A circular dichroism study of the structure of *Penicillium chrysogenum* mycovirus

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ABSTRACT

We have examined the absorption and circular dichroism spectra of intact *Penicillium chrysogenum* virus, empty capsid particles, and isolated double-stranded RNA. The absorbance at 260 nm of intact virus was less than 4% hypochromic relative to the absorbances of the free double-stranded RNA and free viral protein, indicating very little change in the base stacking interactions of the RNA. Circular dichroism studies of intact virus indicate that the capsid protein consists of 45% α -helix. Empty capsids, containing a protein of the same molecular weight as intact virus protein, were found to have 30% α -helix, suggesting a conformational change in the capsid upon assembly with RNA. The conformation of double-stranded RNA in the virus was slightly altered from the solution structure of the RNA in 0.01 M Na⁺ and resembled the conformation of double-stranded RNA partially bound with spermidine. However, the virus does not appear to contain polyamines. Electrophoretic experiments indicate a pH- and salt-titratable RNA binding site on the capsid protein in virus disrupted by urea or non-ionic detergents. The results are consistent with significant ionic interactions between the RNA and the capsid protein in the virus.

INTRODUCTION

The packaging of double-stranded (ds) DNA into Sd and T-even bacteriophage particles results in dramatic changes in the optical properties of DNA. First, the absorbance of intraphage DNA is 11% hyperchromic relative to free ds DNA.^{1,2} Second, the circular dichroism (CD) spectrum of intraphage DNA shows a decrease in the positive band at 280 nm, a change originally thought to be diagnostic of C-form DNA although X-ray diffraction data indicate that intraphage DNA is in the B conformation.^{3,4,5} Third, suspensions of bacteriophage scatter circularly polarized light of one sense to a greater extent than circularly polarized light of the other sense, complicating the interpretation of CD data.^{2,6,7} Tikchonenco *et al.*⁸ concluded from a study of the reaction of formaldehyde with Sd and T2 phages that these optical changes in ds DNA are due to the interaction of about 30% of intraphage DNA with viral protein.

In this report, we examine the optical properties of two ds RNA viruses isolated from different species of the fungus Penicillium. At present, there is almost no information on the structure of ds RNA in these viruses, and this is the first instance in which circular dichroism has been used to study the in vivo conformations of ds RNA and viral capsid protein.

The viruses used in this study, Penicillium chrysogenum virus (PcV) and Penicillium brevi-compactum virus (PbcV), are serologically similar, and their physical properties have been well characterized. They both possess segmented genomes, and each ds RNA segment is encapsidated in a separate particle by a protein shell of icosohedral symmetry.^{9,10,11,12,13}

MATERIALS AND METHODS

Penicillium chrysogenum was obtained from the American Type Culture Collection, Rockville, MD (ATTC #9480). A culture of Penicillium brevi-compactum⁹ was provided by Dr. R.F. Bozarth (Department of Life Sciences, Indiana State University, Terre Haute, Indiana). The fungi were grown at 22°C in a medium containing in one liter of water: 3.5 g corn steep liquor (Anheuser Busch), 22.5 g peptone, 2.5 g tryptone, 1 g yeast extract, 5 g glycerol, 5 g glucose, 5 g NaCl, and 1 g K₂HPO₄.

Virus was isolated from the fungi by a procedure similar to that used by Wood et al.⁹ After about 7 days of growth in one-liter flasks, each containing 200 ml of medium, the surfaces were covered by fungal mats. The mats were collected on cheesecloth and washed with 0.1 M K⁺ (phosphate buffer, pH 7.1). The cells were disrupted by grinding either with alumina in a mortar and pestle on ice or with 1 mm glass beads in a Braun homogenizer cooled with liquid CO₂. The lysate was then suspended in 0.1 M K⁺ (phosphate buffer, pH 7.1), clarified by a chloroform extraction, and subjected to two rounds of differential centrifugation to remove cellular debris. Virus from the supernatant of the last low-speed centrifugation was banded by equilibrium centrifugation in self-forming CsCl (Bethesda Research Laboratories, ultra pure) density gradients. The CsCl solutions were adjusted to an average density of 1.37 g/cm³ and were centrifuged in a Ti 50.2 rotor at 30,000 rpm for 48 h at 20°C. PbcV, which is easily disrupted by CsCl, was purified by isopycnic centrifugation in sucrose gradients. Aliquots of 2.0 ml were layered on preformed, linear 30-60% sucrose (E. Merck Laboratories, RNase-free) gradients and centrifuged at 22,500 rpm for 48 h in an SW 27 rotor at 4°C. All virus samples were dialyzed exhaustively against 0.01 M Na⁺ (phosphate buffer, pH 7.0). Dialysis of samples from CsCl

gradients was done through a series of decreasing NaCl concentrations to avoid osmotic shock.

Isolation of RNA and Protein

Double-stranded RNA was extracted from virus with buffer-saturated, redistilled phenol and was dialyzed against 0.01 M Na^+ (phosphate buffer, pH 7.0) to remove the phenol. The RNA was banded in self-forming Cs_2SO_4 (E. Merck Laboratories, suprapur) density gradients, initially at a density of 1.65 g/cm^3 , by centrifuging in a Ti 50.2 rotor at 30,000 rpm for 60 h at 20°C . The purified RNA was dialyzed twice against 100 volumes of 0.01 M di-Na EDTA, 0.01 M Na^+ (phosphate buffer, pH 7.0); then twice against 0.01 M NaCl, 0.01 M Na^+ (phosphate buffer, pH 7.0); and then four times against 0.01 M Na^+ (phosphate buffer, pH 7.0). The extinction coefficients for both PcV RNA and PbcV RNA were taken to be the same as that reported for PcV RNA, $7200 \text{ liter} \cdot \text{mole}^{-1} \cdot \text{cm}^{-1}$ at 258 nm.¹²

Denatured viral protein was isolated by incubating a PcV suspension in 0.4 M NaOH at 37°C for 18 h and dialyzing against 0.01 M Na^+ (phosphate buffer, pH 7.0) to remove the ribonucleotides.

Polyamine Analysis

Polyamines were assayed by the fluorimetric procedure of Seiler and Wiechmann¹⁴ as described by Dion and Herbst.¹⁵ Virus suspensions (0.5 ml) were incubated with 3% (v/v) perchloric acid at 4°C for 30 min. The precipitated proteins and nucleic acids were removed by centrifugation for 5 min in an Eppendorf Model 5412 centrifuge. The supernatant was adjusted to a pH of 8.5 with Na_2CO_3 and incubated in the dark for 18 h with an equal volume of an acetone solution of 1-dimethylamino-naphthalene-5-sulfonyl chloride (30 mg dansyl chloride per ml). Excess dansyl chloride was then converted to dansyl proline by an additional incubation of 30 min with 0.1 ml proline (100 mg/ml). The dansylated amines were extracted from the reaction mixture by shaking with 0.5 ml benzene, and aliquots of the benzene phase were spotted on plastic Silica G-250 chromatographic plates (Eastman). The standards, putrescine, cadaverine, spermidine, and spermine (purchased as the free base from Sigma), were dansylated and chromatographed at concentrations comparable to those found in the samples to be analyzed. The quantities of the polyamine standards were accepted as labeled by the manufacturer and were consistent with each other in the expected concentrations of the amine groups available for dansylation. The solvents used for chromatography were (a) ethyl acetate: cyclohexane (2:3), and (b) benzene:triethylamine (5:1) (all solvents were Eastman chromatographic grade). After chromatography, the plates were

sprayed with 10 ml of isopropanol:triethanolamine (4:1), and the amines were recognized by their yellow fluorescence when exposed to UV light of 350 nm. For quantitation, the plates were photographed with a Polaroid camera, and the negatives were scanned with a Joyce-Loebl densitometer. In a control experiment, standards were added to a virus sample prior to polyamine extraction and dansylation. The amount of polyamine recovered was greater than 85% for each of the standards. To test the possibility that intraviral polyamines were coprecipitating with protein or RNA, aliquots of a virus preparation were incubated either with 0.4 M NaOH at 37°C for 18 h or with 6 M HCl at 150°C for 18 h prior to extraction with 3% (v/v) perchloric acid. These treatments, however, did not increase the amounts of polyamines detected in the virus samples.

RNA and Protein Determinations

Virus samples were incubated in 0.4 M NaOH at 37°C for 18 h. An equal volume of 6% (v/v) perchloric acid was added, and the samples were chilled on ice for 10 min and then filtered through 0.45 μ m Millipore filters (HAWP 02500). The RNA concentration was determined from the absorbance at 260 nm of the filtrate. The extinction coefficient of the ribonucleotides in acid, determined by alkaline hydrolysis of the isolated RNA, was 64% higher than the extinction coefficient of native RNA at 260 nm.

Protein concentrations were measured by the Lowry assay on aliquots of the alkali-treated virus using bovine serum albumin (Sigma) as the standard.¹⁶

Spectroscopic Methods

Absorption spectra were recorded on either a Cary Model 14 or a Cary-Varian Model 118C UV-Vis absorption spectrometer. Virus samples exhibited scattering and typically had an $A_{320}:A_{260}$ absorbance ratio of 0.07. Virus absorption spectra were corrected for light scattering by extrapolating a linear regression of a log-log plot of optical density versus wavelength from 350 nm to 314 nm, as described by Englander and Epstein.¹⁷

CD measurements were obtained on a Cary Model 61 circular dichrometer calibrated with d-10-camphorsulfonic acid (Aldrich Chemical Co.) to give a value within 2% of 0.336° ellipticity at 290.5 nm for a 0.1% solution at 20°C, as recommended by Chen and Yang.¹⁸ The ratio of ellipticity at 192.5 nm to that at 290.5 nm for d-10-camphorsulfonic acid was 2.08 ± 0.05 . The sample temperature, controlled thermoelectrically, was $20 \pm 0.5^\circ\text{C}$. Where applicable, the spectra were computer-smoothed with a sliding 13-point convoluting quadratic (or cubic) function as described by Savitsky and Golay.¹⁹

CD spectra of PcV and PbcV were fit from 300 nm to 200 nm at 2 nm intervals with a set of reference spectra to determine the percentages of α -helix, β -sheet, β -turns, and random-coil protein structures, and the amount of RNA in the viruses. The RNA reference CD spectra are from this work, and the protein reference CD spectra (calculated from the CD spectra of 15 proteins of known secondary structure by Chang *et al.*²⁰) were provided by Dr. J.T. Yang and his collaborators (Cardiovascular Research Institute and Department of Biochemistry and Biophysics, University of California, San Francisco).

The fitting program used for the CD analyses simultaneously minimized at 51 wavelengths the deviations between the unknown spectrum and summed fractions of the library reference spectra. This computer program was written by Dr. F.S. Allen (Department of Chemistry, University of New Mexico, Albuquerque) and is based on the same linear programming techniques previously used in our work.²¹

The analysis of the average PcV CD spectrum in the long-wavelength region (305 nm to 255 nm) was performed at 1-nm intervals using only the RNA reference spectra. To quantitate differences in spectral shape alone, standard deviations s_{RNA} and s_{PcV} of the RNA and virus spectra, respectively, were calculated by the equation

$$s = \left[\sum_{i=1}^n (\theta_i/f_i - \theta_{\text{Ave}})^2 / (n-1) \right]^{1/2}.$$

The normalization factor f_i was determined by fitting the unsmoothed *i*th spectrum with the corresponding average spectrum at each wavelength from 305 nm to 255 nm. The total standard deviation (s_{total}) is then

$$s_{\text{(total)}} = [s_{\text{RNA}}^2 + s_{\text{PcV}}^2]^{1/2}.$$

Electrophoresis

Proteins were analyzed on SDS-polyacrylamide (Eastman) slab gels (7% w/v acrylamide) according to the procedure of Laemmli.²² The gels were stained with 2.5 mg/ml Coomassie Blue R-250 dissolved in methanol:acetic acid:water (5:1:5) and destained in methanol:acetic acid:water (3.3:1:9). The proteins (Sigma) used for molecular weight markers were ovalbumin (43,000), bovine serum albumin (68,000), phosphorylase b (94,000), and β -galactosidase (116,000) (molecular weights given in parentheses are from Weber and Osborn,²³ except for galactosidase which is from Fowler and Zabin²⁴). The estimated error in determining the molecular weight of viral protein relative to these standards was 5%.

Isoelectric focusing was performed in 4% polyacrylamide tube gels con-

taining 2% Nonidet P-40, 9.3 M urea, and 2% ampholytes (Biorad, pH 3-10), as recommended by Biorad. The cathode solution was 0.4% triethanolamine (degassed), and the anode solution was 0.2% H_2SO_4 . PcV was disrupted for isoelectric focusing by incubating in 4% Nonidet P-40, 9.5 M urea, and 5% β -mercaptoethanol. After electrophoresis for 18 h at 400 volts, the gels were stained with Coomassie Blue as described above. To determine the pH gradient, slices (0.5 cm) of parallel gels containing no sample were incubated with 1.0 ml 0.1 M KCl for 1 h, and the pH was measured by a pH electrode. The pH of the gels ranged from 4 to 8.

Double-stranded RNA was separated by electrophoresis in 0.7% (w/v) agarose (Seakem ME) slab gels at 2.0 volts per cm for 18 h. Tube gels were electrophoresed for the times indicated. Electrophoresis buffer was 0.001 M di-Na EDTA, 0.05 M K^+ (phosphate buffer, pH 7.1). After electrophoresis, the gels were stained with ethidium bromide and were photographed under UV illumination with a Polaroid camera. The three double-stranded RNA segments from the bacteriophage Phi 6 with molecular weights of 2.01, 2.76, and 4.36×10^6 (personal communication, Dr. D. Lang and H.T. Steely, Programs in Biology, The University of Texas at Dallas) were used as molecular weight standards. RNA-spermidine complexes were prepared for electrophoresis by incubating PcV RNA with HCl-titrated spermidine (pH 6) for 15 min. Virus samples were disrupted by incubating PcV for 15 min with solutions of urea (Baker) that had been deionized on a mixed-bed ion-exchange column (Biorad AG 501-X8[D]).

RESULTS

Equilibrium Density Separation of Virus Components

Preparations of Penicillium chrysogenum virus (PcV) contain four components with densities of 1.39, 1.36, 1.30, and 1.27 g/cm^3 ($\pm 0.01 \text{ g}/\text{cm}^3$) in isopycnic CsCl gradients. The component which banded at 1.27 g/cm^3 lacked RNA, as indicated by the absence of nucleic acid absorption and CD bands. However, as observed by electron microscopy, this component contained virus-like particles morphologically similar to PcV except that they were all penetratable by phosphotungstic acid negative stain. Thus, this component appears to contain empty capsids.

No major differences in composition or optical properties were detected among the other three more dense components; each contained all four RNA segments (see below) and the same molecular weight capsid protein. Since each component maintained its original density when recentrifuged in CsCl, contrary to the data of Wood and Bozarth,¹⁰ the components probably reflect

small structural differences in the virus particles. The results on the virus to be described below were obtained with the predominate component (1.36 g/cm^3) after purification to homogeneity in CsCl. Only one RNA-containing component, with a density of 1.19 g/cm^3 , was observed upon isopycnic sucrose gradient centrifuge of virus preparations.

The serologically related virus from Penicillium brevis-compactum, PbcV,¹⁰ possessed only one component in CsCl and sucrose density gradients. The density of PbcV was 1.40 g/cm^3 in CsCl and 1.20 g/cm^3 in sucrose.

Protein and RNA Composition of Viruses

More than 98% of the PcV protein appeared in one band upon SDS-polyacrylamide gel electrophoresis (PAGE), and only one band with a pI of 6.3 was detected by isoelectric focusing of disrupted PcV. The molecular weights of PcV and PbcV capsid protein were estimated by SDS-PAGE to be 125,000. PcV empty capsid protein had the same molecular weight in SDS-PAGE as intact viral protein, and these empty capsids may therefore be identical to one of the PcV empty capsid particles reported by Buck and Girvan.¹³

Both PcV and PbcV consist of $18 \pm 2\%$ (w/w) double-stranded RNA, as determined by UV absorption measurements and the Lowry assay (see Materials and Methods). The CD analysis (see below) yielded a value of $19 \pm 4\%$ (w/w) RNA for PcV and $16 \pm 2\%$ (w/w) RNA for PbcV. The thermal denaturation profile of PcV RNA was similar to that reported by Burnett *et al.*²⁵ with a melting temperature (T_m) of 78°C in 0.01 M Na^+ . PbcV RNA had a T_m of 84°C in 0.01 M Na^+ , and both RNA species exhibited a premelting region extending to 20°C below the T_m .

Double-stranded RNA from PcV was determined by agarose gel electrophoresis to have molecular weights of 2.37, 2.17, 2.10 and 1.94×10^6 . The same distribution of RNA molecular weights was found in each of the three RNA-containing density components of PcV from CsCl gradients. Double-stranded RNA from PbcV had only three electrophoretic species with molecular weights of 2.37, 2.17, and 2.10×10^6 . The molecular weights of PcV and PbcV RNAs are estimated to be accurate to within $\pm 5\%$.

Electrophoretic properties of RNA released from PcV

Incubation of PcV with 6M urea, or with Triton X-100 or Brij 35 in the presence of β -mercaptoethanol, released ds RNA with an apparent retarded electrophoretic mobility relative to that of purified ds RNA (Figure 1, b & e). Denatured capsid protein remained at the top of the gel, as demonstrated by staining with Coomassie Blue. The mobility of viral-released RNA could be restored to that of purified ds RNA by the addition of 0.1 M NaCl to the

Figure 1. Agarose gel electrophoresis of PcV and PcV RNA. (a) PcV; (b) PcV plus 6 M urea; (c) PcV in 0.1 M NaCl; (d) PcV in 0.1 M NaCl plus 6 M urea; (e) PcV RNA; (f) PcV RNA plus spermidine at N:P of 200:1.

incubation mixture (Fig. 1,d). As shown in Fig. 1,f, spermidine can cause a similar reduction in the apparent mobility of purified RNA at a nitrogen-to-phosphate (N:P) ratio of 200:1. At lower spermidine concentrations, down to a 50:1 N:P ratio, the RNA was distributed between the freely migrating RNA and the retarded species with very little RNA of an intermediate mobility. The RNA-spermidine interaction was also salt-dependent (data not shown). We therefore investigated PcV and PbcV for the presence of polyamines, but less than one molecule per virus particle was detected (Table 1).

As demonstrated in Figure 2, the electrophoretic velocity (i.e., distance migrated per unit time) of the RNA-spermidine complex and of the viral-released RNA is the same as that of native RNA, but the entry of the RNA into the gel has been retarded by 7 h relative to isolated ds RNA. A possible explanation of the delayed entry is that the RNA is trapped at the top of the

Table 1.
Molecules of polyamines per
Penicillium chrysogenum virus particle

Stage of purification ^a	Putrescine	Spermidine	Spermine
1	10	4	< 0.01
2	1	0.5	< 0.01
3	0.5	0.2	< 0.01
4	0.5	0.2	< 0.01

^aStages of purification: 1 and 2 are first and second high speed centrifugations, respectively; 3 and 4 are density gradient centrifugations with sucrose and CsCl, respectively.

gel in an insoluble complex with the spermidine or, in the case of viral-released RNA, with the capsid protein. This was suggested by the ability of spermidine at high concentrations to precipitate ds RNA, as evidenced by an increase in light scattering. Because of their inherent ability to self-associate, viral capsid proteins could also serve to non-covalently crosslink nucleic acids if the protein possessed nucleic acid binding properties.

The delay of entry of RNA into the gel exhibits characteristics that are

Figure 2. Migration of second largest PcV RNA segment in agarose tube-gel electrophoresis: isolated RNA (●); isolated RNA plus spermidine at N:P of 200:1 (Δ); RNA from PcV disrupted with 6 M urea (○). The pH of the cathode buffer is also shown (x).

typical of ionic interactions since the electrophoretic effect is both salt- and pH-dependent. As shown in Figure 2, the RNA is released from the complex with viral protein, or the complex with spermidine, and enters the gel when the pH of the cathode buffer reaches the pK of the amine group (10.8). In a separate experiment, recirculation of the electrolyte buffer to maintain a constant pH at the cathode completely restrained the RNA from entering the gel. The results suggest that amine groups of the viral capsid protein could provide charge neutralization of the RNA in the virus. Support for this notion was obtained from a study of the CD spectrum of the intact virus (see below).

Absorption Spectra

The $A_{260}:A_{280}$ absorbance ratios were 1.54 for PcV and PbcV, and 2.16 for the RNAs. The absorption spectrum of alkali-denatured PcV protein (shown in Figure 3) matched a calculated spectrum (not shown) based on the molar absorption spectra of the individual amino acids²⁶ and the amino acid composition reported by Buck and Girvan.¹³ The absorption spectra of PcV (corrected for scattering) and PcV RNA are also shown in Figure 3. The absorption spectrum of the intact virus can be approximated by the sum of the absorbances of

Figure 3. Absorption spectra of: intact PcV at 1 mg/ml (—); PcV RNA at 0.17 mg/ml (----); denatured PcV protein at 0.83 mg/ml (- -). Sum of component RNA and protein spectra at 17% (w/w) RNA (x). All samples were in 0.01 M Na⁺ (phosphate buffer, pH 7.0). Extinction coefficient and % RNA of virus were determined from the CD analysis. Similar results were obtained for a second virus preparation containing 19% (w/w) RNA.

Figure 4. Molar CD spectra of: PcV RNA (average of four preparations) (x); PcV RNA plus spermidine at a N:P of 1:1 (average of two experiments) (•). Error bars are $\pm$ one standard deviation.

denatured protein and native RNA, showing that there is less than a 4% change in the absorbance at 260 nm of the viral components upon assembly of PcV.

CD Spectra

Figure 4 shows the average molar CD spectrum of four PcV RNA preparations. The error bars indicate $\pm$ one standard deviation and include the error in determining the RNA concentration from the absorption spectrum. The CD spectrum of PbcV RNA was very similar to that of PcV RNA.

Figure 5. Measured CD spectrum (—) of intact PcV (average of 8 preparations); calculated spectrum (x) from fit with ref. set 1 (RNA in 0.01 M Na^+ and protein reference spectra); calculated spectrum (o) from fit with ref. set 2 (RNA in 0.01 M Na^+ , RNA bound with spermidine, and protein reference spectra). Error bars are $\pm$ one standard deviation of the PcV spectra, normalized at 260 nm.

The solid line in Figure 5 is the average CD spectrum of PcV from eight different viral preparations. The error bars represent the standard deviations from the average spectrum, after normalizing the spectra at 260 nm. The viral spectrum is approximately the sum of the CD of the RNA and protein components and does not exhibit any differential scattering of circularly polarized light as is found for the bacteriophage T2.² This permits an evalu-

ation of the RNA and protein conformations in the virus.

The x's in Figure 5 denote the calculated virus CD spectrum based on the native RNA spectrum shown in Figure 4 and the empirical protein reference spectra of Chang *et al.*²⁰ The amount of RNA calculated for PcV from the CD spectrum is $19 \pm 4\%$ (w/w). The calculated values for the protein conformations are 45% α -helix, 14% β -sheet, 16% β -turns and 25% random coil (see Table 2).

An examination of the long-wavelength region of the CD spectrum of PcV reveals a small but significant deviation from the CD spectrum of native ds RNA in 0.01 M Na^+ (see Figures 4 and 5). For example, the crossover point for the RNA is at 289.3 ± 0.2 nm (4 preparations) while the CD spectrum of the virus has a crossover at 291.1 ± 0.7 nm (8 preparations). Similar spectral changes for PcV RNA have been found in solutions of high salt concentrations,²⁵ in ethanolic solutions (personal communication, H.T. Steely, Programs in Biology, The University of Texas at Dallas), and in the presence of spermidine.

Figure 4 shows the effect of spermidine on the CD spectrum of PcV RNA at an equimolar nitrogen-to-phosphate ratio. There was an increase in the magnitude of the negative band at 210 nm and of the positive band above 260 nm. The presence of spermidine also caused a shift in the positive peak from 261 to 263 nm and in the long-wavelength crossover point from 289 to 292 nm. No further change in the CD spectrum of the RNA was observed until

Table 2.
Results of CD analyses^a

Reference Spectrum	PcV ^b	PbcV ^c	Capsid ^d	Protein ^e
RNA	19 ± 4	16 ± 2	0	0
α -helix	45 ± 7	46 ± 11	30 ± 1	6
β -sheet	14 ± 6	16 ± 8	23 ± 11	22
β -turns	16 ± 5	17 ± 2	17 ± 5	28
random coil	25 ± 5	21 ± 6	30 ± 6	45

^a% RNA in virus, or % peptide in specified conformation, $\pm$ one standard deviation.

^baverage of 8 PcV preparations

^caverage of 4 PbcV preparations

^daverage of 2 PcV empty capsid preparations

^eone preparation of denatured PcV protein

the N:P ratio exceeded 100:1. At these higher spermidine concentrations, the RNA precipitates out of solution and the CD spectrum simply decreases in magnitude.

Inclusion of the RNA-spermidine spectrum from Figure 4 in the reference set used for the viral CD analysis improved the spectral fit in the long wavelength region (see Figure 5) but did not significantly alter the calculated values for the RNA content or the protein conformations. As seen in Figure 6, the residuals of the PcV spectrum fitted with only the native RNA spectrum from 305 to 255 nm exceeded the standard deviation (s_{total}) of the measurements. The residuals could be reduced to a low frequency noise within the total standard deviation of the measured spectra by the addition of the RNA-spermidine spectrum to the reference set. The amount of RNA calculated to be in a conformation similar to that induced by the binding of spermidine to native RNA was 50% of the viral RNA when the CD analysis of PcV was performed over the entire wavelength range (300-200 nm) and 33% when the analysis was restricted to the long-wavelength region (305-255 nm). The CD spectrum of PcV was also analyzed using a reference set that included spectra of RNA perturbed in ethanol and NaCl (spectra provided by H.T. Steely). These RNA spectra, however, did not provide as good a fit to the observed virus spectrum as did the spectrum of RNA perturbed by spermidine.

In Figure 7, the CD spectrum of PcV empty capsids is compared with the

Figure 6. Residuals (measured minus calculated CD values) of the average CD spectrum of PcV fit from 305 nm to 255 nm with: the spectrum of RNA in 0.01 M Na⁺ (●); the spectra of RNA in 0.01 M Na⁺ and RNA bound with spermidine (x). Stippled area represents $\pm$ one standard deviation (see Materials and Methods). RNA reference spectra are shown in Figure 4.

Figure 7. CD spectra of PcV capsid ($\Delta\epsilon_{\text{PcV}} - \Delta\epsilon_{\text{RNA}}$) (—); PcV empty capsid (average of 2 preparations) (----); denatured PcV protein (— · —). Peptide concentrations were determined from CD analyses.

calculated CD spectrum of intact virus capsids ($\Delta\epsilon_{\text{PcV}} - \Delta\epsilon_{\text{RNA}}$), calculated by subtracting the spectrum of the isolated RNA from the spectrum of the intact virus, both in 0.01 M Na^+ (phosphate buffer, pH 7.0). The α -helical content of the empty capsids was estimated to be 30% (Table 2). The extinction coefficient at 280 nm of empty capsid protein determined by CD was $165 \text{ liter} \cdot \text{mole}^{-1} \cdot \text{cm}^{-1}$, close to the value calculated for PcV capsid protein from the amino acid composition,^{13,26} and the absorption spectrum of PcV empty capsids was identical in shape to that of denatured PcV protein (shown in Figure 3). Figure 7 also shows the CD spectrum of denatured PcV protein, which has almost no α -helical structure (Table 2). The extinction coefficient at 280 nm of denatured viral protein determined by CD was $129 \text{ liter} \cdot \text{mole}^{-1} \cdot \text{cm}^{-1}$, 17% lower than that calculated from its amino acid composition. Neither denatured PcV protein nor the empty capsids possessed CD bands above 250 nm (detection limits for denatured protein and empty capsids were 1.5 and 0.5%, respectively, of the ellipticity at 220 nm).

The CD spectrum of the ds RNA virus from *P. brevis-compactum*, PbcV, was fitted with the protein reference spectra of Chang *et al.*²⁰ and a PbcV RNA spectrum. As shown in Table 2, an analysis of the optical properties of PbcV gave very similar results to those of PcV.

DISCUSSION

The optical properties of the double-stranded RNA viruses PcV and PbcV can be ascribed to the secondary structures of their protein and RNA constituents. The absorbance of intact PcV at 260 nm is less than 4% hypochromic relative to the absorbances of isolated RNA and viral protein indicating very little change in the base stacking interactions of the RNA upon assembly of PcV. In contrast, the ds DNA of the T-even and Sd bacteriophages is 11% hyperchromic relative to free DNA.^{1,2}

PcV and PbcV do not differentially scatter left and right circularly polarized light as do the elongated T bacteriophages and the isometric Sd bacteriophage.^{3,8} Since "ghosts" of T2 do not exhibit differential scattering, this phenomenon is probably due to the condensation of DNA in the phage head.² Maestre and Reich²⁷ have shown that tightly packed helical arrays of nucleic acid can produce anomalous reflection bands in the CD spectrum at absorbing wavelengths and can differentially scatter circularly polarized light at long wavelengths. However, tertiary structure need not be invoked to explain the CD spectra of PcV and PbcV; rather, the CD spectra of these viruses can be attributed to the secondary structures of RNA and protein. Thus, the arrangement of ds RNA in these viruses must be considerably different from the ordering of ds DNA in Sd and the T bacteriophages.

An analysis of the CD spectrum of PcV indicates that the virus consists of 19% RNA (w/w), in good agreement with the value of 18% determined by biochemical analyses and with the value of 18.8% reported for PcV by Buck and Girvan (1977). The capsid protein of PcV was estimated by CD analysis to contain approximately 45% α -helix, and PcV empty capsids had less α -helix (30%) than intact PcV, suggesting a change in the conformation of the capsid upon packaging of ds RNA.

A statistically significant deviation was observed between the CD spectra of PcV and of isolated RNA in the wavelength region above 250 nm. Although denatured PcV capsid protein and empty capsid protein did not possess detectable CD bands above 250 nm, tryptophan residues could contribute to the optical activity of the intact virus in this wavelength region.²⁸ However, we believe that the change in the virus CD spectrum above 250 nm reflects a perturbation of the intraviral RNA from the solution structure in 0.01 M Na⁺. This was suggested by similarities in the electrophoretic properties of viral-released RNA and of isolated RNA bound with spermidine and by similarities in the long-wavelength CD spectra of PcV and of isolated RNA bound with spermidine.

Since PcV does not contain appreciable amounts of polyamines, some other aspect of the intravirus environment, such as RNA-protein interaction or decreased solvation of intraviral RNA, must be responsible for the conformational change. We propose that basic amino acids of the capsid protein interact with viral RNA and that this interaction is responsible for the alterations in the CD spectra of the viral capsid and ds RNA. This is supported by electrophoretic experiments which demonstrate a pH- and salt-dependent RNA-binding site on PcV capsid. Since PcV capsid protein is an acidic protein and has a net negative charge at neutral pH, the interaction between the RNA and the capsid is not merely of a non-specific electrostatic origin; rather, the basic amino acids of the capsid protein that interact with the RNA must form a specific RNA-binding site.

To determine the fraction of intraviral RNA that could be in contact with the capsid protein, we have calculated the internal radius of PcV, assuming that the virus is a sphere of 36 nm diameter¹³ and consists of 82% capsid protein with a partial specific volume of 0.73 cm³/g. We calculate that all of the RNA could be accommodated in a single spherical layer covering the inner capsid wall. Therefore, RNA-capsid interactions in the virus are possible in numbers consistent with our proposed explanation for the observed CD and electrophoretic results.

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