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Isolation and characterization of virus-specific double-stranded DNA from tissues infected by bean golden mosaic virus

(circular replication intermediate/geminivirus/whitefly-transmitted plant virus/endonucleases)

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ABSTRACT **A double-stranded (ds) DNA which may be a replication intermediate was isolated from bean (*Phaseolus vulgaris* L. "Top Crop") leaves systemically infected with bean golden mosaic virus, a whitefly-transmitted plant virus with a genome of circular single-stranded (ss)DNA. The isolation method used phenol/chloroform extraction, hydroxyapatite column chromatography, and rate-zonal centrifugation. The dsDNA had sequences complementary to those of viral DNA. The guanine-plus-cytosine content was 35%, and the sedimentation coefficient in alkaline sucrose density gradients was similar to that of viral ssDNA. Digestion of the dsDNA by *Hha* I endonuclease produced fragments that corresponded exactly in number and size with those produced by complete digestion of circular viral ssDNA by *Hha* I, when the fragments were denatured and analyzed on polyacrylamide gels. The dsDNA molecule was a circular structure with one discontinuity in one strand; hybridization results suggest that some of the dsDNA has a discontinuity in the viral strand and some has a discontinuity in the nonviral strand. On the basis of these structures for the dsDNA, a preliminary model for replication of viral DNA is discussed.**

Bean golden mosaic virus (BGMV) (1, 2) is one of a newly recognized group of plant viruses called geminiviruses (3, 4) which contain single-stranded (ss) DNA (2, 5, 6). BGMV virions are geminate particles measuring about 18 × 30 nm (7), and each particle contains a single molecule of circular ssDNA of M_r 800,000 (8, 9). The results of restriction endonuclease analysis and infectivity-dilution kinetics suggest that BGMV may contain a divided genome with genome components of the same physical size but differing in nucleotide sequence (10). In this paper, we describe the purification and some properties of a double-stranded (ds) DNA from BGMV-infected tissue that contains a DNA strand complementary to viral DNA and that may be a viral replication intermediate.

MATERIALS AND METHODS

Virus and Viral DNA Purification. An isolate of BGMV from Puerto Rico was maintained in and purified from bean (*Phaseolus vulgaris* L. "Top Crop") leaves as described (7). Viral DNA was labeled *in vivo* by immersing the petioles of trifoliolate leaves cut from plants 5–8 days after inoculation of primary leaves in a 150-μl drop of carrier-free $H_3{}^{32}PO_4$(8 mCi/ml; 1 Ci = 3.7×10^{10} becquerels) adjusted to pH 7 with 1M Tris (pH 9.5). After uptake of the isotope, leaves were maintained on a balanced inorganic salt solution (11) under humid conditions either for 24 hr for isolation of virus-specific dsDNA or for 4 days for preparation of viral DNA. Leaves labeled with ^{32}P were frozen at −80°C for at least 12–18 hr before being used for DNA purification. Labeled viral DNA was purified by alkaline sucrose density gradient and phenol/chloroform extraction or from partially purified virus preparations made as described (9, 10).

Isolation of Virus-Specific dsDNA. Frozen leaf tissue (labeled or unlabeled) was homogenized in 0.1 M Tris·HCl/0.1 M NaCl/0.01 M EDTA, pH 7.0, with 100 μg of proteinase K (Merck) per ml and 1% $NaDodSO_4$, and was incubated at room temperature for 10–15 min. Nucleic acids were extracted by using the phenol/chloroform method [^{N}phenol/$CHCl_3$, 8:2, (vol/vol)] (12). The aqueous phase, which was reextracted twice, was adjusted to 0.1 M in potassium acetate, and the nucleic acid was concentrated by ethanol precipitation and then fractionated on a Sephadex G-75 column (0.8 × 20 cm) equilibrated and eluted with 0.12 M sodium phosphate (pH 6.8). The nucleic acids in the void volume were collected and used for dsDNA purification by hydroxyapatite (HA) chromatography (13). The HA (Bio-Gel HTP, Bio-Rad) was equilibrated in a column (1.5 × 3 cm) with 0.12 M sodium phosphate (pH 6.8). The nucleic acid preparation was loaded and the column was washed with 0.12 M sodium phosphate (pH 6.8) until no absorbance at 260 nm and 280 nm was detected in the eluant. This was achieved after passing 100–150 ml of buffer for every 2–5 g of tissue. Elution of dsDNA was with 0.24 M sodium phosphate (pH 6.8); the fractions containing DNA were combined and dialyzed overnight against distilled water at 4°C. DNA was precipitated with ethanol and redissolved in 0.2 ml of 10 mM Tris·HCl/10 mM NaCl/1 mM EDTA/0.1% $NaDodSO_4$, pH 7.0. The DNA preparation was layered on a 12-ml linear 5–20% (wt/vol) sucrose gradient in 10 mM Tris·HCl/10 mM NaCl/1 mM EDTA/0.1% $NaDodSO_4$ (pH 7.0) and centrifuged at 22,000 rpm for 16.5 hr or 25,000 rpm for 17 hr in a Beckman SW 41 rotor. Gradient fractions were assayed by Cerenkov radiation, and the DNA in those containing ^{32}P-labeled DNA was concentrated by ethanol precipitation. In the case of unlabeled DNA, the fractions that corresponded in position to the ^{32}P-labeled DNA were pooled.

Analysis of Virus-Specific dsDNA. *Nuclease treatments.* The material to be tested (approximately 1000 cpm in 50 μl) was incubated with 0.5 μg of DNase I (Sigma), 0.2 μg of bovine pancreatic RNase A (Worthington), or 0.1–0.2 unit of nuclease S1 (Calbiochem). Treatments were at pH 5.0 in 10 mM sodium acetate/5 mM magnesium acetate (nuclease S1 treatments also included 0.5 mM $ZnCl_2$). Phosphodiesterase I (*Crotalus atrox* venom; P-L Biochemicals) was used for experiments at pH 8.0 in 67 mM Tris·HCl/0.67 mM $MgCl_2$/1 mM 2-mercaptoethanol. All nuclease treatments were at 37°C for 1 hr and were terminated by the addition of 1.0 ml of 10% (wt/vol) trichloroacetic acid; bovine serum albumin (75 μg) was added as a carrier. After 30 min at 0°C, the precipitates from the trichloroacetic acid so-

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Abbreviations: ds, double stranded; ss, single stranded; BGMV, bean golden mosaic virus; HA, hydroxyapatite; RF, replicative form.

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lution were collected on GF/A filters and washed four times with 5% trichloroacetic acid and twice with 5 ml of ether. The filters were then assayed by liquid scintillation spectrometry in a toluene-based scintillant.

Isopycnic density gradient centrifugation. The material isolated by neutral sucrose density gradient centrifugation was also analyzed by isopycnic centrifugation in CsCl. The starting density of the CsCl solution was 1.680 g/cm^3. The gradients were centrifuged for 40–65 hr at 40,000 rpm at 20°C in either a Beckman Spinco type 65Ti or the Beckman SW 50.1 rotor. Gradients were fractionated and counted by Cerenkov radiation. BGMV DNA was used as a buoyant density standard (1.717 g/cm^3) (2).

Hybridization analysis with ^{32}P-labeled BGMV DNA. The solution to be tested (40 μl) was heated at 100°C for 10 min in a microcentrifuge tube and then quickly cooled in a bath of dry ice/methanol. The denatured DNA was incubated in the presence of 1 μl of ^{32}P-labeled BGMV DNA (approximately 1000 cpm/μl) at 69°C for 25 hr. Unfractionated ^{32}P-labeled BGMV DNA, containing both circular and linear molecules, was used as the hybridization probe; restriction endonuclease analysis (14) showed that these topological forms contain the same nucleotide sequences. Hybridization was terminated by chilling the microcentrifuge tube on ice. To the solution was added 400 μl of 30 mM sodium acetate, pH 4.6/50 mM NaCl/1 mM $ZnSO_4$/5% (vol/vol) glycerol containing 40 μg of heat-denatured calf thymus DNA per ml (15). Two 200-μl samples were taken, and to one was added 0.2 unit of nuclease S1. Both samples were then incubated at 37°C for 30 min. Bovine serium albumin (75 μg) was added as carrier, and the mixtures were precipitated with trichloroacetic acid and assayed for radioactivity.

Alkaline sucrose density gradient centrifugation. The ethanol-precipitated DNA was suspended in 0.3 M NaOH/10 mM NaCl/1 mM EDTA, and the suspension was left at room temperature for 10 min. It was then layered on linear gradients of sucrose (5–20%) in 0.3 M NaOH/10 mM NaCl/1 mM EDTA and was centrifuged for 16.5 hr at 22,000 rpm in a Beckman SW 41 rotor. Gradients were fractionated and assayed by Cerenkov radiation.

Restriction endonuclease digestions. Endonucleases *Hae* III, *Bal* I, *Alu* I, and *Hha* I were purchased from Bethesda Research Laboratories (Rockville, MD). Digestions were carried out at 37°C for 16 hr, unless otherwise stated, in the buffers specified for each enzyme by the supplier.

After digestion, the reaction mixture was extracted with 2 vol of 1:1 phenol/chloroform. DNA was precipitated with 3 vol of ethanol, dissolved in 12 M urea/18% sucrose/0.06% xylene cyanol/0.06% bromphenol blue, heated at 90°C for 1 min, and analyzed by electrophoresis in a 7 M urea/4% (wt/vol) polyacrylamide gel.

Polyacrylamide gel electrophoresis under denaturing conditions. Ethanol-precipitated samples were suspended in 10 μl of 10 mM Tris·HCl/10 mM EDTA, pH 7.0, placed in a boiling water bath for 10 min, and quickly cooled in a dry ice/methanol bath. After addition of 60 μl of 90% (vol/vol) formamide, 30 μl of glycerol, and 2 μl of marker dye, each sample was loaded on 3.5% polyacrylamide gel containing 90% formamide and 20 mM sodium phosphate (pH 7.0) (16). The 0.7 × 8.0 cm cylindrical gels were run for 14 hr at 100 V or for 20 hr at 70 V. Gels were stained with 0.1% ethidium bromide in 50 mM Tris·HCl, pH 7.8/1 mM EDTA for 30 min.

In some experiments, circular and linear forms from virus-specific dsDNA were recovered from gel segments by electroelution into a dialysis bag (17). Electroelution was at constant voltage (100 V) for 24 hr; upper and lower buffer chambers held 50 mM Tris·borate, pH 8.3/1 mM EDTA/0.01% sodium lauroyl sarcosine. DNA fractions thus obtained were extracted by the phenol/chloroform method, precipitated with ethanol, and suspended in 0.18 M NaCl/10 mM Tris·HCl, pH 7.0/1 mM EDTA/0.05% $NaDodSO_4$ for hybridization analysis.

RESULTS

Purification of BGMV-Specific dsDNA. The dsDNA isolated by HA chromatography from unlabeled BGMV-infected tissues, after heat denaturation, proved to contain sequences that hybridized with ^{32}P-labeled BGMV ssDNA, whereas the heat-denatured dsDNA from uninfected tissues did not hybridize with ^{32}P-labeled viral DNA (data not shown).

When ^{32}P-labeled dsDNA purified from virus-infected tissues by HA chromatography was subjected to neutral sucrose density gradient analysis, one somewhat broad peak was obtained that sedimented slightly faster than the BGMV ssDNA marker; some radioactivity was also detected on the bottom of the tube (Fig. 1A). On the other hand, when HA-purified ^{32}P-

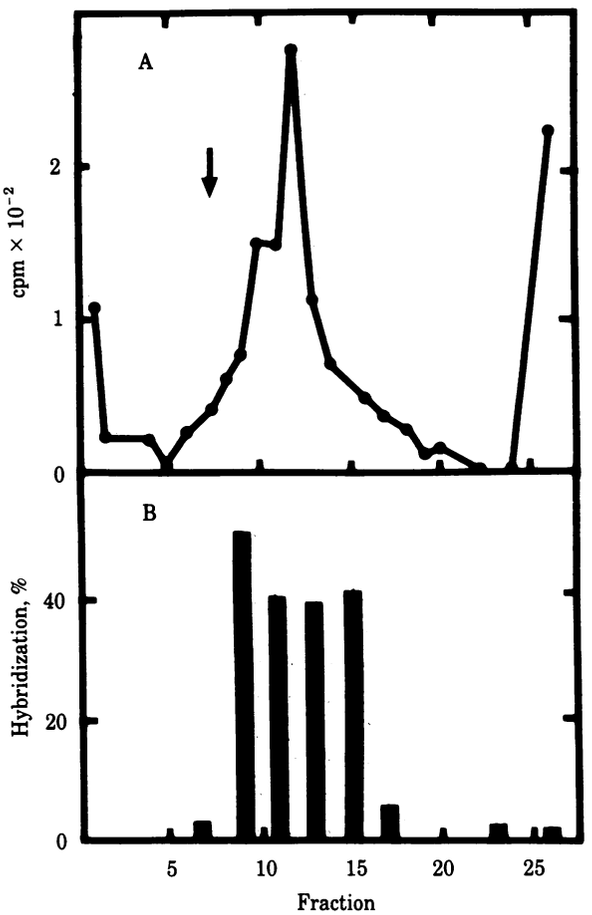

FIG. 1. (*A*) Sedimentation analysis on neutral sucrose density gradient of ^{32}P-labeled dsDNA isolated by HA chromotography from BGMV-infected tissue. The linear gradient (5–20%) was centrifuged at 22,000 rpm for 16.5 hr in an SW 41 rotor. Sedimentation was from left to right. To 10 μl from each fraction (0.5 ml), 75 μg of bovine serum albumin and 0.5 ml of 10% trichloroacetic acid were added. After 30 min at 0°C, the insoluble material was collected on filters and assayed by liquid scintillation spectrometry. Arrow, position of BGMV ssDNA in a parallel gradient. (*B*) Hybridization analysis of ^{32}P-labeled BGMV ssDNA with fractions of unlabeled dsDNA that have been centrifuged in a neutral sucrose density gradient identical to that described in *A*. From each fraction (0.5 ml), 80 μl was adjusted to 0.18 M NaCl, heat-denatured (100°C for 10 min), and incubated in the presence of ^{32}P-labeled BGMV ssDNA (868 cpm) for 24 hr at 69°C. Material resistant to nuclease S1 was collected on filters, and the radioactivity was determined. The percentage of hybrid formation was calculated from data for duplicate samples incubated either in the presence or absence of nuclease S1.

Table 1. Effect of nucleases on BGMV-specific dsDNA*

Treatment	Radioactivity,† cpm			
	1	2	3	4
None	1084	966	894	935
Phosphodiesterase I	ND	—	891	916
DNase I	—	116	—	—
Nuclease	865	1058	—	—
RNase A	997	738	—	—
Heated (100°C, 10 min), quick-cooled, then nuclease S1	72	91	—	—
Heated (100°C, 10 min), quick-cooled, then RNase A	980	801	—	—

ND, not determined.

* Nucleic acids in fraction 9–14 in Fig. 1 were pooled, precipitated with ethanol, suspended in an appropriate buffer, and tested as described.

† Radioactivity in trichloroacetic acid-precipitated material after nuclease treatment (four experiments).

labeled material from healthy leaves was subjected to the neutral sucrose density analysis identical to that described above, radioactivity was only detected on the bottom of the tube (data not shown).

The dsDNA isolation experiment was repeated with dsDNA from unlabeled infected tissues. DNA samples from each fraction after sucrose density gradient centrifugation were heat-denatured and incubated in the presence of ^{32}P-labeled BGMV DNA under hybridization conditions. Material resistant to nuclease S1 was collected on filters, and the radioactivity was determined. The results of such experiments (Fig. 1*B*) showed that the DNA molecules sedimenting in the middle of the gradient hybridized with labeled viral DNA. In contrast, ^{32}P-labeled viral DNA was not resistant to nuclease S1 when it was incubated under appropriate conditions with the faster sedimenting DNA. This larger DNA is probably host DNA. The results of these experiments suggested that the smaller dsDNA

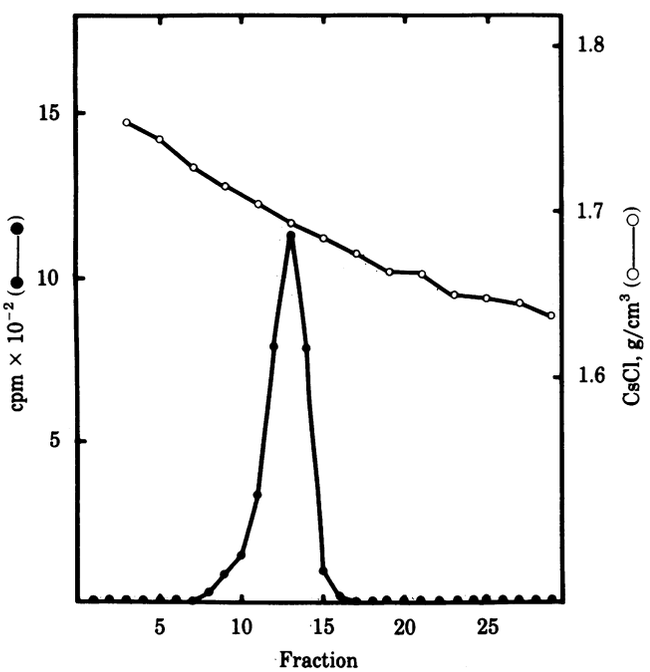

FIG. 2. Isopycnic centrifugation in CsCl of BGMV-specific dsDNA. The dsDNA was dissolved in 5 ml of 0.15 M NaCl/15 mM sodium citrate, pH 7.0, containing 4.6 g of CsCl. The final density of solutions was approximately 1.680 g/cm^3. Centrifugation was at 20°C for 45 hr at 40,000 rpm in a Beckman 65 Ti rotor.

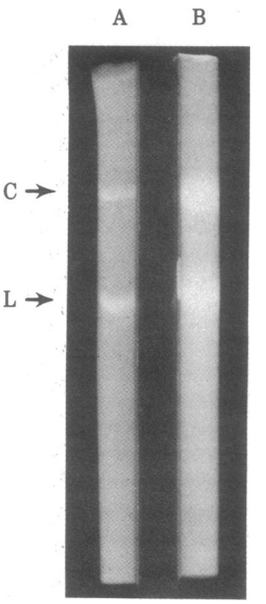

FIG. 3. Polyacrylamide gel electrophoresis in formamide of heat-denatured BGMV ssDNA (A) and BGMV dsDNA (B). Electrophoresis was for 18.5 hr at 70 V. Arrows, positions of circular (C) and linear (L) DNAs purified from BGMV particles.

molecules were virus-specific and contained BGMV DNA and its complementary strand. The yield of virus-specific dsDNA was from 1.0 to 1.5 μg per g of tissue in different experiments. The dsDNA concentration was estimated by UV spectrophotometry ($A^{0.1\%}_{260nm}$ = 20) The specific activity of such DNA was 35,000–40,000 cpm/μg.

Properties of BGMV-Specific dsDNA. The data indicated that BGMV-specific material has some properties of a double-stranded DNA molecule. This was confirmed by a series of additional tests. The nucleic acid was hydrolyzed by DNase I but was highly resistant to digestion by nuclease S1 and RNase (Table 1). After heat denaturation, it was almost completely digested by nuclease S1 but not by RNase. The possibility that the nucleic acid contains DNA·RNA hybrid molecules seems to be ruled out because such hybrids would not, after melting, be over 90% resistant to RNase. That BGMV dsDNA was resistant to hydrolysis by the 3′→5′ attack of exonuclease (Table

Table 2. Hybridization analysis of circular and linear ssDNA from virus-specific dsDNA with ^{32}P-labeled BGMV DNA

Hybridization component	Nuclease S1 resistance,* % of control	
	Exp. 1	Exp. 2
Circular ssDNA†	51	66
Linear ssDNA†	36	28
Nil	6	1

* Forty microliters of the solution containing circular ssDNA (8 μg), linear ssDNA (8 μg), or no ssDNA was incubated in the presence of 2 μl of ^{32}P-labeled BGMV ssDNA (6910 cpm in experiment 1 and 1262 cpm in experiment 2) at 67°C for 17 hr. Samples were divided into aliquots and one portion was treated with nuclease S1. Enzyme-resistant DNA was precipitated with trichloroacetic acid, collected on filters, and assayed for radioactivity. The percentage of hybrid formation was calculated from a comparison of data from duplicate samples incubated either in the presence or absence of nuclease S1.

† Circular and linear ssDNAs from virus-specific dsDNA were obtained by electrophoresis from polyacrylamide gels containing formamide.

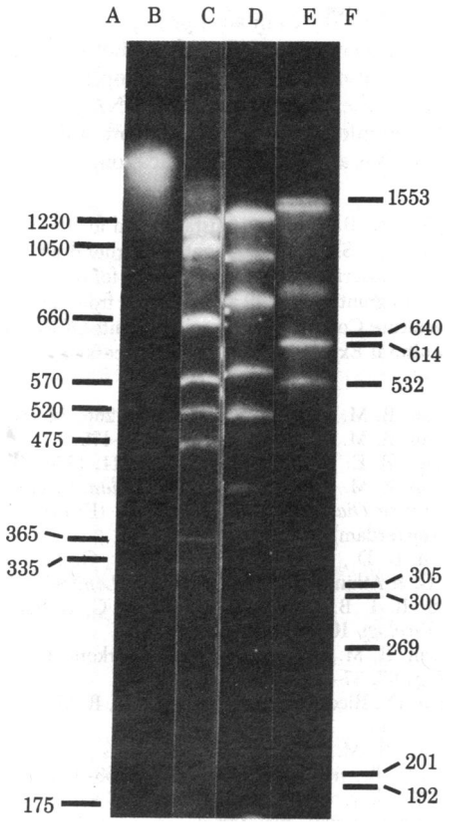

FIG. 4. Analysis of BGMV dsDNA by restriction endonucleases. Lanes: A, size markers were restriction fragments produced by cleavage of BGMV ssDNA with *Hha* I (size values from ref. 10); B, 2000 cpm of ^{32}P-labeled BGMV dsDNA was incubated with 0.1 units of *Bal* I for 1 hr at 37°C; C, 2000 cpm of ^{32}P-labeled BGMV ds DNA was incubated with 20 units of *Hha* I; D, 2000 cpm of ^{32}P-labeled BGMV dsDNA was incubated with 10 units of *Alu* I; E, 2000 cpm of ^{32}P-labeled BGMV dsDNA was incubated with 35 units of *Hae* III; F, size markers were restriction fragments produced by cleavage of ϕX174 RF with *Hha* I (size values from ref. 21). After incubation, the samples were extracted with phenol/chloroform; the ethanol-precipitated DNA was dissolved in 12 M urea/18% sucrose/0.06% xylene cyanol/0.06% bromophenol blue. Each sample was heated at 90°C for 1 min and examined on 4% acrylamide/7 M urea slab gels containing 50 mM Tris·borate (pH 8.3) and 1mM EDTA. Electrophoresis was at 200 V (origin at top of lanes) until the xylene cyanol dye reached the bottom of the gel. The gel was dried and exposed to x-ray film (Du Pont Cronex) at −80°C for 5 days against a Du Pont Cronex intensifying screen.

1) suggested that it was probably circular, although the results do not rule out the possibility of the 3′ end of a linear molecule being blocked (18).

The BGMV-specific dsDNA formed a single, somewhat diffuse band at equilibrium in CsCl (Fig. 2). Some equilibrium experiments were run for 45 hr and others for 65 hr; no differences in banding behavior or position were observed. The calculated buoyant density of the DNA was 1.694 g/cm^3, which corresponds to a guanine-plus-cytosine content of 35% for dsDNA (19). This guanine-plus-cytosine content was the same as that found in viral DNA (10). The possibility that the nucleic acid contains DNA·RNA hybrid molecules also seems to be ruled out because such hybrids would have a density higher than 1.694 g/cm^3 (20).

The size and structure of the ss components of the dsDNA were investigated by two methods. When the purified dsDNA treated with 0.3 M NaOH was subjected to alkaline sucrose density gradient centrifugation, only one peak was observed,

Table 3. Summary of restriction fragment sizes of BGMV dsDNA (Fig. 4) listed in order of increasing electrophoretic mobility

Treatment	Restriction sites	Fragment size,* bp	Fragment lengths,* bp
Bal I	0	—	—
Hha I	9	1350, 1030, 650, 520, 460, 420, 340, 320, 190	5280
Alu I	8	1300, 960, 710, 535, 465, 390, 355, 330	5045
Hae III	6	1480, 1380, 750, 585, 510, 290	4995

* Sizes based on interpolation with ϕX174 RF DNA *Hha* I fragments as standards (21); bp, base pairs.

and its position coincided with that of BGMV ssDNA purified from virus particles (data not shown). This would be the expected result if denaturation of BGMV dsDNA by treatment with NaOH led to the conversion of all molecules to the ss form with the same sedimentation coefficient as that of viral DNA. Moreover, when the dsDNA was heat-denatured and analyzed by gel electrophoresis after denaturation with formamide, two major species approximately equal in amount were observed. One of these comigrated with viral circular molecules, and the other coincided with viral linear molecules in the gels (Fig. 3). This result is consistent with a circular structure having one discontinuity in one of the strands. Further investigation will be required, however, to determine whether the discontinuity is random or at a specific site in the nucleotide sequence. Results of hybridization analysis with ^{32}P-labeled BGMV DNA (Table 2) showed that both circular and linear molecules hybridized with labeled viral DNA; viral DNA appears to hybridize with circular molecules more than with linear molecules. These results suggested that some of the dsDNA molecules are discontinuous in the viral DNA strand and others in the strand complementary to viral DNA.

Digestion of BGMV dsDNA with *Hha* I, *Alu* I, and *Hae* III yielded 9, 8, and 6 fragments respectively (Fig. 4, Table 3). Using the nucleotide sequence lengths of ϕX174 RF *Hha* I fragments as size standards (21, 22), we calculated the total length of the BGMV dsDNA fragments to be approximately 5280 nucleotide pairs for *Hha* I fragments, 4995 for *Hae* III fragments, and 5045 for *Alu* I fragments. These sizes were approximately twice what was expected (2510 nucleotide pairs) on the basis of the physical size of the molecule (8). These results extend and appear to confirm previous indications that BGMV may contain a divided genome (10). BGMV dsDNA was not digested by *Bal* I (Fig. 4, Table 3).

DISCUSSION

We conclude that the DNA molecules described here are specific to BGMV and presumably play some role in viral DNA replication. Several observations are consistent with this idea. (*i*) The DNA contains sequences complementary to those in BGMV viral DNA as demonstrated by results of hybridization analysis (Fig. 1). (*ii*) The guanine-plus-cytosine content of approximately 35% is the same as that found in viral DNA. (*iii*) It had a sedimentation coefficient similar to that of viral DNA when analyzed by alkaline sucrose density gradient centrifugation. (*iv*) It was absent in healthy leaves. (*v*) The fragments produced by *Hha* I digestion corresponded exactly in number and size with those produced by the complete digestion of cir-

cular viral ssDNA with *Hha* I when analyzed on denaturating polyacrylamide gels containing urea (Fig. 4).

This molecule, which we presume to be a replicative form (RF), is a dsDNA structure. Evidence for this conclusion comes from (*i*) elution properties of the DNA from HA, (*ii*) loss of resistance to digestion with nuclease S1 after heating to 100°C followed by rapid cooling (Table 1), and (*iii*) a buoyant density in CsCl much lower than would be expected for RNA or a RNA·DNA hybrid (Fig. 2).

We have not yet examined the dsDNA by electron microscopy, but the results of restriction analysis, the mobility in formamide gels, and the sedimentation coefficient of the DNA after denaturation with alkali clearly show that the size of the dsDNA is that expected of a double-stranded form of the viral DNA.

Denaturation and analysis of the dsDNA by gel electrophoresis produced evidence for a circular and linear molecule. This suggests that the RF molecule has a circular structure with one discontinuity in one of the strands. The circular structure of the RF molecule was indicated also by its resistance to exonuclease (Table 1). The size of the discontinuity in the RF is unknown, but it may be 150 nucleotides or less because circular and linear molecules from denatured RF comigrate with viral circular (2510 nucleotides) and linear (2360 nucleotides) molecules (8), respectively. Although the significance or location of the discontinuity is not yet known, it is unlikely that it is produced from covalently closed circular dsDNA during DNA purification. In control experiments (data not shown), the same techniques were used for isolation of RF from unlabeled BGMV-infected tissue mixed with ^{32}P-labeled RFI (covalently closed circular molecules) of the bacteriophage M13 prepared as described (23), and the results showed that the RFI DNA survived the purification process.

The isolation and partial characterization of a replication intermediate in BGMV DNA synthesis is the first step in understanding DNA replication of geminiviruses, the only circular ssDNA viruses known in eukaryotic organisms. We have looked for but did not find virus-specific dsDNAs that were larger or smaller than the viral DNA. Thus, these unit-size, relaxed circular molecules appear to be the intermediates that accumulate during virus synthesis. Their presence suggests a mechanism for DNA replication employing circular rather than linear templates. The other known eukaryotic viruses with ssDNA genomes possess linear viral DNA replicated through linear intermediates (24).

We postulate that upon infection, an early event, probably with a cellular RNA polymerase, results in partial or complete transcription of the viral DNA. Infectivity of phenol- or alkali-purified DNA (1, 2) implies that no virion proteins are involved in this process. An RNA transcript, or its translation product, is presumably involved in the priming of DNA replication.

The RF of ϕX174 is infectious (25, 26). In preliminary experiments, we have detected infection by using an indirect immunofluorescence assay 24 hr after protoplasts were inoculated with RF. This observation suggests that whether or not BGMV ssDNA, which is also infective in the protoplast system (10), is first converted to the ds form by cellular DNA polymerases, the RF isolated from infected cells is competent both for transcription of viral mRNA and for DNA replication.

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1. Goodman, R. M. (1977) *Nature (London)* **266**, 54–55.
2. Goodman, R. M. (1977) *Virology* **83**, 171–179.
3. Matthews, R. E. F. (1979) *Intervirology* **11**, 133–135.
4. Goodman, R. M. (1981) in *Handbook of Plant Viral Diseases and Comparative Diagnosis*, ed. Kurstak, E. (Elsevier/North Holland, Amsterdam), in press.
5. Harrison, B. D., Barker, H., Bock, K. R., Guthrie, E. J., Meredith, G. & Atkinson, M. (1977) *Nature (London)* **270**, 760–762.
6. Francki, R. I. B., Hatta, T., Boccardo, G. & Randles, J. W. (1980) *Virology* **101**, 233–241.
7. Goodman, R. M., Bird, J. & Thongmeearkom, P. (1977) *Phytopathology* **67**, 37–67.
8. Reisman, D., Ricciardi, R. P. & Goodman, R. M. (1979) *Virology* **97**, 388–395.
9. Goodman, R. M., Shock, T. L., Haber, S., Browning, K. S. & Bowers, G. R., Jr. (1980) *Virology* **106**, 168–172.
10. Haber, S., Ikegami, M., Bajet, N. B. & Goodman, R. M. (1981) *Nature(London)* **289**, 324–326.
11. Aoki, S. & Takebe, I. (1969) *Virology* **39**, 439–448,
12. Condit, C. & Fraenkel-Conrat, H. (1979) *Virology* **97**, 122–130.
13. Markov, G. G. & Ivanov, I. G. (1974) *Anal. Biochem.* **59**, 555–563.
14. Goodman, R. M. (1981) *J. Gen. Virol.*, in press.
15. Gould, A. R. & Symons, R. H. (1977) *Nucleic Acids Res.* **4**, 3787–3802.
16. Duesberg, P. H. & Vogt, P. K. (1973) *J. Virol.* **12**, 594–599.
17. Browning, K. S. (1980) Dissertation (Univ. Illinois, Urbana, IL).
18. Burton, A. & Sinsheimer, R. L. (1963) *Science* **142**, 962–963.
19. Schildkraut, C. L., Marmur, J. & Doty, P. (1962) *J. Mol. Biol.* **4**, 430–443.
20. Birnie, G. D. (1972) *FEBS Lett.* **27**, 19–22.
21. Sanger, F., Coulson, A. R., Friedman, T., Air, G. M., Barrell, B. G., Brown, N. L., Fiddes, J. C., Hutchinson, C. A., III, Slocombe, P. M. & Smith, M. (1978) *J. Mol. Biol.* **125**, 224–246.
22. Air, G. M., Sanger, F. & Coulson, A. R. (1976) *J. Mol. Biol.* **108**, 519–533.
23. Messing, J., Gronenborn, B., Müller-Hill, B. & Hofschneider, P. S. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3642–3646.
24. Ward, D. C. & Tattersall, P. (1978) *Replication of Mammalian Parvoviruses* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
25. Sinsheimer, R. L., Starman, B., Nagler, C. & Guthrie, S. (1962) *J. Mol. Biol.* **4**, 142–160.
26. Burton, A. & Sinsheimer, R. L. (1965) *J. Mol. Biol* **14**, 327–347.