Factors Affecting Dissociation of Southern Bean Mosaic Virus

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ABSTRACT

Treatment of southern bean mosaic virus (SBMV) with a dissociative medium (DM) comprising of an appropriate buffer (0.1 M), pH 7.5 to 10.0, 1% sodium dodecyl sulfate (SDS), 1 mM ethylenediaminetetraacetic acid (EDTA) and bentonite (50 μg/ml) resulted in its rapid dissociation into coat protein and ribonucleic acid (RNA). These virus components were then separated by density-gradient sedimentation. Presence of a buffer of suitable ionic concentration and pH, and SDS in DM were essential for virus disassembly. Absence of EDTA at pH 8.5 and 9.1 impaired SBMV dissociation but not at pH 7.5. Pretreatment of SBMV with spermine, formaldehyde, formamide, Mg^{++}, or several reducing agents or their presence in DM did not affect virus dissociation at pH 7.5. SBMV protein prepared by the virus dissociation method between pH 7.5 and 9.1, and sedimented by density-gradient centrifugation possessed E280/E260 ratio of 1.4 and was non-infectious. The specific infectivity of SBMV-RNA prepared by this procedure was superior to RNA isolated from SBMV by the conventional SDS-phenol method.

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Additional key words: southern bean mosaic virus protein, southern bean mosaic virus ribonucleic acid.

A useful procedure for isolating ribonucleic acid (RNA) from small quantities of wheat streak mosaic virus (WSMV) was developed by Brakke & Van Pelt (3). In this method WSMV was incubated overnight at 2 C in 0.1 M ammonium carbonate buffer, pH 9.0, containing 0.001 M ethylenediaminetetraacetic acid (EDTA) 1% sodium dodecyl sulfate (SDS) and bentonite followed by rate zonal sucrose density gradient centrifugation. Dissociation of southern bean mosaic virus (SBMV) into its coat protein and RNA was achieved by a modification of this method (13). This procedure proved to be superior (8) to the SDS-phenol method (4) for quantitative recovery of RNA from SBMV. Furthermore, macromolecular RNA was isolated from SBMV treated with nitrous acid by this method, whereas, extensive fragmentation of RNA occurred upon emulsification of nitrous acid-treated SBMV with SDS and phenol (8). After SBMV dissociation, the capsid protein and RNA can be separated by sucrose density-gradient sedimentation, passage through a Sephadex G-200 column (13) or gel electrophoresis (8). Factors affecting dissociation of SBMV have now been examined and some properties of the isolated capsid protein and RNA are described in this paper.

MATERIALS AND METHODS.–SBMV was purified from *Phaseolus vulgaris* L. 'Bountiful' by the procedure of Wells & Sisler (16). After the last purification step, virus was dissolved in 0.01 M phosphate buffer, pH 7.0, and dialyzed (4 to 6 hr, 5 C) against the same buffer. SBMV-RNA was isolated by SDS-phenol method (4) in presence of purified bentonite (6). Infectivity assays were performed on primary leaves of *P. vulgaris* 'Pinto' (13). The

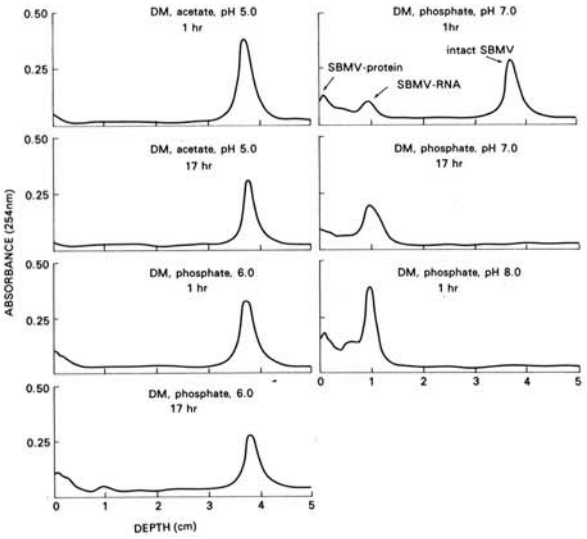

Fig. 1. Effect of pH of the dissociative medium (DM) on the disassembly of southern bean mosaic virus (SBMV).

concentrations of SBMV and SBMV-RNA were determined spectrophotometrically (7, 11).

The virus dissociative medium (DM) contained 0.2 M of an appropriate buffer and desired pH, 2% SDS, 2 mM EDTA, and purified bentonite (100 μg/ml). The following conditions were standardized for SBMV dissociation. One volume of SBMV (500 μg/ml) was precooled to 5 C and to it an equal volume of DM (at 20 C) was added. The suspension was briefly shaken, and unless otherwise stated, was stored for 1 hr at 5 C. Samples (equivalent to 75 μg of SBMV) were layered on 5 to 30% sucrose gradients (prepared in 0.02 M phosphate buffer, pH 7.0) and centrifuged at 160,000 *g* (Rotor No. SB-206, I. E. C. Ultracentrifuge Model B-35) for 2 hr at 5 C. For complete separation of virus protein from RNA, the centrifugation period was prolonged to 6 hr. Density-gradient columns were fractionated and monitored in the same manner as described previously (14).

The dissociation of SBMV at varying concentrations (10, 5, 2.5 mg/ml) was examined by removing aliquots of the DM-treated (1 to 3 hr) virus, diluting in 0.01 M phosphate buffer, pH 7.0, and analyzing by density gradient centrifugation.

The effect of formaldehyde, formamide, Mg^{++} β-mercaptoethanol, thioglycollic acid, and dithiothreitol (Clelands' reagent) on the integrity of SBMV and its subsequent sensitivity to dissociation was evaluated as follows. One volume of SBMV (1 mg/ml) was mixed with the same volume of the above reagents (dissolved in 0.01 M phosphate buffer, pH adjusted to 7.0, or in deionized water, pH adjusted to 7.0), and maintained for 1 to 16 hr at 5 C. The solutions were either dialyzed (4 to 5 hr, 5 C) against the phosphate buffer (or deionized water), or to the undialyzed samples, an equal volume of phosphate, pH 7.5-DM, was added. The samples were kept at 5 C for 10 to 15 min and then analyzed by sucrose density-gradient centrifugation.

Reconstitution of SBMV was attempted by the procedure developed for tobacco mosaic virus (TMV) (5) or cucumber mosaic virus (9). Fractions containing SBMV protein and RNA after dissociation in phosphate, pH 7.5-DM, and density-gradient centrifugation were dialyzed against 0.01 M phosphate buffer, pH 7.0, to remove sucrose. SBMV protein (four parts) and RNA (one part) were then mixed in presence of the appropriate reconstitution mixture. Aliquots of these samples were assayed directly or after incubation with pancreatic ribonuclease (0.1 μg/ml, 25 C, 1 hr). Samples without incubation with ribonuclease were infectious but those treated with the enzyme were non-infectious. Under the conditions examined, the virus protein and RNA apparently did not reconstitute to form a ribonuclease-resistant entity.

RESULTS AND DISCUSSION.—The effect of pH on the disassembly of SBMV is shown in Fig. 1. The virus concentration in these experiments was 250 μg/ml and the concentrations of the various components of DM were, 0.1 M buffer of desired pH, 1% SDS, 1 mM EDTA, and 50 μg/ml of bentonite. SBMV did not dissociate after incubation for 1-17 hr in acetate pH 5.0-DM. Similarly, it remained intact when treated for 1 hr with phosphate, pH 6.0-DM, but some dissociation occurred after exposure for 17 hr. Incubation for 1 hr in phosphate, pH 7.0-DM, resulted in dissociation of ca. 30% of the virions but all the virus was fully dissociated after 17 hr of

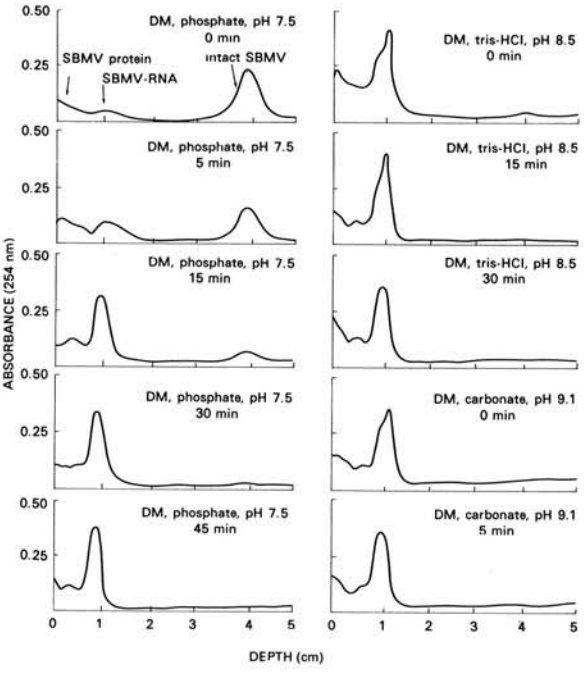

Fig. 2. Dissociation of southern bean mosaic virus (SBMV) treated with dissociative media (DM) at pH 7.5, 8.5, and 9.1 for varying periods.

treatment. Sedimentation behavior of RNA, however, indicated that it did not achieve a compact conformation when liberated at this pH. Upon exposure to phosphate, pH 8.0-DM for 1 hr, SBMV was completely dissociated and the freed RNA had sedimented sharply. Similarly, Tris [tris(hydroxymethyl) amino methane] pH 8.5-DM, and carbonate, pH 9.1 or 10.0-DM, were equally effective in the dissociation of SBMV. These results suggested that disassembly of SBMV was highly pH-dependent and alkaline buffers were superior to the neutral or acidic buffers. Degradation of bromegrass mosaic virus (BMV) with the consequent release of its RNA occurred following exposure of virions to 0.02 M Tris buffer, pH 7-9, containing 0.001 M EDTA, bentonite, and 0.10 M NaCl, overnight, at 2 C (1).

Disassembly of SBMV started within 5 min of treatment with phosphate, pH 7.5-DM, and all the virions were dissociated after 30 min (Fig. 2). Exposure to Tris, pH 8.5-DM, or carbonate pH 9.1-DM, caused instantaneous dissociation of SBMV. Furthermore, it appears that SBMV dissociation was a spontaneous process without the formation of stable intermediary nucleoproteinaceous entities.

The effect of varying ionic concentration of buffers in DM on SBMV dissociation is shown in Fig. 3. Complete dissociation of SBMV occurred after treatment for 1 hr with 0.05 M or 0.01 M carbonate, pH 9.1-DM. The 0.05 M carbonate caused more efficient dissociation as indicated by the sedimentation profile of liberated RNA than did 0.01 M carbonate. Similarly, 0.05 M phosphate, pH 7.5-DM, was more efficient than 0.01 M phosphate pH 7.5-DM in SBMV disassembly but some particles remained undissociated at this pH. Apparently, the appropriate ionic concentration of buffer in DM at pH 7.5 was more critical than at pH 9.1 for SBMV dissociation.

When treated for 1 hr with phosphate, pH 7.5-DM, complete dissociation of SBMV up to a concentration of 2.5 mg/ml occurred but at higher concentrations (10 and 5 mg/ml), appreciable amount of the virus remained intact (Fig. 4). Prolonging the treatment period to 2 to 3 hr caused all the virions to dissociate even at higher concentration levels. Treatment of SBMV (10, 5 and 2.5 mg/ml) with Tris,

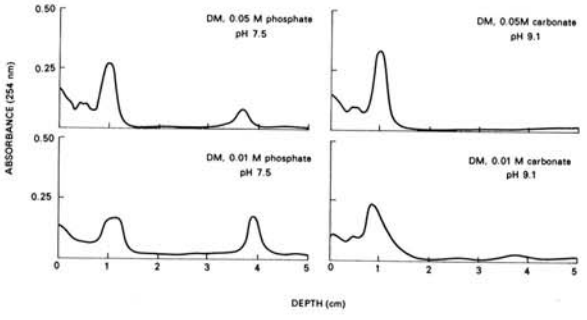

Fig. 3. Effect of ionic concentrations of buffers in the dissociative medium (DM) at pH 7.5 and 9.1 on the disassembly of southern bean mosaic virus (SBMV).

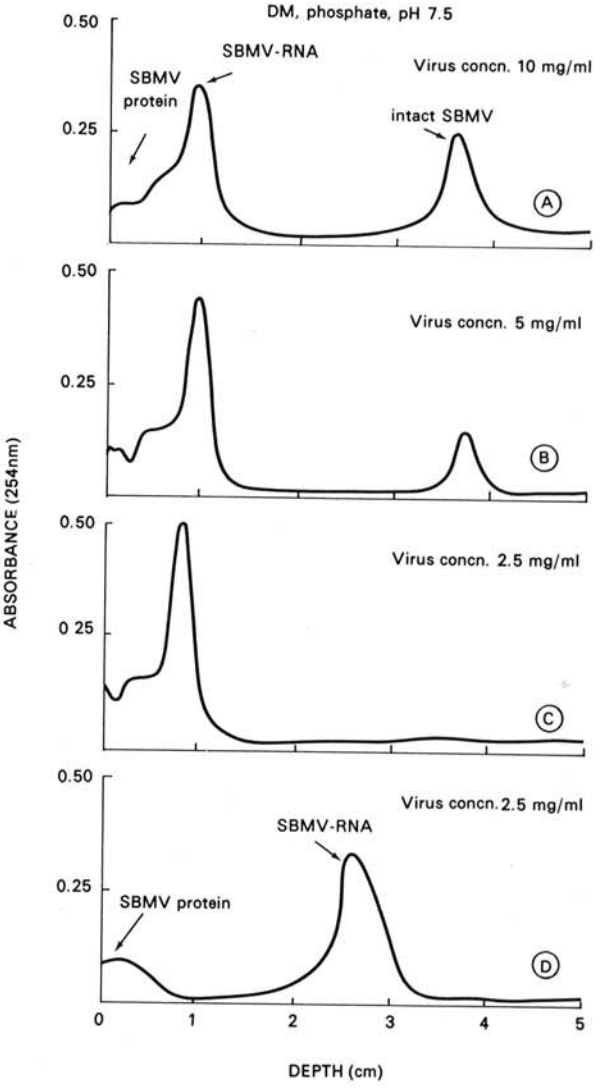

Fig. 4. Disassembly of southern bean mosaic virus (SBMV) at various concentrations following exposure to phosphate pH 7.5 dissociative medium (DM). **A-C)** sedimentation patterns after centrifugation at 160,000 *g* for 2 hr. **D)** centrifugation at 160,000 *g* for 6 hr.

pH 8.5-DM or carbonate, pH 9.1-DM caused its complete dissociation within 1 hr. These data indicated absence of virions in a population of SBMV particles that were refractory to dissociation.

The effect of various components of DM on SBMV dissociation is shown in Fig. 5. In complete DM, within 1 hr, SBMV dissociated equally well at pH 7.5, 8.5, and 9.1. By elimination of the appropriate buffer, only a small fraction of the virus dissociated at pH 7.5 and 8.5, but more dissociation occurred at pH 9.1. Absence of bentonite at any of these pH levels, did not affect SBMV disassembly. EDTA was essential for virus dissociation at pH 8.5 and 9.1 but not at pH 7.5. Wells & Sisler (16),

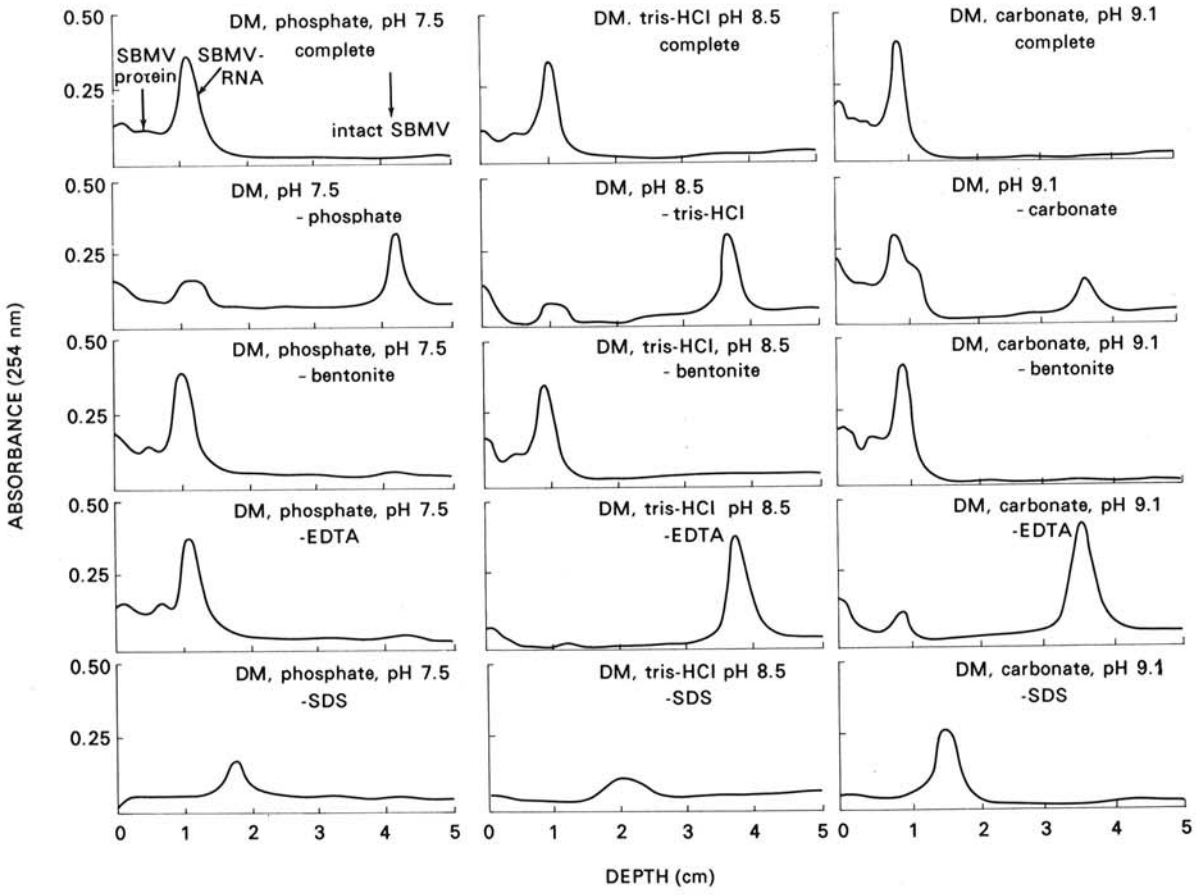

Fig. 5. Effect of various components of the dissociative medium (DM) at pH 7.5, 8.5, and 9.1 on the disassembly of southern bean mosaic virus (SBMV).

similarly, reported that presence of EDTA (0.004 M) in 0.1 M carbonate buffer, pH 9.3, was necessary for complete degradation (25 C, 16 hr) of SBMV. Absence of SDS at pH 7.5, 8.5, and 9.1 impaired the virus disassembly mechanism and caused formation of a subvirus entity sedimenting faster (47 to 50 S) than the intact SBMV-RNA (25 S).

Treatment of SBMV with spermine (0.01 M) formaldehyde (0.02%), formamide (10%), Mg^{++} (0.01 M), β-mercaptoethanol (0.02%), thioglycollic acid (0.02%), and Clelands' reagent (10 mM) did not affect its sedimentation in sucrose density-gradient columns. Furthermore, prior treatment of SBMV or the presence of these reagents in the DM did not affect its dissociation at pH 7.5. Brakke & Van Pelt (2) and Brakke (1) reported, however, that treatment of BMV or TMV with Mg^{++} and two polyamines (bis-3-amino propyl amine and spermidine) inhibited virus degradation. Exposure to spermidine stabilized BMV more than Mg^{++}.

The ultraviolet light absorption spectra of SBMV protein (13) released from the virus by treatments with DM at pH 7.5, 8.5, and 9.1, and separated by density-gradient sedimentation were identical. The E280/E260 ratios of these preparations were 1.4. When assayed at a concentration of ca. 1 mg/ml, these preparations were noninfectious. SBMV protein, like TMV protein (1) was not adsorbed by bentonite. Contrastingly, coat protein of BMV following dissociation of virions was adsorbed by bentonite and removed by centrifugation (1).

SBMV-RNA isolated by treating virus with DM at pH 7.5, 8.5, and 9.1, possessed E260/E230 ratio of 2.4 and exhibited ultraviolet light absorption spectra similar to RNA isolated by the SDS-phenol treatment of SBMV (4). The specific infectivities of RNA isolated at these three pH levels were similar and greater than the phenol prepared SBMV-RNA. For instance, DM-isolated SBMV-RNA assayed at a concentration of 2 μg/ml induced 60 to 69 lesions per primary leaf of 'Pinto' vs. 20 to 25 lesions by the phenol prepared RNA. SBMV-RNA prepared by both these procedures was inactivated by incubation with ribonuclease (0.1 μg/ml) for 1 hr at 25 C.

Alkaline degradation of TMV results in the formation of stable intermediary nucleoproteinaceous entities and a significant fraction of the virus remains resistant to degradation (10). This observation and other studies (14, 15) suggest existence of stable bonds between virus RNA and coat protein in TMV.

Dissociation of SBMV in DM, a spontaneous and rapid process, does not involve formation of stable intermediates detectable in the present study. Release of a few protein subunits from the capsid probably results in its rapid depolymerization with the consequent release of intact SBMV-RNA. These studies together with data on the reaction of intraparticle SBMV-RNA with nitrous acid (14) and ultraviolet light-irradiation of intact virus (13) suggests that stability of SBMV results primarily from protein:protein interaction with a minimal contribution by forces involved in protein:RNA copolymerization.

Presence of SDS in DM, pH 7.5 - 9.1, was essential for SBMV dissociation. In its absence, an entity sedimenting slightly faster (47 to 50 S) than SBMV-RNA (25 S), but much slower than the intact virus (115 S) was formed. SDS binds with proteins and alters their conformation (12). Apparently, in absence of SDS, the necessary conformational change in SBMV favoring complete release of viral RNA does not occur. Additional investigations on the properties of this subvirus entity can yield useful information on the disassembly mechanism of SBMV and the role of SDS in this process.

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