Effect of Protein Cross-linking Reagents and Sodium Dodecyl Sulfate on Southern Bean Mosaic Virus

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ABSTRACT

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Sodium dodecyl sulfate (SDS) sensitivity of the "native" and divalent cation-free virions of southern bean mosaic virus (SBMV) was examined under the various conditions. In the absence of SDS (25 C, 1 hr), "native" virions sedimented at 115S between pH 2.5 and 9.5; at pH 10.0–10.5, SBMV was converted progressively into a 60–65S entity; but it was degraded into a 35–40S nucleoproteinaceous product at pH 11.0. In the presence of 0.1% SDS, SBMV dissociated readily at pH 2.5–3.0 or pH 10.5–11.0; an increasing proportion of the virions were rendered SDS-sensitive at pH 9.5–10.0, but they were stable in the presence of SDS within the pH range of 3.5 and 9.0. Virions were dissociated with 2% SDS at pH 5.5 if exposed at 60–70 C for 10 min, but remained structurally stable in 0.5 M NaCl plus SDS. Divalent cation-free SBMV in the "swollen" form (100 S) at pH 7.5 or in the compact conformation (115S) at pH 5.5 was sensitive to SDS. When treated with the protein cross-linking reagents viz, formaldehyde or dimethyl adipimidate, "swollen" SBMV at pH 7.5 was transformed into a sharply sedimenting 105–107S form, but remained sensitive to SDS. With additional stabilization of the viral capsid by reducing the pH to 5.5, however, such virions sedimented uniformly at 115S and also became resistant to SDS. These results are discussed relative to the available information on SBMV-stabilizing interactions.

Additional key words: protein:protein bonds; protein:RNA linkages; virus stability.

Southern bean mosaic virus (SBMV) virions have been the objects of detailed biophysical and biochemical investigations. X-ray crystallography and small-angle neutron scattering analysis have yielded useful information on the conformation of SBMV structural units, capsid topography, in situ location of the viral ribonucleic acid (RNA), and on the contact sites between the coat protein and the genome (9,10). The structural stability of SBMV and turnip rosette mosaic virus (TRoSV, a member of the SBMV group [7]) depends upon the presence of divalent cation-mediated interactions (4,6,20,25), pH-sensitive bonds (6,29), and salt linkages between the protein and RNA (6,7). Controlled disassembly of the

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SBMV and TRoSV yielded a subviral entity (50S) comprised of the intact RNA and ~1/3 of the capsid protein (5,8,28). Apparently, SBMV and TRoSV virions are stabilized by strong inter-protein bonds supplemented by the protein:RNA linkages.

Kaper (12,13) proposed that the isometric plant viruses may be classified in two groups depending upon the nature of the forces governing virus stability. I—Viruses stabilized by protein: RNA interactions, (eg, cucumber mosaic virus [CMV])and II—Viruses stabilized by strong protein:protein bonds (eg, turnip yellow mosaic virus [TYMV]). This classification was based upon several physico-chemical properties of which virion sensitivity to sodium dodecyl sulfate (SDS) was most striking. Viruses stabilized by protein:RNA interactions were dissociated easily by SDS (0.005–0.05%), but the capsid-stabilized viruses were largely stable in SDS (1–5%) The behavior of several plant viruses in SDS under a variety of conditions was examined by Ronald and Tremaine (20) and Tremaine and Ronald (32). Several interesting observations emerged from those studies: viz, the rate at which viruses are dissociated by SDS may differ, strains of a virus may show differential sensitivity to SDS under identical conditions, and most SDS-sensitive viruses also are susceptible to proteases at a slightly alkaline pH. It was suggested, however, that SDS sensitivity along with other physico-chemical parameters are sufficiently reliable criteria for grouping viruses and viral strains (20,31).

Several factors influence the stability of viruses in SDS and these are not well understood (2). Furthermore, the stabilizing interactions in macromolecules are thermodynamic in nature (17,30) while their sensitivity to reagents such as SDS is a kinetic phenomenon (19). To improve our understanding of the detergent-virus interactions, I have examined the effect of pH, ionic concentration, and ethylenediaminetetraacetate (EDTA)-induced changes on virus interactions with SDS. SBMV is uniquely suited for such investigations because it "swells" when the capsid-associated divalent cations are removed with EDTA at pH 7.5–8.0 (4,6,18,20). Subsequently, such virions can be compacted by reducing the pH to 5.0–6.4 (7,29). Further, SBMV treated with EDTA at pH 6.0 remains compact but it "swells" if the pH is increased to 7.5–8.0 (7,29). The effect of selected protein cross-linking reagents (16) namely, formaldehyde or dimethyl adipimidate (DMA) on the SDS-sensitivity of the divalent cation-free SBMV also was examined. Our previous results (25,26) demonstrated that these reagents effectively induced chemical cross-links between the coat protein subunits of the "native" SBMV. SBMV.

MATERIALS AND METHODS

Ultrapure grades of sucrose (ribonuclease-free) and Tris-(hydroxymethyl)-aminomethane (Tris) were obtained from Schwarz/Mann (Orangeburg, NY 10962); SDS, glycine (ammonia-free), and β-mercaptoethanol were purchased from Isolab (Akron, OH 44321); and DMA was obtained from Pierce Chemical Co., (Rockford, IL 61105). All other reagents (analytical grade) were supplied by Fisher Scientific Co., (St. Louis, MO 63132).

SBMV (bean strain) was purified from frozen leaves of *Phaseolus vulgaris* L.'Bountiful' (26) and maintained in 0.02 M sodium phosphate buffer, pH 7.0. SBMV concentration was estimated spectrophotometrically (4). Infectivity assays were performed on primary leaves of *P. vulgaris* 'Pinto' in the presence of 50 mg/ml Celite (22). In each assay one primary leaf was inoculated with SBMV maintained in 0.1 M sodium phosphate buffer (pH 6.0) to serve as the control (infectivity, 100%) and the opposite leaf was inoculated with the SDS-treated virus. These experiments were repeated four times with 55–65 primary leaves per treated sample.

The buffer solutions (0.1 M) were prepared according to Dawson et al (3) and included: glycine-HCl, pH 2.5–3.5; sodium acetate-acetic acid, pH 4.0–5.5; sodium phosphate, pH 6.0–7.5; Tris-HCl, pH 7.5–8.5; and sodium carbonate-bicarbonate, pH 9.0–11.0. pH measurements were made with a Beckman Zeromatic II pH meter calibrated with standard buffer solutions (pH 4.0 and 7.0). SDS or EDTA was dissolved in the appropriate buffer (0.1 M) and the pH was adjusted to the desired level. DMA was dissolved in 0.1 M phosphate buffer, pH 7.5. Formaldehyde (2%) solution was prepared by diluting a stock preparation in 0.1 M phosphate buffer, pH 7.5, and adjusting the pH. All solutions were freshly prepared.

The following procedure was used for treating SBMV with 0.1% SDS at the various pH levels: 5 μl of the SBMV preparation (5 mg/ml) was diluted in 0.40 ml of the appropriate buffer and the samples were incubated at 25 C for 30 min; 4 μl of 10% SDS solution was introduced into the samples that received the detergent treatment and the samples were maintained at 25 C for an additional 30 min. No pH changes were detected during this period. SBMV treatment with 1–10% SDS at pH 5.5 or 7.5 was performed by introducing the virion suspension directly into the buffered SDS solutions and maintaining the samples at 25 C for 30 min.

Divalent cation-free SBMV (1 mg/ml) was prepared by treating it (5 C, 1 hr) with 10 mM EDTA in 0.1 M Tris-HCl buffer, pH 7.5. The sample was divided into two equal parts; one sample was dialyzed (5 C, 48 hr) against 2 L of 0.1 M Tris-HCl buffer, pH 7.5 to maintain virions in the "swollen" state, and the other sample was dialyzed against 0.1 M sodium acetate-acetic acid buffer, pH 5.5, for virion compaction. Similarly, SBMV was treated with EDTA at pH 5.5 and dialyzed at pH 5.5 to maintain virions in the compact state or at pH 7.5 for SBMV "swelling". For treating SBMV with the protein cross-linking reagents, equal volumes of the virion suspension (2 mg/ml in 0.1 M phosphate buffer, pH 7.5) and of formaldehyde (2%) or DMA (2 mg/ml) were mixed, the samples were stirred constantly (2 hr, 25 C), and the pH was readjusted to 7.5, if necessary. These samples were dialyzed (5 C, 20 hr) against 0.1 M phosphate buffer, pH 7.5. The SDS sensitivity of these virions was tested after treatment with 10 mM EDTA dissolved in 0.1 M phosphate buffer, pH 7.5. The divalent cation-free SBMV (1 mg/ml) at pH 7.5, from which EDTA was removed by dialysis, was treated similarly with the protein cross-linking reagents. These samples were dialyzed (5 C, 20 hr) against 0.1 M phosphate buffer pH 7.5, or 0.1 M acetate buffer, pH 5.5, and then analyzed with density gradient sedimentation with or without SDS treatment.

Linear sucrose density gradients were prepared with layering 1.0 ml each of the 40, 30, 20, and 10% sucrose (dissolved in the appropriate buffer) solution in an ultracentrifuge tube (13×51 mm), and tubes were sealed with Parafilm and kept at 5 C for 18 hr. The following schedule was followed for sedimenting the SDS-treated SBMV in sucrose gradient columns at the various pHs: virions treated at pH 2.5–3.5 were centrifuged in the pH 3.5 gradients; those treated at pH 4.0 to 6.0, in the pH 5.5 gradients; those treated at pH 6.5 to 7.0, in the pH 7.0 sucrose gradients; those treated at pH 7.5 to 8.5, in the pH 8.5 gradients; and those treated at pH 9.0–11.0, in the pH 9.0 sucrose gradients. The sedimentation markers used were tobacco mosaic virus (TMV) RNA (30S), MS2 bacteriophage (80S), and TMV virions (190S). Preliminary tests showed that these markers remained stable between pH 5.5 to 8.5. Consequently, in most experiments, a sample containing these markers was sedimented routinely in the pH 7.0 gradients. SBMV treated with the protein cross-linking reagents was sedimented in the sucrose gradients prepared with 0.1 M phosphate buffer, pH 7.5, or 0.1 M acetate buffer, pH 5.5. In experiments involving estimations of the S values of treated SBMV, MS2, and/or TMV, virions were co-sedimented in the same gradient columns. This method provided a more precise assessment of the sedimenting behavior of SBMV, especially after the EDTA treatment.

SDS-polyacrylamide gel electrophoresis was performed according to Sehgal and Hsu (26). Uranyl-formate-stained samples were examined with a JEOL 100 B electron microscope (4). Details of other methods are given under the individual experiments.

RESULTS

SDS-sensitivity of SBMV over a range of pH levels. *Sedimentation behavior.* In the absence of SDS, SBMV maintained between pH 2.5 and 9.5 sedimented uniformly at 115S; at pH 10.0–10.5 it was converted progressively into a 60–65S entity,

but was degraded to a 35–40S product at pH 11.0 (Fig. 1). When treated with SDS at pH 2.5–3.0 or 10.5–11.0, SBMV was dissociated and released intact RNA (25S); an increasing proportion of the virus was rendered SDS-sensitive at pH 9.5–10.0, but it proved to be stable in SDS within the pH range 3.5–9.0. The 60–65S SBMV entity or the 35–40S product appeared to be nucleoproteinaceous since these yielded intact (25S) RNA in the presence of SDS. SBMV treated with SDS between pH 3.5 and 9.0 yielded 25S RNA when exposed to the virion dissociative medium (23) which indicated that SDS treatment within that pH range caused no structural damage to the viral genome in situ.

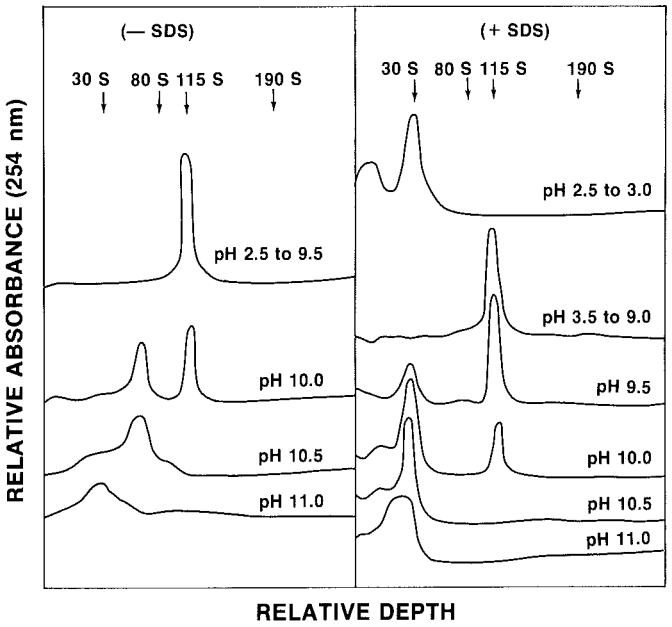

Fig. 1. Rate zonal sucrose density gradient sedimentation profiles of southern bean mosaic virus maintained in the various buffer solutions in the absence or presence of 0.1% sodium dodecyl sulfate. The treated virions (~25 μg in 0.4 ml) were layered on linear 10–40% sucrose gradients and centrifuged (5 C) at 5,000 rpm for 5 min and then at 40,000 rpm for 60 min in an SW 50.1 rotor in a Beckman L3-50 ultracentrifuge. The gradients were fractionated with an ISCO Model D fractionator and monitored at 254 nm.

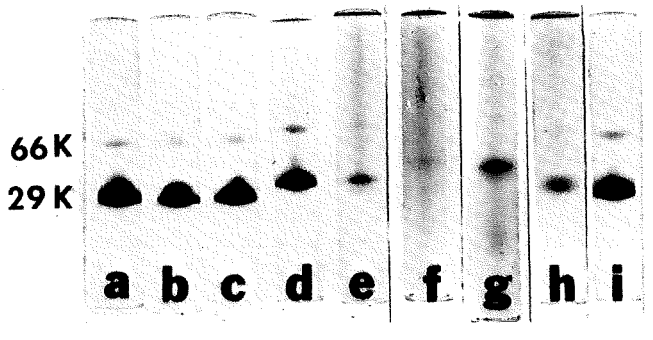

Fig. 2. Polyacrylamide gel electrophoresis of southern bean mosaic virus exposed to 0.1% sodium dodecyl sulfate (SDS) at the various pH levels. Electrophoresis was performed (3 hr, 7.5 mA/gel, 25 C) in 5% polyacrylamide gels containing 0.1% SDS at pH 7.0. (a) Control, virions dissociated with heating (100 C, 5 min) in 1% SDS and 0.1% β-mercaptoethanol at pH 7.0; (b) virions treated (25 C) with SDS at pH 2.5; (c) SDS-treated virions at pH 3.0; (d) pH 3.2; (e) pH 3.5; (f) pH 4.0–9.0; (g) pH 9.5; (h) pH 10.0; and (i) pH 10.5. After electrophoresis, the gels were stained with Coomassie Brilliant Blue and then destained with methanol:acetic acid:water (5:1:5, v/v) mixture. The 66-K protein band represents the *stable* coat protein dimers, whereas the 29-K fraction represents the monomers (26). Note the presence of undissociated SBMV at the origin in (d) through (h).

Polyacrylamide gel electrophoresis. Estimates of the stability of SBMV in 0.1% SDS between pH 2.5 and 10.5 obtained with gel electrophoresis were consistent with those based upon the density gradient centrifugation (Fig. 2).

Serology. SBMV (100 μg/ml) was exposed to 0.1% SDS in the various buffers, and then reacted against anti-SBMV serum with the Ouchterlony double diffusion method. In the absence of SDS, SBMV remained fully reactive between pH 2.5 and 9.5, showed a marked decline in reactivity at pH 10.0–10.5, and was nonreactive at pH 11.0. In the presence of 0.1% SDS, SBMV was fully reactive between pH 3.5 and 9.0, showed some decline (~1/3) in serological reactivity at pH 9.5, and no reaction was observed at pH 2.5–3.0 or pH 10–11.

Electron microscopy. SBMV maintained at pH 2.5 showed a marked tendency for aggregation, but retained its morphology (Fig. 3A). Exposure to SDS at pH 2.5 caused virions to expand (Fig. 3B) and subsequently to clump into ill-defined tubular structures (Fig. 3C). These results indicate progressive degradation of SBMV exposed to SDS under highly acidic conditions. No structural aberrations were detected if SBMV was treated with SDS at pH 5.5 or 7.5.

Infectivity. In the absence of SDS, SBMV remained fully infectious within the pH range of 2.5 and 9.5, but at pH 10–11, its infectivity declined markedly (Fig. 4). In 0.1% SDS, SBMV retained its full specific infectivity between pH 3.5 and 9.0, but outside this pH range, viral infectivity was reduced drastically.

SDS sensitivity of the conformationally altered and compact SBMV. Fig. 5 shows the results of SDS-sensitivity of the divalent cation-free SBMV in the "swollen" and in the "recontracted" states as well as of the divalent cation-containing virus. The following conclusions can be drawn: divalent cation-containing virions were slightly more stable in 2–5% SDS at pH 5.5 than at pH 7.5; divalent cation-free SBMV in the "swollen" state was readily dissociated by SDS; when treated with EDTA at pH 5.5, SBMV virions remained compact and SDS-resistant, but at pH 7.5, became "swollen" and SDS-sensitive; and divalent cation-free "swollen" SBMV assumed a compact conformation at pH 5.5, but also yielded two aggregated products of unknown nature. In 0.1% SDS, a proportion of the compacted virions were dissociated, but the remainder were aggregated heterogeneously; the latter were transformed into a discrete 80S entity in 2–5% SDS. These results signify that following the pH-induced "recontraction" of "swollen" SBMV, full resistance towards SDS was not regained.

Effect of protein cross-linking reagents on the sedimentation behavior and SDS sensitivity of SBMV. Treating "native" SBMV with formaldehyde or DMA caused no alterations in its sedimentation behavior (Fig. 6I). If exposed to 10 mM EDTA at pH 7.5, the formaldehyde-treated SBMV sedimented uniformly at ~105–107S, while the DMA-treated and the control virions sedimented identically at 100S (Fig. 6 II). In EDTA plus SDS, the formaldehyde-treated SBMV yielded a 60S degraded product, whereas the DMA-treated and control virions dissociated completely and released 25 S RNA (Fig. 6 III). These results demonstrate: that the presence of chemical cross-links does not interfere with virion "swelling" in DMA-treated SBMV, and only partially restricts the expansion of the formaldehyde-treated SBMV; and that SBMV treated with the protein cross-linking reagents is sensitive to SDS in the presence of EDTA.

"Swollen" SBMV at pH 7.5 was treated with formaldehyde or DMA, dialyzed against the pH 7.5 or pH 5.5 buffers, and examined for sedimentation behavior and stability towards SDS (Fig. 7). Exposing "swollen" SBMV to these reagents at pH 7.5 transformed it into the 105–107S form which is SDS-sensitive. However, at pH 5.5, virions treated with the cross-linking reagents sedimented uniformly at 115S and also became SDS-resistant.

The formaldehyde- or DMA-treated divalent cation-free SBMV at pH 7.5 or 5.5 was disrupted with heating (100 C, 5 min) in 1% SDS plus 0.1% β-mercaptoethanol and analyzed with gel electrophoresis. The types of the polymerized coat protein products were qualitatively and quantitatively similar to those formed when the "native" SBMV was treated with these cross-linking reagents (25,26).

SDS sensitivity of SBMV at pH 5.5 in the absence or presence of NaCl. Since the "native" SBMV at pH 5.5 proved stable in 0.1–10% SDS at 25 C, its sensitivity to 2% SDS was examined at elevated temperatures in the presence or absence of 0.5 M NaCl (Fig. 8). SBMV proved to be stable in SDS up to 50 C both in the absence or presence of NaCl. In the absence of salt, SBMV was dissociated at 60–70 C but remained structurally stable at these temperatures in NaCl plus SDS. At 80–85 C, virions were dissociated by SDS even in the presence of NaCl. SBMV proved to be fully stable if exposed at 80 C (10 min) in the absence or presence of NaCl.

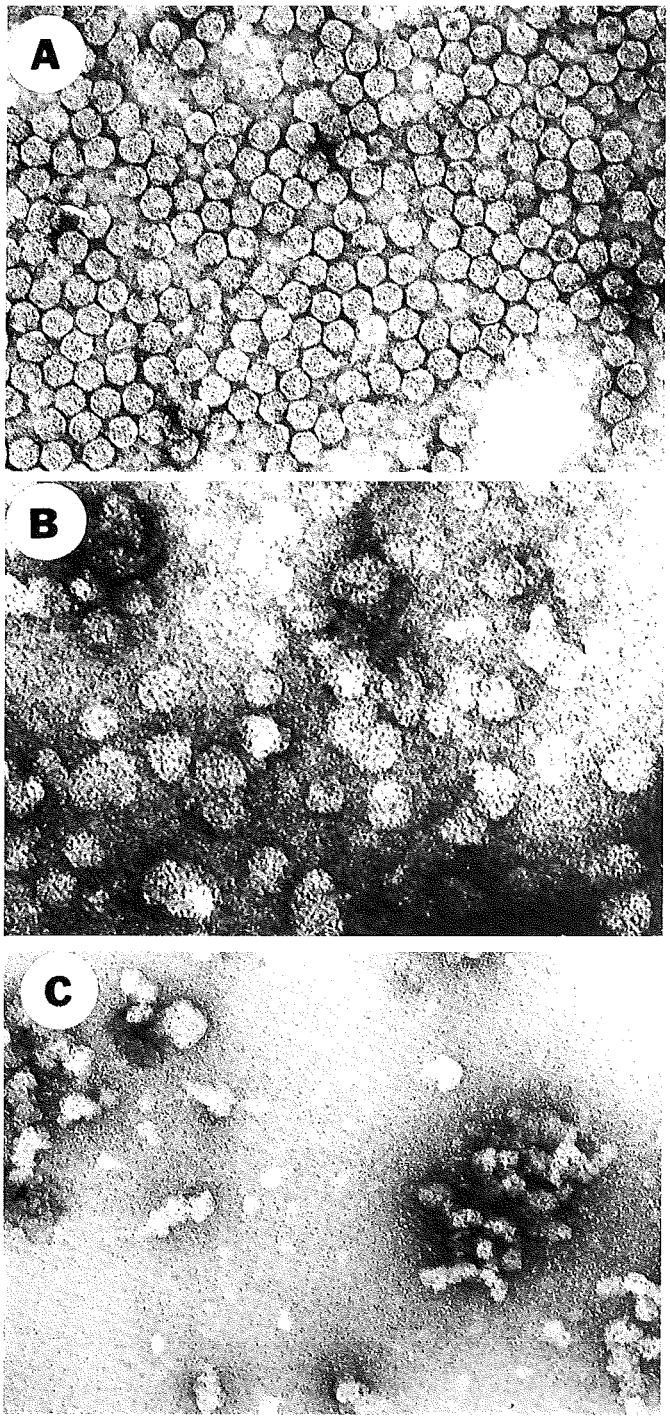

Fig. 3. Electron micrographs of southern bean mosaic virus maintained in 0.1 M glycine-HCl buffer, pH 2.5. **A,** Control; **B,** treated with 0.1% SDS for 10 min; **C,** treated with 0.1% SDS for 30 min. After deposition of the samples on the electron microscope grids they were washed carefully with the deionized water and then stained with uranyl formate. Magnification, × 155,250.

DISCUSSION

SBMV exhibits a remarkable structural and biological tolerance to a wide range of hydrogen ion concentrations (viz, pH 2.5–9.5), as do several animal picornaviruses (21). SBMV degradation at pH 11 into the 35–40S nucleoproteinaceous complex, however, differs from the alkali-sensitivity of picornaviruses which dissociate completely, releasing RNA (13). The observed pattern of SBMV degradation suggests a more intimate binding of some coat protein subunits to the genome compared to the bulk of the capsid protein and supports our earlier conclusion which was based upon SBMV sensitivity to EDTA plus NaCl (5).

Insofar as SBMV sensitivity towards SDS was concerned, interesting differences were observed at the extreme pH's. Under the highly alkaline conditions (pH 10.0–10.5), a structural change was essential for rendering virions sensitive to SDS. This clearly was evident from the observation that, at pH 10.0, only the transformed 60–65S virions proved to be SDS-sensitive while the residual 115S particles remained SDS-resistant. Contrastingly, under the acidic conditions (ie, pH 2.5–3.0) no structural change was detected, but SBMV dissociated readily in SDS. Effects of EDTA treatment or freezing damage on SBMV and TRoSV suggests that a conformational change is necessary to render these viruses sensitive to SDS (4,6,24). Although it is unclear why SBMV in an apparently compact state (115S) at pH 2.5–3.0 was dissociated with SDS, it may have been due to a more effective binding of the detergent molecules to the capsid. Bovine β-lactoglobulin binds significantly more SDS at pH 3.5 than at pH 5.4 or 7.0 (11). Alternatively, SBMV structural transition at pH 2.5–3.0 may have been too subtle to be detected with the analytical methods that were employed. Exposing proteins to acidic or alkaline conditions causes ionization of the buried groups, hydration shells develop around these groups, and proteins unfold (17). SDS can then bind to the receptive sites causing irreversible denaturation (14). The mode or extent of SDS binding with SBMV remains to be determined. However, it is apparent that within the pH range 3.5–9.0, SBMV behavior in SDS resembles that of the capsid-stabilized viruses, but outside that pH range it is similar to that of the viruses stabilized with protein:RNA bonds (12).

The fact that the divalent cation-free "swollen" SBMV at pH 7.5 was transformed into an apparently compact state at pH 5.5 but remained SDS-sensitive suggests that SBMV recontraction was not fully reversible under those conditions. The pH-induced "swelling" of brome mosaic virus (BMV) can be reversed largely by reducing the pH from 7 to 6 (15) but full contraction of the capsid fails to occur (33). Treating "swollen" SBMV with the protein cross-linking reagents at pH 7.5 caused a limited compaction with virions sedimenting at 105–107S but in that state also the virions were SDS-sensitive. Observations based upon the gel electrophoresis indicates that the concentrations of formaldehyde or DMA were adequate to induce chemical cross-links in the SBMV capsid. With an additional strengthening of the pH-

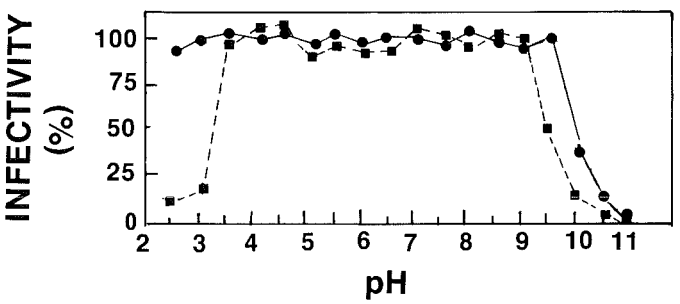

Fig. 4. Infectivity of southern bean mosaic virus at a range of pHs in the absence (●——●) or presence (■----■) of 0.1% sodium dodecyl sulfate. Virions were treated at a concentration of 100 μg/ml, diluted with 0.1 M sodium phosphate buffer, pH 6.0 (final concentration 0.1 μg/ml), and then assayed on the primary leaves of *Phaseolus vulgaris* 'Pinto.'

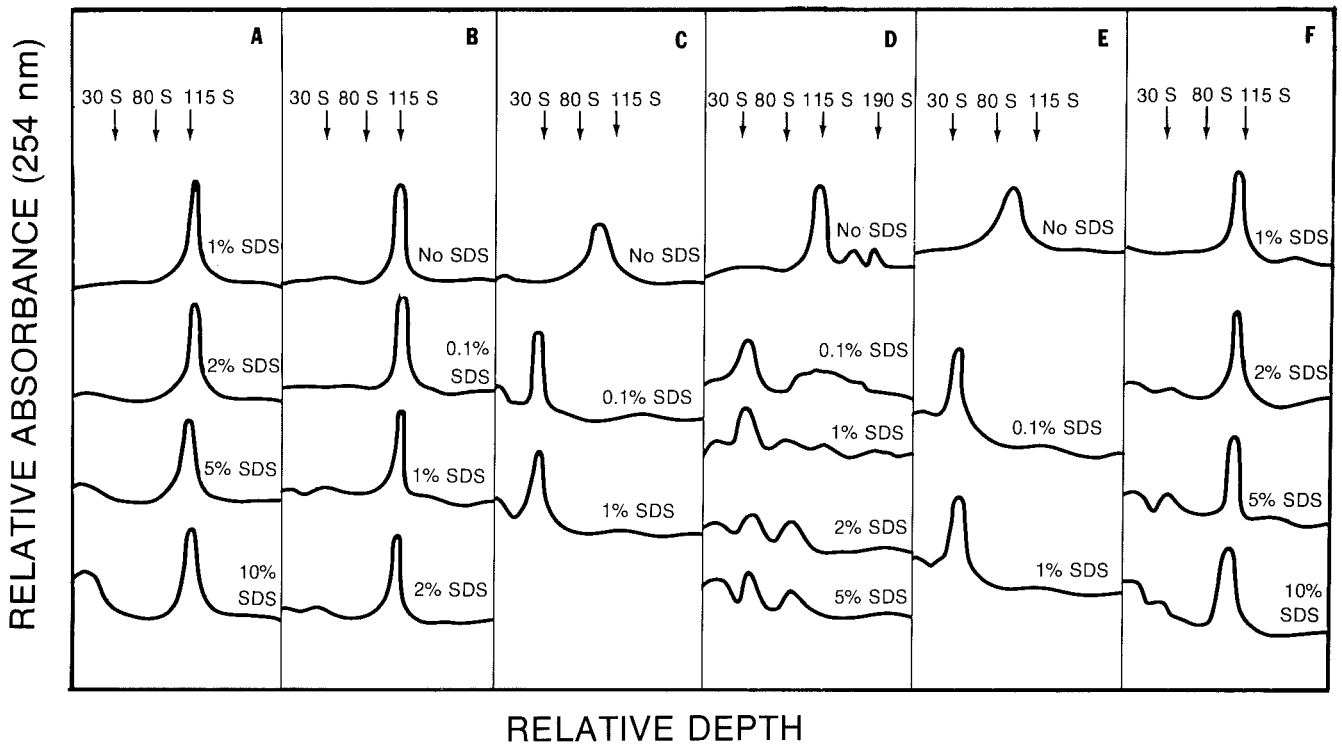

Fig. 5. The sensitivity of the "native" or ethylenediaminetetraacetate (EDTA)-treated southern bean mosaic virus (SBMV) to sodium dodecyl sulfate (SDS) at 25 C. **A,** "native" SBMV in 0.1 M acetate buffer, pH 5.5; **B,** SBMV treated with EDTA at pH 5.5, and dialyzed at pH 5.5; **C,** SBMV treated with EDTA at pH 5.5, and dialyzed against 0.1 M Tris-HCl buffer, pH 7.5; **D,** SBMV treated with EDTA at pH 7.5, and dialyzed against 0.1 M acetate buffer, pH 5.5; **E,** SBMV exposed to EDTA at pH 7.5, and dialyzed at pH 7.5; **F,** "native" SBMV in 0.1 M Tris-HCl buffer, pH 7.5. For (A), (B), and (D), the treated virus was sedimented in the pH 5.5 sucrose density gradients; in (C), (E), and (F) the virions were sedimented in pH 7.5 gradients. The conditions for virion sedimentation were as given in the caption in Fig. 1.

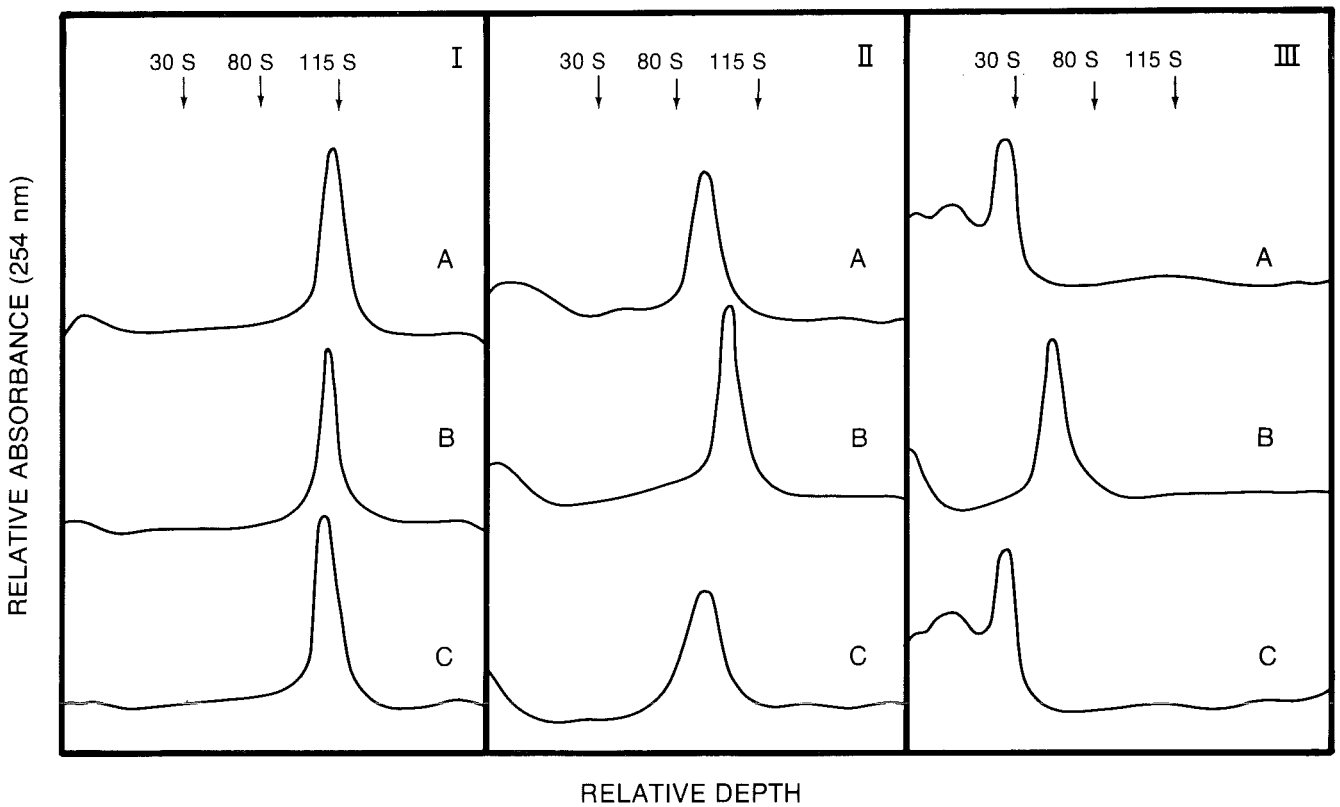

Fig. 6. Effect of the protein cross-linking reagents on the sedimentation behavior and sodium dodecyl sulfate (SDS) sensitivity of southern bean mosaic virus (SBMV) at pH 7.5. Untreated SBMV as well as virions treated with formaldehyde or dimethyl adipimidate (DMA) were dialyzed against 0.1 M phosphate buffer, pH 7.5. After the appropriate treatment, the virions were sedimented in pH 7.5 sucrose density gradients. Sedimentation curves are those of SBMV samples suspended in: **I,** buffer alone; **II,** 10 mM ethylenediaminetetraacetate (EDTA); or **III,** EDTA in the presence of 0.1% SDS. Within each group of curves: **A,** untreated control; **B,** treated with formaldehyde; or **C,** treated with DMA. The conditions for virion sedimentation were as given in the Fig. 1 caption.

sensitive bonds in the capsid with lowering the pH to 5.5 (6), however, the formaldehyde- or DMA-treated SBMV became refractory to SDS. These results signify a mutualistic role of the various interactions that confer stability to SBMV virions. Bancroft and Smith (1) reported that DMA in the presence of magnesium was effective in stabilizing expanded virions of BMV and cowpea chlorotic mottle virus (CCMV), but that DMA alone was ineffective. Treating CCMV with formaldehyde at pH 7.0 partially stabilized it against the dissociative effect of SDS at pH 7.0 or 5.5 (20).

SBMV proved stable in 2% SDS in the presence of NaCl at 60–70 C, but was dissociated in the absence of salt. The presence of salt, under such conditions, lowers the critical micelle concentration (19) and consequently, the effective SDS monomer level might have been too low for interaction with the SBMV capsid. Under the moderately acidic conditions, NaCl stabilizes the hydrophobic interactions (14). A possibility exists, therefore, that increased strengthening of such interactions with NaCl may have enhanced SBMV resistance to SDS. Preliminary evidence exists (24,25) that hydrophobic interactions are involved in SBMV capsid stabilization.

It is rather significant that TYMV, a capsid-stabilized virus, fails to bind SDS while CMV which is stabilized with protein:RNA linkages binds SDS effectively (2). Virions stabilized with strong interprotein bonds resist attack by enzymes and protein denaturants which suggests a highly ordered and compact capsid organization. It is likely that for such viruses, the SDS-sensitive sites are masked or are otherwise inaccessible. SBMV resists attack by the proteases and denaturants in the "native" state, but becomes susceptible in the "expanded" form (4,27,32). Observations based upon the behavior of the "native" and conformationally-altered bromoviruses and members of the SBMV group suggest that, under conditions which stabilize virions with strong protein: protein bonds, SDS insensitivity is due to the unavailability of the receptive sites on the viral capsid.

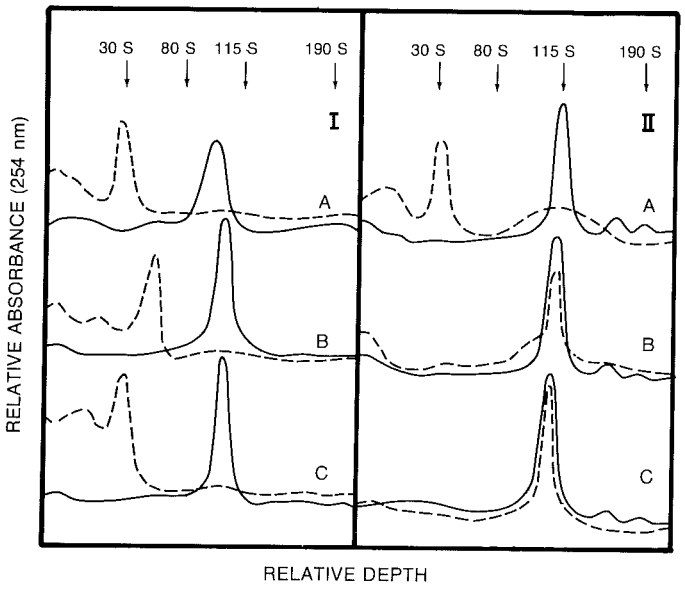

Fig. 7. Effect of the protein cross-linking reagents on the sedimentation behavior and sodium dodecyl sulfate (SDS) sensitivity of the divalent cation-free southern bean mosaic virus (SBMV). The virions were exposed to ethylenediaminetetraacetate at pH 7.5, dialyzed against 0.1 M phosphate buffer, (pH 7.5), and treated with formaldehyde or dimethyl adipimidate (DMA). Then these samples as well as the untreated "swollen" SBMV were dialyzed against 0.1 M phosphate buffer, pH 7.5, or 0.1 M sodium acetate buffer, pH 5.5. Sedimentation curves are those of SBMV samples treated as described above and: **Group I,** SBMV at pH 7.5 and sedimented in the pH 7.5 sucrose gradients or **Group II,** SBMV at pH 5.5 and sedimented in the pH 5.5 gradients. Within groups: **A,** control; **B,** formaldehyde-treated; **C,** DMA-treated; ———, untreated; and -----, 1% SDS. Conditions for SBMV sedimentation were as given in the Fig. 1 caption.

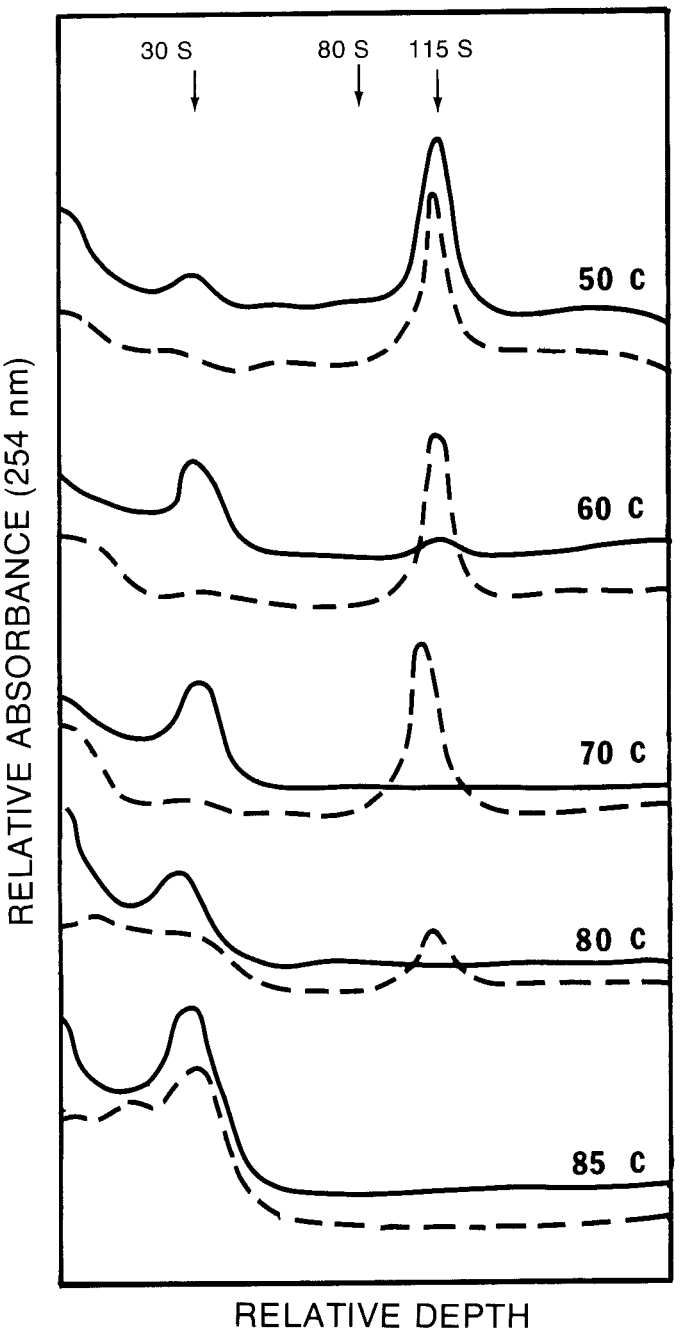

Fig. 8. The sensitivity of southern bean mosaic virus at pH 5.5 to sodium dodecyl sulfate (2%) at the indicated temperatures in the absence (———) or presence (- - -) of 0.5 M NaCl. The conditions for virion sedimentation were as given in the Fig. 1 caption.

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