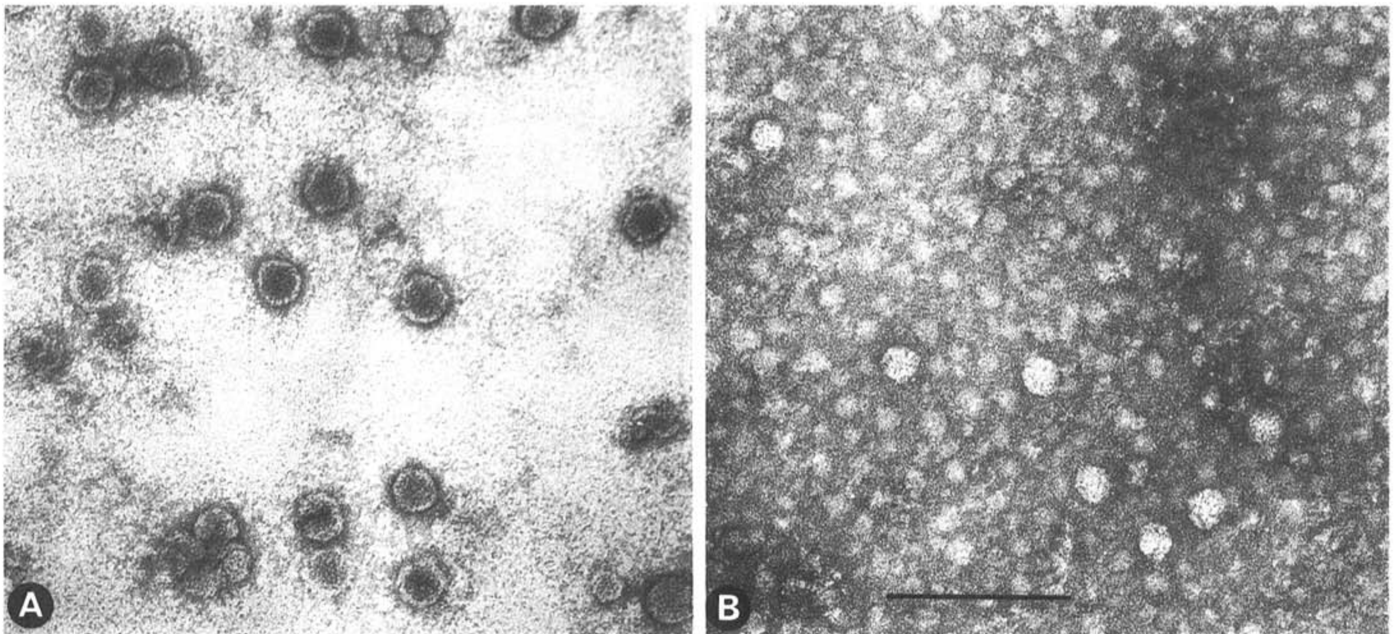


Fig. 4. Electron micrographs of southern bean mosaic virus treated with various concentrations of sodium dextran sulfate (NDS). **A,** Sucrose density gradient fraction from the center of the peak treated at an 8% NDS/virus ratio in Fig. 3. **B,** Fraction from the minor peak treated at a 4% NDS/virus ratio in Fig. 3. Particles were negative stained with 2% uranyl acetate. Both micrographs are at the same magnification. Bar indicates 100 nm.

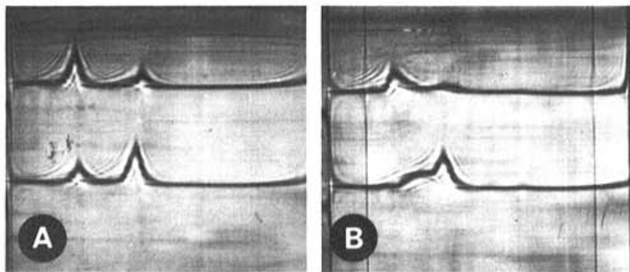


Fig. 5. Schlieren patterns from analytical ultracentrifugation of viruses treated with sodium dextran sulfate (NDS). **A,** top: southern bean mosaic virus (3 mg/ml) treated at 4% NDS/virus ratio; and bottom: at a 2% NDS/virus ratio; **B,** top: sowbane mosaic virus (3 mg/ml) treated at a 4% NDS/virus ratio; and bottom: at a 2% NDS/virus ratio. The photographs were taken 8 min after attaining the speed of 35,600 rpm. Sedimentation is from left to right.

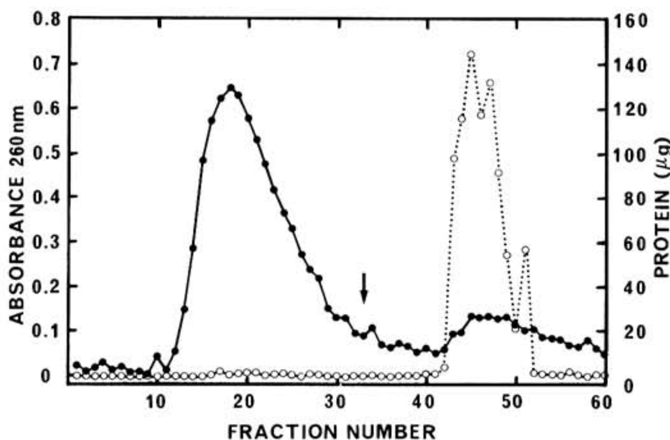


Fig. 6. Affinity chromatography of 1 mg of brome mosaic virus on dextran sulfate-agarose column equilibrated in 0.01 M tris acetate buffer pH 7.5, containing 0.1 M NaCl (TAN 7.5). At the arrow 0.2 mg/ml sodium dextran sulfate in TAN 7.5 was used to elute the column. The 1.6 × 43 cm column was pumped at 7.1 ml/hr and 0.5-ml fractions were collected. •——• = absorbance at 260 nm, o⋯⋯o = micrograms of protein per fraction determined by dye binding method.

gradient of the 20% NDS/virus preparation, but both 20- and 30-nm particles were found on examination of the unfractionated preparation. These particles were probably pelleted in the sucrose gradient.

The experiment was repeated and the number of protein molecules per SBMV RNA molecule was determined and is presented in Table 1. The protein content of the slower component decreased as the NDS/virus ratio increased.

Sedimentation rates of the products were determined in an experiment with SBMV at 3 mg/ml. At a 20% NDS/virus ratio and an 8% NDS/virus ratio, single components were observed sedimenting at 25S and 30S, respectively. Two components in varying proportions were observed at 4 and 2% NDS/virus ratios and their sedimentation rates were 98S and 50S, and 98S and 57S, respectively (Fig. 5A).

Dissociation of TRosV. The sedimentation patterns in sucrose density gradients and protein analyses obtained with TRosV were very similar to those shown for SBMV (Fig. 3). Examination of a 4% NDS/virus preparation in the electron microscope showed amorphous masses of material, 20- and 30-nm isometric particles, and deformed particles.

An experiment was designed to locate the TRosV protein in a 20% NDS/virus mixture. Three samples containing 1.6 mg of virus in NDS were centrifuged at 20,000 rpm for 5, 10, and 20 min, respectively, on sucrose gradients in an SW 39 rotor. The gradients were fractionated into 1-ml fractions and protein was not detected in any of the fractions. The experiment was repeated, the contents of the centrifuge tubes were drained, and the dye-binding reagent (2) was added to the empty tube. The brown reagent reacted, and the blue pigment formed was attached to the bottom of the tube and remained attached on draining the reagent from the tube.

Dissociation of SoMV. The sedimentation patterns in sucrose gradients obtained with SoMV differed from those of SBMV in Fig. 3. A single heterogenous band with maximum absorbance was observed at 63, 66, and 68% of the distance moved by native virus, at 4, 2, and 1% NDS/virus ratios, respectively (*unpublished*). The patterns of 20 and 8% NDS/virus were similar to those of SBMV at these NDS/virus ratios in Fig. 3.

Sedimentation rates of the products were determined in an experiment with SoMV at 3 mg/ml. At 2% NDS/virus ratio heterogenous components were observed with sedimentation rates ranging from 41 to 84S (Fig. 5B). Two heterogenous components of

51 and 88S were observed at a 4% NDS/virus ratio (Fig. 5B).

Electron micrographs of 4% NDS/virus preparation showed similar particle sizes and shapes as found with TRosV, but SoMV had a greater proportion of 20-nm-diameter particles.

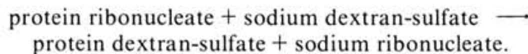
Affinity chromatography. One milligram of BMV in 0.4 ml of TAN 7.5 was applied to the 9.0 ml NDS-agarose column and RNA was eluted after 6 ml and continued for 9 ml (Fig. 6). The protein was eluted with 0.2 mg NDS per milliliter in TAN 7.5. In another experiment, 5 mg of BMV was applied, the column became saturated and a mixture of native virus and RNA was eluted in TAN 7.5. The protein was eluted from the column with 1 M NaCl in TAN 7.5. Similar overloading was obtained when 1 mg of SBMV in TAN 7.5 and 10 mM EDTA was applied to the column equilibrated in TAN 7.5 and 10 mM EDTA. Apparently the affinity of the column for BMV protein was much greater than for SBMV protein.

The NDS-agarose column acted as an ion-exchanger when 1 mg of BMV in NAN 5 was used. The virus, which is cationic at pH 5.0 (1), was retained on the column and eluted intact after 27 ml.

DISCUSSION

We have studied the effect of NDS on the interaction between the anionic RNA and the cationic side chains of the viral coat protein. In addition to providing information on structure, the NDS stability of each particular virus could be used to assess similarities and differences for taxonomic or identification purposes.

The NDS had no effect on CPMV and TYMV or on the other viruses unless they were in the swollen state and presumably stabilized predominantly by interactions between the RNA and protein. The NDS appeared to compete with the RNA for the protein. The reaction could be written:



protein ribonucleate + sodium dextran-sulfate ⟶
protein dextran-sulfate + sodium ribonucleate.

The affinity of the RNAs for the coat proteins of BMV, CRSV, and the sobemoviruses appeared to be less than that of the NDS because these viruses were almost completely dissociated at a 20% NDS/virus ratio. At this ratio the amount of NDS was approximately equal to the amount of RNA present in the virus particle. The affinity of the RNAs for the coat proteins of TBSV and TCV (or their particle stabilities) appeared to be greater because TBSV and TCV were not completely dissociated at a 20% NDS/virus ratio. These results were similar to the dissociation of these viruses in 1 M NaCl; BMV, CRSV, and the sobemoviruses are completely dissociated in 0.1 M tris-HCl buffer, pH 7.5, containing 30 mM EDTA and 1 M NaCl (1,9, *unpublished*). Less than a half of the virions in TBSV or TCV preparations dissociate in this medium; some TBSV particles remain as superswollen particles. We have been unable to completely dissociate TBSV, but TCV dissociates completely at pH 9.0 (*unpublished*).

The dissociation of sobemoviruses at NDS/virus ratios less than 20% differs from that of the other viruses. The loss of protein subunits from the RNAs of BMV and CRSV appeared to be an all or none phenomenon. Apparently no stable soluble intermediates formed in detectable quantities. The removal of a few protein subunits probably weakened the whole virion. The dissociation of SBMV by NDS appeared to be complex. The components that sedimented at rates intermediate between those of the swollen virus and RNA (Fig. 3) were probably intact virus RNA with some protein subunits attached, and the number of protein subunits per RNA molecule decreased as the NDS/virus ratio increased (Table 1). These components were probably similar to the ribonucleoproteinaceous components (RNPCs) that were prepared by the disassembly of swollen SBMV in 0.4, 0.3, and 0.2 M NaCl (13). These RNPCs sedimented similarly in density gradients and were stable in solution. Electron microscopy of these RNPCs revealed only individual amorphous masses with highly irregular outlines (13) and similar material was also found with NDS-treated SBMV (Fig. 4B). The smaller particles in Fig. 4B were probably virus protein assembled around smaller RNA pieces (subgenomic RNAs). It was possible that some of these particles have NDS as a nucleating agent (9).

In Table 1, analyses of protein and RNA in density gradient fractions from the swollen virus peaks yielded variable and unusual values. Deformed particles similar to those seen in Fig. 4A were found in these fractions. These and some soluble NDS-protein complexes may account for the unusual analyses.

The effect of NDS on SBMV was studied in the analytical ultracentrifuge at higher virus concentrations and at 20 C. However, the components formed were similar to those shown in the density gradients (Fig. 3).

When treated with NDS, SoMV and TRosV also formed stable soluble intermediates that sedimented at rates between those of the swollen virus and RNA. This similar reaction with NDS supports their grouping in the sobemovirus group.

The dissociation of all the viruses by NDS was quite different from their previously reported (7) dissociation by sodium dodecyl sulfate (SDS) at room temperature. SDS is a smaller molecule with a hydrophobic alkane group and one hydrophilic sulfate group. Presumably, the alkane group reacts with a hydrophobic side chain on the protein, and denatures the protein. SDS is difficult to remove from proteins. NDS is a hydrophilic polymer resembling RNA and competed with the RNA for the viral coat protein subunits. It can be separated from protein in 1 M NaCl (*unpublished*). The portion of the virus coat protein involved in the interaction with the nucelic acid or the NDS is probably the highly basic regions found in BMV (10), SBMV (3,11), SoMV (12), TRosV, TCV, CRSV, and TBSV (*unpublished*).

The amounts of NDS and SDS required to dissociate some viruses are similar. For example a 0.01% SDS treatment used in previous studies (7) is actually an SDS/virus ratio of 14%. SDS acts on BMV, CRSV, and TCV at pH 5 (7), but NDS does not. The smaller genome components of CRSV (8) and BMV (7) are released at lower SDS levels than are the larger genome components. These components were released equally at lower NDS/virus ratios.

The NDS-virus reaction could be used to separate RNA from virus protein. Most of the proteinaceous products at 20% NDS/virus ratios were not soluble and could be removed by a low-speed centrifugation into a sucrose pad. However, about 4% of the coat protein remained attached to the RNA; this is similar to 1 M NaCl methods (9,13). There may be NDS present in such RNA preparations and this could interfere with the intended use of the RNA. An alternative method for RNA isolation is NDS-agarose affinity chromatography. However, the capacity of our column for coat protein was much lower than anticipated since the 9 ml of gel contains 4.5–5.4 mg of immobilized NDS and should bind the protein from 25 mg of BMV, CRSV, and sobemoviruses. The amount of NDS available for interaction with virus protein may be much lower because of steric hindrances. The capacity of the gel for protein might be increased by the use of higher molecular weight NDS or spacer arms. The NDS-agarose column could also be useful in the separation of virus coat protein from pure viruses or possibly plant material.

LITERATURE CITED

1. Bancroft, J. B. 1970. The self-assembly of spherical plant viruses. Adv. Virus Res. 16:99-133.
2. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.
3. Hermodson, M. A., Abad-Zapatero, C., Abdel-Meguid, S. S., Pundak, S., Rossmann, M. G., and Tremaine, J. H. 1982. Amino acid sequence of southern bean mosaic virus coat protein and its relation to the three dimensional structure of the virus. Virology 119:133-149.
4. Hull, R. 1977. The stabilization of the particles of turnip rosette virus and other members of the southern bean mosaic group. Virology 79:58-66.
5. Kaper, J. M. 1975. The chemical basis of virus structure, dissociation and reassembly. In: Frontiers of Biology, vol. 39. A. Neuberger and E. L. Tatum, eds. North-Holland, Amsterdam-Oxford.
6. Markham, R. 1962. The analytical ultracentrifuge as a tool in the investigation of plant viruses. Adv. Virus Res. 9:241-270.

7. Ronald, W. P., and Tremaine, H. H. 1976. Comparison of the effects of sodium dodecyl sulfate on some isometric plant viruses. Phytopathology 66:1302-1309.
8. Tremaine, J. H., and Ronald, W. P. 1976. Differential effects of sodium dodecyl sulphate on strains of carnation ringspot virus. J. Gen. Virol. 30:299-308.
9. Tremaine, J. H., and Ronald, W. P. 1977. Assembly of particles from southern bean mosaic virus and sowbane mosaic virus components. Can. J. Bot. 55:2274-2277.
10. Tremaine, J. H., Ronald, W. P., and Agrawal, H. O. 1977. Some tryptic peptides of bromovirus proteins. Virology 83:404-412.
11. Tremaine, J. H., Ronald, W. P., and Kelly, E. M. 1980. Chemical and serological properties of a cyanogen bromide peptide of southern bean mosaic virus protein. Can. J. Microbiol. 26:1450-1459.
12. Tremaine, J. H., Ronald, W. P., and Kelly, E. M. 1981. A highly basic cyanogen bromide peptide from sowbane mosaic virus protein. Virology 114:282-285.
13. Tremaine, J. H., Ronald, W. P., and Kelly, E. M. 1982. Intermediates in the *in vitro* assembly and dissassembly of the southern bean mosaic virus. Virology 118:35-44.
14. van Kammen, A., and de Jager, C. P. 1978. Cowpea mosaic virus. Descriptions of Plant Viruses. No. 197. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England.
15. Verduin, B. J. M. 1978. Characterization of cowpea chlorotic mottle virus and its assembly. Mededeling No. 134. Laboratorium voor Virologie, Wageningen, The Netherlands.