Inactivation of Southern Bean Mosaic Virus and its Ribonucleic Acid by Nitrous Acid and Ultraviolet Light

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SUMMARY

Upon treatment with nitrous acid, southern bean mosaic virus (SBMV) was inactivated twice as fast as its isolated RNA. Nitrous acid treatment of SBMV resulted in appreciable binding of the virus protein to the nucleic acid. The extent of deamination of aminoribonucleotides, of intraparticle RNA and isolated SBMV-RNA by nitrous acid was identical. SBMV and SBMV-RNA were inactivated at the same rate following exposure to u.v. light. Furthermore, the extent of photoreactivation of the u.v.-irradiated SBMV and SBMV-RNA was similar. U.v. irradiation did not induce any detectable binding of virus protein to RNA in SBMV.

INTRODUCTION

Encapsidated RNA of southern bean mosaic virus (SBMV) is rapidly inactivated upon treatment with nitrous acid. Furthermore, the aminoribonucleotides of intraparticle SBMV-RNA are readily deaminated by nitrous acid. These results indicated that a majority of amino groups of in situ SBMV-RNA existed free with little association with the coat protein (Sehgal & Soong, 1972). In order to investigate the rapid loss of infectivity and obtain additional information on the extent of RNA and protein interaction in SBMV, a comparative study was made of the reactivities with nitrous acid of encapsidated and isolated SBMV-RNA. Furthermore, as some measure of protection conferred by the capsid to the virus genome can be obtained from u.v. irradiation experiments (Siegel, Wildman & Ginoza, 1956; Goddard et al. 1966; Streeter & Gordon, 1968); a comparison was also made of the relative u.v. sensitivities of intact SBMV and its isolated RNA.

METHODS

Purification of SBMV and SBMV-RNA. SBMV was purified from Phaseolus vulgaris L. 'Bountiful' by the procedure of Wells & Sisler (1969). SBMV-RNA was routinely isolated from the virus by the method of Diener (1965) in the presence of purified bentonite (Fraenkel-Conrat, Singer & Tsugita, 1961). SBMV-RNA prepared by this procedure had an $E_{260}/E_{230}$ ratio of 2.3 to 2.4, its infectivity was approximately 25 to 30% that of the intact virus, and its yield was between 65 and 70%. The concentrations of SBMV and SBMV-RNA were determined spectrophotometrically (Price, 1965; Ghabrial, Shepherd & Grogan, 1967).

Treatment with nitrous acid. SBMV (2 mg/ml) and SBMV-RNA (400 µg/ml) were reacted with nitrous acid (0.25 M-sodium nitrite, 0.25 M-acetate buffer, pH 4.2) at 25 °C. Sodium nitrate was substituted for sodium nitrite in control experiments. Other conditions during the treatment were the same as described before (Sehgal & Krause, 1968). Samples of the
treated SBMV or SBMV-RNA removed periodically (0 to 60 min) from the reaction mixtures were appropriately diluted in cold 0.02 M-phosphate buffer, pH 7.0, for infectivity tests.

The base composition of SBMV following treatment with nitrous acid was determined by the procedure described previously (Sehgal & Soong, 1972). The nitrous acid-treated and nitrate-treated SBMV-RNA were characterized by the following method. Treated RNA (3 to 4 mg) was removed at intervals (0 to 96 h) from the reaction mixture and immediately diluted in 2 vol. of cold phosphate buffer, pH 7.0. Samples containing 80 to 100 µg of RNA were analysed by rate zonal sucrose density-gradient sedimentation. The remaining RNA was precipitated by 3 vol. of cold 95% ethanol and centrifuged at 5000 g for 15 min. The RNA was washed 3 to 4 times with ethanol, dissolved in 0.02 M-neutral phosphate buffer and dialysed (4 to 6 h) at 2 to 3 °C against the same buffer. Part of this samples was characterized spectrophotometrically and the remainder was lyophilized. The RNA was subsequently hydrolysed (1 N HCl at 100 °C, 1 h) and its base composition was determined (Sehgal & Soong, 1972).

Irradiation with u.v. The source of u.v. was a low-pressure argon–mercury discharge lamp, which, according to the manufacturer’s (Ultraviolet Products, Inc., San Gabriel, California) specification, had an intensity (at 2537 Å) of 190 µW/cm² at a distance of 18 in. Samples of SBMV (1 mg/ml) or SBMV-RNA (200 µg/ml) contained in 5 cm diam. glass dishes were irradiated 18 in from the u.v. source while being constantly agitated at room temperature. Portions of the treated (0 to 15 min) samples were appropriately diluted in cold 0.02 M-phosphate buffer, pH 7.0, for bioassay. Samples of irradiated SBMV or SBMV-RNA (0 to 60 min) were similarly prepared for biophysical characterization.

Infectivity assays were performed on primary leaves of 14- to 16-day-old plants of P. vulgaris ‘Pinto’. Two days prior to inoculation the apical part of plants comprising the trifoliate leaf and terminal bud was removed for uniform expansion of the primary leaves. The test plants were given a pre-inoculation dark treatment for 12 to 15 h. All inocula contained Celite (50 mg/ml) and were kept ice-cold during inoculation. In preliminary studies, SBMV and SBMV-RNA were assayed at concentrations of 0.5 µg/ml and 2.0 µg/ml, respectively. In subsequent studies, depending upon the duration of exposure to nitrous acid or u.v., SBMV was assayed at 0.5 to 4.0 µg/ml and SBMV-RNA at 2 to 20 µg/ml. In assay schedules, zero-time (100% survival) sample was inoculated on one primary leaf and the opposite leaf was inoculated with the treated preparation. The inoculated leaves were immediately rinsed with water. In experiments to study the photoreactivation of u.v.-irradiated SBMV and SBMV-RNA, one set of plants following inoculation was placed on the greenhouse bench while a comparable set was given a post-inoculation dark treatment for 24 h. The dark-treated plants were subsequently returned to the normal light conditions of the greenhouse. Local lesions were counted 6 to 7 days later. The percentage survival following treatment with nitrous acid or u.v. irradiation was estimated from comparisons with the infectivity of zero-time sample.

Dissociation of SBMV into its coat protein and RNA moieties was achieved by incubating the virus for 1 h at 4 °C in a medium containing 0.1 M-carbonate, 1% SDS, 10⁻³ M-EDTA and bentonite, pH adjusted to 9.1. The two virus components were then easily separated by rate zonal sucrose density-gradient sedimentation (Brakke & Van Pelt, 1970). The specific infectivity of SBMV-RNA prepared by this procedure was similar to that isolated by the SDS:phenol method (Diener, 1965). The nitrous acid or nitrate-treated SBMV was extensively dialysed against 0.02 M-neutral phosphate buffer and concentrated by ultracentrifuging (Sehgal & Soong, 1972) before incubation in the dissociative medium.

Estimation of protein-RNA binding in treated virus. The nitrous acid and nitrate-treated
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SBMV or the u.v.-irradiated virus (6 mg/ml) was dissociated into its protein and RNA components as described above. In a few preliminary experiments, the virus protein was precipitated with 33% ammonium sulphate and removed by centrifuging, while the RNA in the supernatant fluid was passed through a Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) column. The salt precipitation procedure prior to gel chromatography resulted in a considerable reduction in the yield of virus RNA, especially of the nitrous acid-treated SBMV samples. Furthermore, no significant improvement in the quality of RNA was indicated (judged by $E_{260}/E_{280}$ ratio) by including the ammonium sulphate precipitation step prior to gel chromatography. Consequently, the salt precipitation step was omitted from further experimentation. The samples of the dissociated virus were directly applied to a Sephadex G-200 column (30 × 2.5 cm) equilibrated with 0.05 M-carbonate buffer, pH 9.1, containing $10^{-4}$ M-EDTA and eluted with the same buffer (flow rate 0.33 ml/min) at room temperature. The peak fractions containing the RNA were collected and it was precipitated by 95% ethanol and a few drops of 3 M-sodium acetate. Following low-speed centrifuging (5000 g, 15 min) the RNA was washed 3 to 4 times with ethanol and dissolved in buffer to a concentration of approximately 500 µg/ml. The RNA and protein contents of these samples were estimated colorimetrically (Shatkin, 1969), using highly purified yeast RNA (Schwarz/Mann, Orangeburg, New York) and bovine serum albumin (Nutritional Biochemicals Corporation, Cleveland, Ohio) as standards.

Rate zonal density-gradient sedimentations for intact SBMV were done in 10 to 50% sucrose (ribonuclease-free, Schwarz/Mann, Orangeburg, N.Y.) gradients (prepared in 0.02 M-neutral phosphate buffer) at 160000 g (rotor No. S.B. 206, L.E.C. Ultracentrifuge, Model B-35) for 2.5 h at 4 °C. The density-gradient columns were fractionated and analysed as before (Sehgal & Soong, 1972). Sucrose density-gradient sedimentations for SBMV-RNA or the dissociated virus were done in 5 to 30% gradients at 160000 g for 4 to 8 h. Those fractions containing the virus protein were pooled, dialysed (2 to 3 h) against 0.02 M-neutral phosphate buffer and characterized spectrophotometrically. The fractions containing the virus RNA were combined, sucrose was removed by dialysis and the RNA was precipitated with ethanol and finally dissolved in 0.02 M-phosphate buffer, pH 7.0, for spectrophotometric characterization.

Electrophoresis of SBMV (150 to 200 µg) was done on 1.5% agarose (Sigma Chemical Co., St Louis, Missouri) gels, 0.7 × 7.0 cm, at constant current (3 mA/gel) for 150 min in 0.05 M-tris-HCl buffer, pH 8.6, containing 0.02 M-sodium acetate. The gels were stained overnight with Coomassie Brilliant Blue and subsequently destained by several changes of 1:1 solution of 7% acetic acid and methanol (Lesnaw & Reichmann, 1969).

Serological tests using SBMV-antiserum (American Type Culture Collection, Rockville, Maryland) were done in Ouchterlony gel diffusion plates and developed at 25 °C.

RESULTS

Treatment of SBMV and SBMV-RNA with nitrous acid

Intact SBMV and its isolated RNA, upon treatment with nitrous acid, showed a rapid and exponential decrease in infectivity (Fig. 1). No loss of infectivity was observed in the nitrate-treated samples. Intact SBMV was consistently inactivated slightly faster (approx. × 2) than the isolated SBMV-RNA.

Nitrous acid-treated (0 to 96 h) SBMV when reacted against SBMV-antiserum formed sharp precipitin bands in the immunodiffusion tests, similar to the nitrate-treated or un-
treated SBMV, indicating no alterations in its serological properties. Similarly, no differences were observed between the electrophoretic mobilities of nitrous acid-treated, nitrate-treated or untreated virus when electrophoresed on 1.5% agarose gels. These results confirm our previous findings based on rate zonal sucrose density-gradient or isopycnic CsCl sedimentations and electron microscopy that SBMV remained physically intact even during prolonged periods of exposure to nitrous acid (Sehgal & Soong, 1972).

Analyses by sucrose density-gradient sedimentation showed that nitrate-treated or nitrous acid-treated (0 to 96 h) SBMV-RNA sedimented as single (25 S) component. These results indicate that the physical integrity of SBMV-RNA was fully maintained during such treatments. No significant alterations occurred in the u.v. absorption spectrum of SBMV-RNA upon treatment with nitrous acid.

The faster inactivation of intact SBMV in comparison to isolated SBMV-RNA by nitrous acid suggested that besides lethal deaminations, other factors were probably involved in the inactivation of virus. To examine if genomic cleavage was involved in this process, attempts

Fig. 1. Inactivation of southern bean mosaic virus and its isolated RNA by nitrous acid. The points on the graph indicate average values obtained in 18 to 21 bioassays in four separate experiments.

- O-O, SBMV (0.25 M-NO₂, pH 4.2); ■■■■, SBMV-RNA (0.25 M-NO₂, pH 4.2); control, ○-○, SBMV, or □-□, SBMV-RNA (0.25 M-NO₂, pH 4.2).
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Table 1. Some properties of SBMV-RNA isolated from NaNO₃ or nitrous acid-treated SBMV*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Yield (%)</th>
<th>$E_{260}/E_{230}$</th>
<th>μg virus protein/100 μg of SBMV/RNA†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (untreated)</td>
<td>85 to 90</td>
<td>2.3 to 2.4</td>
<td>5.5</td>
</tr>
<tr>
<td>NaNO₃-treated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 h</td>
<td>85 to 90</td>
<td>2.3</td>
<td>5.2</td>
</tr>
<tr>
<td>96 h</td>
<td>80 to 83</td>
<td>2.1</td>
<td>6.0</td>
</tr>
<tr>
<td>Nitrous acid-treated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 h</td>
<td>85 to 90</td>
<td>2.3 to 2.4</td>
<td>5.9</td>
</tr>
<tr>
<td>1 h</td>
<td>85 to 88</td>
<td>1.9</td>
<td>32.0</td>
</tr>
<tr>
<td>2 h</td>
<td>80 to 83</td>
<td>1.7</td>
<td>49.9</td>
</tr>
<tr>
<td>24 h</td>
<td>70 to 75</td>
<td>1.4</td>
<td>61.0</td>
</tr>
<tr>
<td>96 h</td>
<td>70 to 75</td>
<td>1.4</td>
<td>62.0</td>
</tr>
</tbody>
</table>

* The treated virus (c. 6 mg) was incubated in a medium containing 0.1 M-carbonate, 1 % SDS, 10⁻³ M-EDTA and bentonite for 1 h at 4 °C. The samples were passed through a Sephadex G-200 column. The peak fractions containing RNA were collected, RNA was precipitated with 95 % ethanol, and centrifuged (5000 g, 15 min). The RNA was washed 3 to 4 times with ethanol and finally dissolved in 0.02 M-neutral phosphate buffer.

† Protein and RNA contents of the samples were estimated colorimetrically (Shatkin, 1969) using bovine serum albumin and yeast RNA as standards. These figures represent means of 8 to 10 tests in three separate experiments.

were made to isolate RNA from the nitrous acid-treated SBMV by SDS:phenol method (Diener, 1965). Following emulsification of SBMV (treated with nitrous acid for as short periods as 30 to 60 min; residual infectivity, 0.5 to 0.01 %) with SDS:phenol mixture and low-speed centrifuging, very little RNA (5 to 10 % of expected value) was recovered from the buffer phase by precipitation with ethanol. In comparable experiments, however, the virus RNA was easily released from the nitrate-treated SBMV. Consequently, it became necessary to isolate RNA from the nitrous acid-treated SBMV by an alternative procedure.

Complete dissociation of SBMV into its coat protein and nucleic acid moieties was achieved by incubating virus (500 μg) in a medium containing 0.1 M-carbonate, 10⁻³ M-EDTA, 1 % SDS and bentonite (100 μg), pH adjusted to 9.1, for 1 h at 4 °C. The two virus components were then separated by the rate zonal sucrose density-gradient sedimentation. This procedure resulted in a quantitative release of RNA from SBMV, and made the virus coat protein also available for further characterization. SBMV-RNA purified by Diener's method (1965) and tobacco mosaic virus (TMV) RNA were used as markers. Purified SBMV-RNA, RNA released from untreated or nitrate-treated (0 h, 96 h) virus formed sharp peaks (25 S) in the density-gradient columns. Similarly, RNA released from zero-h nitrous acid-treated SBMV formed a sharp peak. The RNA released from virus that had been treated with nitrous acid for 1 to 96 h, however, formed asymmetric peaks that were markedly skewed towards lower sucrose density but the bulk of RNA sedimented as 25 S component. This sedimentation behaviour of RNA possibly resulted from the presence of some coat protein which caused changes in its tertiary structure; consequently, the RNA was less compact and sedimented somewhat slowly. Analyses of RNA isolated from the nitrous acid-treated virus by polyacrylamide gel electrophoresis gave no indication of fragmentation (J. H. Jean, personal communication). It appeared that prolonged treatment with nitrous acid did not cause any detectable degradation of in situ SBMV-RNA.

The data on some of the properties of SBMV-RNA isolated from the dissociated virus and purified by gel chromatography and ethanol precipitation are presented in Table 1.
Fig. 2. Ultraviolet light absorption profiles of the coat protein isolated from untreated or nitrate-treated (96 h), A, and nitrous acid-treated (96 h), B, southern bean mosaic virus.

Table 2. Base composition of SBMV-RNA isolated from nitrous acid-treated SBMV and treated SBMV-RNA at pH 4.2

<table>
<thead>
<tr>
<th>Bases or nucleotides</th>
<th>Intact SBMV</th>
<th>Isolated SBMV-RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% deamination</td>
<td>% deamination</td>
</tr>
<tr>
<td>Adenine</td>
<td>26.1*</td>
<td>19.8</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>0.0</td>
<td>6.2</td>
</tr>
<tr>
<td>Guanine</td>
<td>24.0</td>
<td>19.4</td>
</tr>
<tr>
<td>Xanthine</td>
<td>0.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Cytidylic acid</td>
<td>22.5</td>
<td>18.4</td>
</tr>
<tr>
<td>Uridylic acid</td>
<td>27.4</td>
<td>31.5</td>
</tr>
</tbody>
</table>

* These values represent means of 12 to 15 analyses in four separate experiments.

Satisfactory yields of the virus RNA were obtained from the untreated, nitrate-treated (0 to 96 h) virus and from SBMV treated with nitrous acid for shorter periods (0 to 2 h). Only a slight reduction in the yield of SBMV-RNA was observed from virus exposed to nitrous acid for prolonged periods (24 to 96 h). The $E_{260}/E_{230}$ ratio of control RNA preparations as well as from zero-h nitrous acid treated SBMV was 2.1 to 2.4 and was similar to the SBMV-RNA isolated from untreated SBMV by SDS:phenol method (Diener, 1965). However, the $E_{260}/E_{230}$ ratios of RNAs isolated from 1 to 96 h nitrous acid-treated SBMV showed significant alterations. Furthermore, while the average amount of the detectable virus protein
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Fig. 3. Inactivation and photoreactivation of southern bean mosaic virus (○, ●) and its isolated RNA (△, ▲) following u.v. irradiation; test plants kept in light (●, ▲) or given a post-inoculation dark treatment for 24 h (○, △). Each point on the curves represents average value of 16 to 19 assays in four experiments.

in the RNA preparations from control samples and zero-h nitrous acid-treated SBMV varied from 5 to 6%, the protein contents of RNA isolated from 1 h, 2 h and 24 to 96 h treated particles were 6, 10 and 12 times greater than the controls.

The u.v. absorption spectrum of the coat protein isolated from the nitrous acid-treated SBMV showed a shift in its maximum extinction from 280 to 270 nm but retained its minimum at 250 nm (Fig. 2). A degree of denaturation of the nitrous acid-treated SBMV-protein was indicated by its relatively higher absorption at 250 nm in comparison to the control samples. The minor shoulder at 292 to 290 nm (due to the presence of tyrosine and tryptophan residues) was present in nitrous acid-treated as well as in nitrate-treated or untreated coat protein. No alterations occurred in the u.v. absorption spectrum of intact SBMV following treatment with nitrous acid; furthermore, in the virus the absorption shoulder at 292 to 290 nm was absent (Price, 1965; Sehgal & Soong, 1972).

The data on the deamination of the virus RNA in situ in comparison to the isolated SBMV-RNA after 0, 48 and 96 h treatments revealed nearly identical reactivities of the
amino-ribonucleotides with nitrous acid (Table 2). These results also indicated quantitative conversions of adenine, guanine and cytidylic acid upon deamination to, respectively, hypoxanthine, xanthine and uridylic acid.

U.v. irradiation of SBMV and SBMV-RNA

Intact SBMV and isolated SBMV-RNA were inactivated at the same rate upon exposure to u.v. (Fig. 3). Furthermore, the extent of photoreactivation \((k_{dark}/k_{light})\) calculated from the relationship, \(p = e^{-kt}\) (Bawden & Kleczkowski, 1955) of u.v.-irradiated SBMV or SBMV-RNA was the same; its value was 1.42 for SBMV or SBMV-RNA inactivated by u.v. irradiation between 80 and 10% level of survival. This value of the photoreactivation of SBMV and SBMV-RNA (1.42) was close to the value (1.35) reported for another isometric plant virus, tobacco necrosis virus, and its isolated RNA (Kassanis & Kleczkowski, 1965). Photoreactivation of u.v.-irradiated SBMV was previously reported by Price (1965).

No alterations occurred in the sedimentation behaviour, as well as in the u.v. absorption spectrum, of SBMV-RNA following u.v. irradiation (0 to 60 min). Similarly, no changes were observed in the sedimentation value, electrophoretic mobility, serological characteristics and u.v. absorption spectrum of irradiated (0 to 60 min) SBMV. After exposure to u.v. for 60 min the infectivity of SBMV-RNA or SBMV was reduced to <0.001%. From the u.v.-irradiated (0 to 60 min) SBMV, the virus RNA was easily released by the SDS:phenol procedure (Diener, 1965), unlike the situation experienced in case of nitrous acid-treated SBMV that was inactivated to a comparable level. The yield and quality of SBMV-RNA released from the irradiated virus were identical when prepared by the SDS:phenol method (Diener, 1965) or incubation in the dissociative medium followed by gel-chromatography and ethanol precipitation. Moreover, u.v. irradiation of SBMV did not cause any cross-linking of the virus protein to the RNA.

DISCUSSION

The macromolecular integrity of SBMV and SBMV-RNA, as ascertained by several analytical criteria, is fully retained during treatment with nitrous acid. Apparently, inactivation of the intact virus or its isolated RNA is not, to any significant degree, due to degradation of these entities. If inactivation of the virus RNA by nitrous acid is primarily due to lethal deaminations (Mundry & Gierer, 1958; Schuster & Wilhelm, 1963; Siegel, 1965), the fact that the intact SBMV is inactivated faster than SBMV-RNA suggests that inactivation of the virus is a somewhat more complex mechanism. Intact poliovirus is also inactivated faster than its RNA by nitrous acid, and a possible role of the virus protein in its inactivation process is implicated (Boey, 1959). Inactivation of intact TMV by nitrous acid, however, is 5 to 6 times slower than TMV-RNA due to protection (by the capsid) of some of the sites on the virus genome involved in lethal conversions (Schuster & Wilhelm, 1963; Siegel, 1965; Sehgal & Krause, 1968).

Our inability to release virus RNA efficiently from SBMV treated with nitrous acid by the SDS:phenol method (Diener, 1965), is similar to the observation reported by Goddard et al. (1966) that phenol released RNA from u.v.-irradiated TMV with difficulty. These investigators presented evidence of u.v.-induced cross-linking between virus RNA and coat protein subunits. In our studies, when it became possible to release RNA from the treated virus particles (by incubation in the dissociative medium), examination of its sedimentation characteristics, u.v. absorption spectrophotometry and colorimetric tests showed presence of a considerable amount of virus protein associated with the RNA. Although the mech-
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An inactivation mechanism contributing to the increased binding of protein to RNA by nitrous acid is not known, possibly, such treatment resulted in strengthening of some of the pre-existing bonds or introduction of new linkages between these two virus components. Nitrous acid-induced cross-linking between RNA and coat protein in viruses has not been reported, although Geiduschek (1961) observed that nitrous acid introduced covalent linkages between complementary chains of a DNA molecule. Besides lethal deaminations of in situ SBMV-RNA, another factor contributing to the inactivation of the virus may have been the inability of some treated particles to adequately uncoat on the leaf surface to initiate infection. Indirect evidence of the involvement of protein:RNA cross-linking in the inactivation process is indicated by u.v. experiments. Isolated SBMV-RNA and intact SBMV are inactivated at the same rate upon u.v. irradiation and no evidence of binding of the virus protein to RNA is indicated.

The identical reactivities of the amino-ribonucleotides of intraparticle versus isolated SBMV-RNA support our earlier finding that most of the amino groups of the virus RNA in situ are not linked with the coat protein. Furthermore, since nitrous acid reacts primarily with the free amino groups, it appears that such groups of the virus ribonucleotides are not preponderantly involved in hydrogen bonding between the complementary bases of SBMV-RNA within the virus. The results of the u.v. irradiation experiments also indicate that in SBMV, as in other isometric viruses (Kassanis & Kleczkowski, 1965; Furuse & Watanabe, 1971), intimate bonding between the virus genome and the capsid is lacking. The presence of some type of interaction between RNA and virus protein in 'native' SBMV especially in the region where these two moieties interpenetrate (Weinbraub & Ragetli, 1970), however, cannot be completely excluded. Nevertheless, if the RNA is linked with the coat protein in SBMV under 'native' conditions, such linkages are considerably less strong than those present in anisometric viruses, like TMV (Schuster & Wilhelm, 1963; Streeter & Gordon, 1968). Alternatively, in SBMV the preponderant interaction between SBMV-RNA and the coat protein may exist in the form of salt linkages between the ionized phosphates of RNA with the cationic centres of the capsid, as postulated for other isometric viruses (Kaper & Geelen, 1971; Tikochonenko, 1969). Or, the stability of SBMV may principally be due to strong protein:protein interactions resulting in the formation of a stable isometric shell which efficiently encapsidates the genome with a minimal degree of binding between RNA and the coat protein.

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