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Protein-RNA Interactions in Belladonna Mottle Virus Investigated by Laser Raman Spectroscopy†

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ABSTRACT: Raman difference spectroscopy of the belladonna mottle virus (BDMV) and its separated RNA and protein components indicates that molecular interaction occurs between the single-stranded RNA genome and capsid subunits. The molecular interactions that stabilize the virion at pH 5.0 are altered or eliminated at pH 8.0, even though release of the RNA from the capsid is prevented by the addition of divalent metal cations (Ca^{2+}). From the perturbations that occur to Raman lines of cytosine and adenine rings of the encapsidated RNA molecule between pH values of 5.0 and 8.0, it is concluded that cytosines are protonated in significant numbers at the conditions which maintain the native virus structure and that the stacking of adenines is altered by changes in pH. The degree of protonation of RNA bases can be reduced by elevation of the pH to 8.0 for encapsidated RNA or by release of the RNA from the capsid at pH 5.0. Although the protein groups that interact with the viral RNA cannot be identified unambiguously from the Raman spectra, it is apparent that the molecular environments of aromatic amino acid side chains are altered with the same changes in pH (from 5.0 to 8.0) that perturb the cytosine and adenine ring structures. No significant change in secondary structures of the capsid subunit can be detected with changes in pH or with RNA release. On the other hand, the characteristic Raman lines of the phosphate groups of packaged RNA differ from those of naked RNA at all pH values examined, most likely as a result of specific electrostatic binding of divalent cations to RNA phosphates within the virus shell.

Belladonna mottle virus (BDMV) is a spherically shaped RNA plant virus of the tymovirus group. The T=3 icosahedral capsid, which has a diameter of 29 nm, contains 180 identical protein subunits of M_r 20 300 and a single RNA strand of M_r 1.9×10^6. The base composition of BDMV RNA, like that of the related TYMV RNA, is especially rich in cytosine residues: 37% C, 23% U, 23% A, and 17% G (Jankulowa et al., 1968). Both BDMV and TYMV are susceptible to loss of RNA when the viruses are isolated from infected plants. In vitro studies show, however, that the two tymoviruses differ significantly in susceptibility to diffusion of packaged RNA out of the capsid.

NMR and analytical ultracentrifugation studies indicate that BDMV RNA undergoes a structural transition within the capsid when the pH is increased from slightly acidic values to near neutrality (Virudachalam et al., 1983a,b). The NMR results suggest that below pH 6.5 the encapsidated RNA is relatively rigid, while above pH 6.8 the RNA achieves significantly greater mobility. A structural transition has also been observed for TYMV RNA over the same range of pH and has been attributed to the loss of specific protein-RNA interactions (Kaper, 1975). Such interactions may be a general feature of RNA plant viruses and may serve to restrict the mobility of the encapsidated RNA genome at lower pH.

At higher pH (>6.8), the RNA is readily released from the capsid of BDMV; however, a much greater increase of pH (>11.5) is required for release of TYMV RNA from its capsid. TYMV also preferentially resists release of its RNA for a variety of other structure-perturbing agents (Lyttleton & Matthews, 1958; Kaper, 1964, 1971; Jonard et al., 1972). The greater stability of TYMV vis-à-vis BDMV has been attributed to the presence of polyamines that are packaged in the native TYMV structure but not in the native BDMV structure. It has been proposed that repulsion between phosphate groups provides the driving force for ejection of RNA from the capsid and that such repulsive forces are neutralized by polyamines (Virudachalam et al., 1983a). This model is supported by the observation that addition of spermine, spermidine, and divalent cations to BDMV render the virus less susceptible to loss of RNA and more nearly identical with TYMV in its retention of RNA with increasing pH or with other structure-perturbing influences (Virudachalam et al., 1983b).

The specific interactions between RNA and protein that stabilize tymovirus structure and provide resistance to disassembly below pH 6.8 are not known. Various models, including abnormally titrating protein and RNA groups, have been proposed to account for the stability of RNA viruses at low pH, and some of these have been tested experimentally (Kaper, 1975). In a previous study of TYMV by Raman spectroscopy the presence of protonated cytidines and adenines (Cyt^+ and Ade^+) in the encapsidated RNA molecule was demonstrated for solution pH values well above the normal pK_a values of the bases (Hartman et al., 1978). Such protonated base residues have the potential for specific electro-

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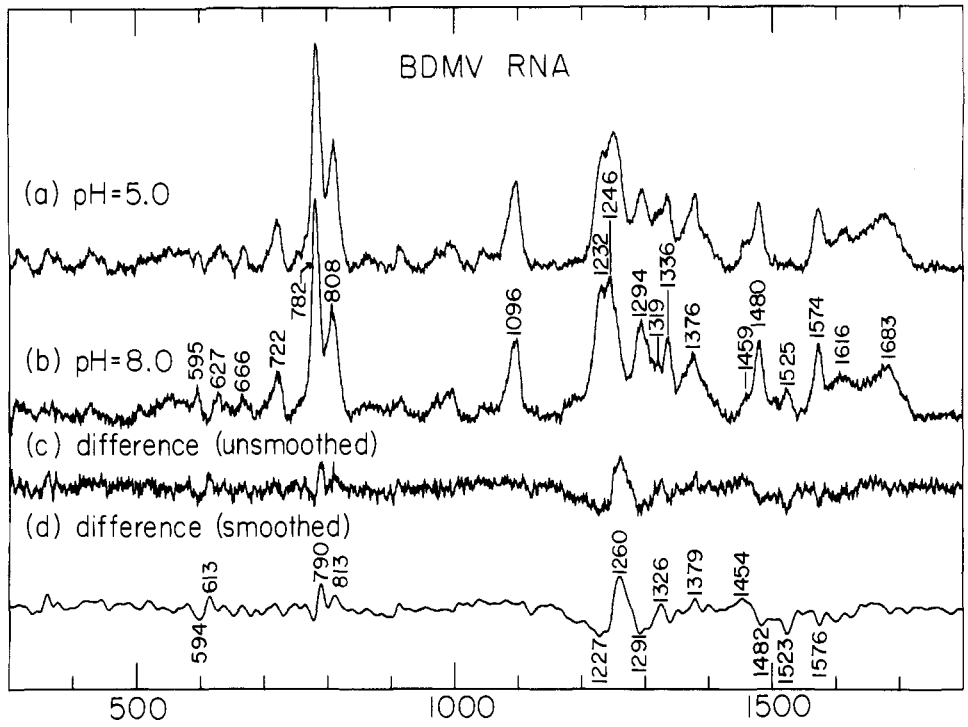

FIGURE 1: Raman spectra of BDMV RNA, 35 mg/mL in 1 mM KCl plus 10 mM $CaCl_2$ plus 10 mM Tris-HCl at 15 °C. (a) pH 5.0. (b) pH 8.0. (c) Computed difference spectrum, pH 5.0 minus pH 8.0 (unsmoothed). (d) Same as (c) but smoothed (15 point, moving average). Each spectrum is the average of eight scans and is corrected for solvent and background scattering as described by Verduin et al. (1984). Prominent Raman lines which differ in (a) and (b) or which are discussed in the text are labeled in cm^{-1} units.

static interaction with ionized carboxyl groups of aspartic and glutamic acid side chains and may also serve to counter repulsive forces between the RNA phosphates. The Raman data, however, have not yet provided direct evidence of such interactions.

In the present study of BDMV, we have applied Raman difference spectroscopy to whole virus, empty shells (capsids), and RNA to compare the structures of fully assembled virus particles and viral components. This study has been carried out over the same range of pH for which a transition in RNA mobility has been demonstrated by NMR methods. We have also used Raman spectra to monitor the state of encapsidated RNA in both the presence and absence of divalent cations. The results are compared with those obtained previously on TYMV (Hartman et al., 1978), and more recently on CCMV (Verduin et al., 1984) which also exhibits sensitivity of virion structure to solution pH.

Experimental Procedures

The strain of BDMV, originally isolated from *Physalis heterophylla*, was purified from the tobacco hybrid *Nicotiana X edwardsonii* by a procedure described previously (Heuss et al., 1981). Empty capsids were separated from intact virus particles in a cesium chloride density gradient centrifugation, and both were stored in the cold in 0.05 M phosphate buffer, pH 5.5, containing 1 mM ethylenediaminetetraacetic acid (EDTA). RNA was isolated from viral protein by phenol extraction and was subsequently ethanol precipitated, rinsed, and stored in 90% ethanol–water in the cold.

For Raman spectroscopy, whole virus and capsids were treated similarly. Each stock solution was dialyzed for 48 h against a pH 5.0 or pH 8.0 buffer containing 1 mM KCl plus 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) or 1 mM KCl plus 10 mM Tris-HCl plus 10 mM $CaCl_2$. The dialysate was centrifuged for 3 h in an airfuge at 160000*g*, and the pellet was redissolved in the appropriate buffer to a final concentration of approximately 80 mg/mL. BDMV RNA was redissolved to 35 mg/mL in the same buffers. Samples were transferred to Raman capillary cells of 10 μL volume and thermostated at 15 ± 1 °C while spectra were recorded (Thomas & Barylski, 1970).

Raman spectra were excited with 400 mW of 514.5-nm radiation from a Spectra Physics Model 171-18 argon laser and were recorded on a Spex Industries Ramalog VI spectrometer interfaced to a North Star Horizon II microcomputer (Li et al., 1981). The spectral resolution was 8 cm^{-1}. Photon counts were taken at intervals of 1 cm^{-1} with integration time of 1 s. To improve the signal-to-noise ratio, each spectrum was scanned repetitively (typically eight scans in the region 300–1800 cm^{-1} and 30 scans in the region 2500–2600 cm^{-1}). The Raman data were corrected by subtraction of Raman scattering of the buffer and by fitting each spectrum to a straight base line, tangent to well-defined minima common to all spectra in the 300–1800-cm^{-1} interval (Benevides et al., 1984).

Results and Discussion

The Raman spectra of BDMV RNA (Figure 1), of capsids devoid of RNA (Figure 2) and of whole virus (Figure 3) were each recorded at conditions that maintain the native virus structure (pH 5.0 buffer plus Ca^{2+}) and at conditions that presumably disrupt protein–RNA interactions but do not cause release of RNA (pH 8.0 buffer plus Ca^{2+}). [In the absence of divalent metal cations, the pH 8.0 state allows diffusion of RNA from the capsid, whereas the pH 5.0 state does not. However, RNA is retained by the particles at pH 8.0 with sufficient divalent cation concentration (10mM)].

(*1*) *Changes in RNA Structures with pH.* Figure 1 shows the effect upon the Raman spectrum of increasing the pH of a solution of protein-free BDMV RNA from 5.0 to 8.0. The positive peaks in the difference spectrum (Figure 1c) identify those Raman lines of RNA that are more intense at pH 5.0, while negative peaks indicate those lines that are more intense at pH 8.0. Comparison with model compounds (Chou &

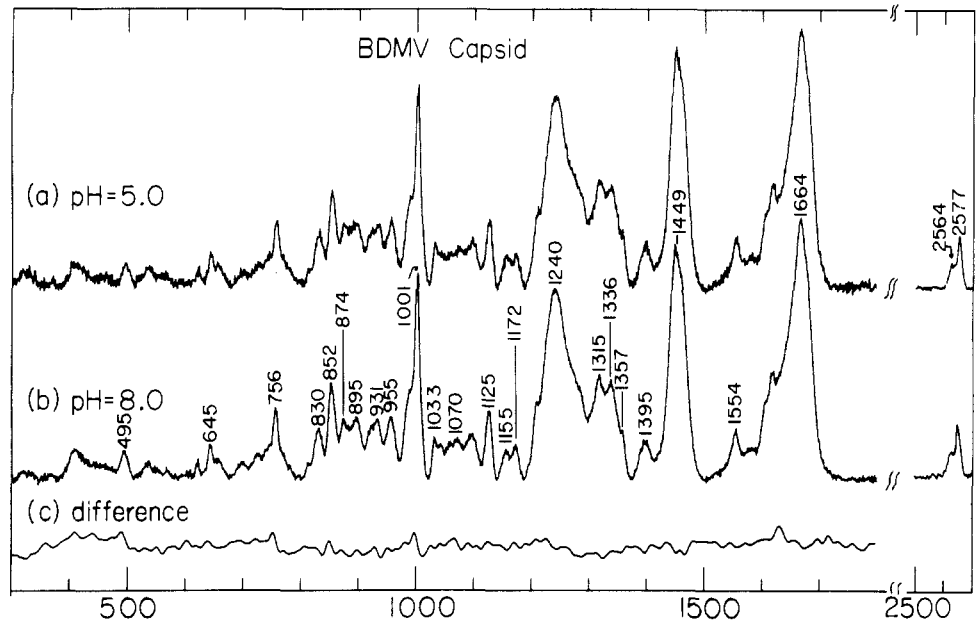

FIGURE 2: Raman spectra of BDMV capsids, 80 mg/mL in 1 mM KCl plus 10 mM $CaCl_2$ plus 10 mM Tris-HCl at 15 °C. (a) pH 5.0. (b) pH 8.0. (c) Computed difference spectrum, pH 5.0 minus pH 8.0. The inset shows the Raman lines of cysteinyl sulfhydryl groups in the 2500–2600-cm^{-1} region. Other conditions are as given in Figure 1.

Thomas, 1977; Hartman et al., 1978) shows that most of the peaks in the difference spectrum are due to vibrations of cytosine (Cyt) and adenine (Ade) rings and that such rings are protonated in significant amounts at pH 5.0 though not at pH 8.0. The magnitudes of the intensity changes in the 790- and 1260-cm^{-1} lines of Cyt indicate that 21 ± 4% of the Cyt residues are protonated at pH 5.0, assuming no protonation at pH 8.0 (Chou & Thomas, 1977; Verduin et al., 1984). An accurate quantitative calculation of the percentage protonation of Ade cannot be made from the data because of overlap of the lines of Ade with those of other bases, particularly guanine near 1480 and 1574 cm^{-1}. However, from the losses of intensity at 1482 and 1576 cm^{-1} (negative peaks in Figure 1c) accompanying decrease of the concentration of neutral Ade residues, we estimate roughtly 5–10% protonation of Ade at pH 5.0.

Figure 1c also shows a small change in intensity of the 813-cm^{-1} line of the RNA phosphodiester group, consistent with a marginal increase (~5%) in ordered A-helix structure at lower pH (Thomas & Hartman, 1973). The Raman data indicate that at least 85% of the nucleotide residues exist in the A geometry at pH 8.0, i.e., containing C3′-endo sugar pucker and anti orientation of the glycosidic bond (Prescott et al., 1984), and at least 90% at pH 5.0.

The present results are qualitatively similar to those obtained in a recent Raman study of cowpea chlorotic mottle virus (CCMV) (Verduin et al., 1984). Here, however, the degree of protonation detected in the protein-free BDMV RNA at pH 5.0 exceeds that observed for CCMV RNA, which is attributed to the greater Cyt content of BDMV RNA (37% vs. 20%). Consistent with this explanation is the fact that comparable protonation of Cyt (~25%) is also observed for TYMV RNA at pH 5.0 (G. J. Thomas, Jr., and B. Prescott, unpublished results). [Protonation of TYMV RNA was not investigated in an earlier study (Hartman et al., 1978), which lacked the precision of computer difference spectroscopy for detecting small Raman intensity changes of RNA].

(2) Changes in Capsid Structure with pH. The Raman spectrum of the BDMV capsid is virtually unchanged by pH (Figure 2), indicating no significant alteration of subunit secondary structure in the absence of RNA over the range 5.0 < pH < 8.0. The data show that the BDMV subunit is richest in β-sheet secondary structure, which is similar to the conclusion reached from Raman spectra of capsids of TYMV (Hartman et al., 1978) and CCMV (Verduin et al., 1984). Comparison of amide I and amide III Raman intensities of the BDMV capsid with those of TYMV and CCMV indicates the same amount of β structure (~50 ± 10%) for all capsid subunits at the conditions of Figure 2. It is noteworthy that the coat proteins of three other spherical plant viruses are also largely β stranded, as determined by X-ray crystallography at atomic resolution (Rossmann et al., 1983 and references cited therein).

The amino acid composition of the capsid subunit of BDMV is known to be similar to that of TYMV (Jankulowa et al., 1968), although the sequence is not known. The one tryptophan residue per subunit is indicated by the Raman lines at 756, 1357, and 1554 cm^{-1} to exist in a hydrophilic environment (Kitagawa et al., 1979; Thomas et al., 1983). The tyrosine doublet at 830 and 852 cm^{-1}, the intensity ratio of which (I_{852}/I_{830} = 1.85 ± 0.05) is informative of the hydrogen-bonding environment of the *p*-hydroxyl groups, indicates that two of the four tyrosines per subunit are acceptors of strong hydrogen bonds from positive donor groups and two are involved in moderate hydrogen bonding as both donor and acceptor (Siamwiza et al., 1975).

The S–H stretching region of the capsid spectrum (2500–2600 cm^{-1} inset, Figure 2) exhibits two Raman lines, indicating two nonequivalent molecular environments for cysteine SH groups. The lower frequency line (2564 cm^{-1}) which is broad is assigned to cysteines involved in hydrogen bonding, while the higher frequency line (2577 cm^{-1}) which is sharp is assigned to buried SH groups. In the absence of more specific information about the subunit sequence and capsid architecture, it is not possible to determine whether the two classes of cysteines correspond respectively to the two residues per subunit or whether a more complex relationship exists. However, since the integrated band intensities are equal, suggesting equal amounts of both types of SH group, it is reasonable to conclude that the two classes exist in each subunit. Finally, we note that the Raman bands of the SH stretching region are unchanged by increasing pH, as well as

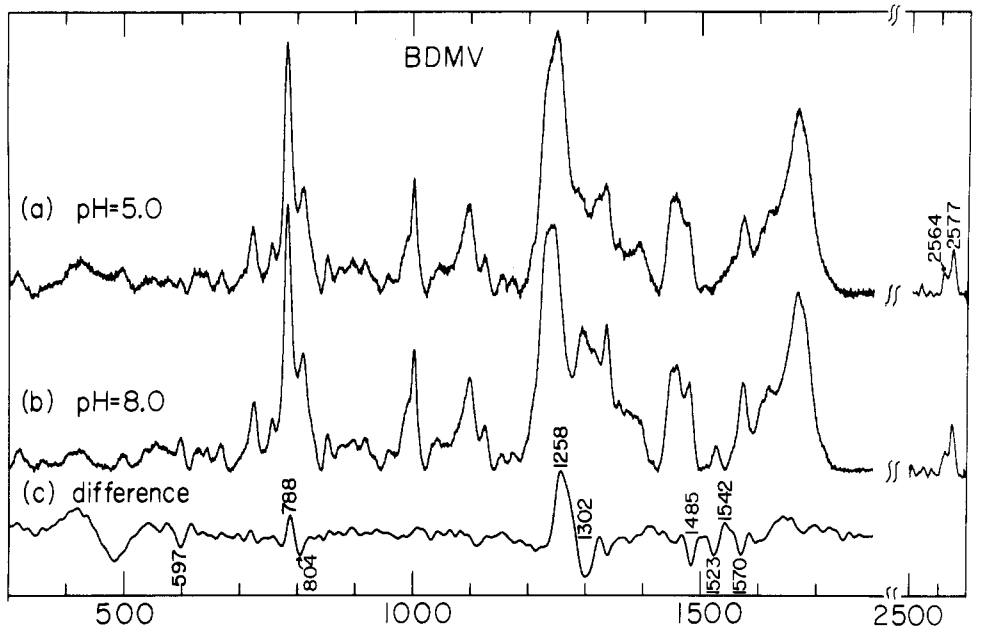

FIGURE 3: Raman spectra of BDMV, 80 mg/mL in 1 mM KCl plus 10 mM $CaCl_2$ plus 10 mM Tris-HCl at 15 °C. (a) pH 5.0. (b) pH 8.0. (c) Computed difference spectrum, pH 5.0 minus pH 8.0. Other conditions are as given in Figure 2.

by packaging of RNA (below), suggesting no significant change in cysteine hydrogen-bonding environment with these effects.

(*3*) *Changes in Virion Structure with pH.* Figure 3 shows the Raman spectra of BDMV at pH 5.0 and pH 8.0 and the corresponding difference spectrum. The Ca^{2+} ions present at pH 8.0 ensure no significant release of RNA from the capsids, even though RNA–protein interactions are altered with elevation of the pH as judged by NMR measurements (Virudachalam et al., 1983b). The native virion (pH 5.0) structure differs significantly from the pH 8.0 structure by virtue of the numerous positive and negative peaks in the difference spectrum that can be interpreted as follows: Most of the features of the difference spectrum of Figure 3c are found also in the difference spectrum of Figure 1c, but with greater amplitudes in the former, indicating greater protonation of Cyt residues in the native virus than in protein-free RNA. The relative intensity change at 1258 cm^{-1} indicates that 30 ± 5% of Cyt residues of encapsidated RNA are protonated at pH 5.0. The intensity changes near 1485 and 1570 cm^{-1} cannot be explained simply in terms of a change in the state of adenine ring protonation but are consistent with less stacking of adenines in encapsidated RNA.

An alternative interpretation of the data of Figure 3 is that the extent of protonation of Cyt residues of encapsidated RNA is the same as in protein-free RNA but that the additional positive intensity at 1258 cm^{-1} is due to a change in the secondary structure of the subunit. This interpretation seems less likely, however, in view of the evident insensitivity of the capid structure (Figure 2c) to the same pH change. Furthermore, the close correspondence between the Raman frequencies and intensities in the spectrum of the virus and those in the sum of spectra of RNA and capsid (section 4 and Figure 4) constitutes evidence that large secondary structure changes of either RNA or protein do not occur upon encapsidation of RNA. Although fortuitous cancellation of changes of opposite sign cannot be ruled out, perfect compensations are unlikely in view of the nature of the Raman spectra of RNA and protein molecules (Thomas, 1976).

A further consideration is the influence of Raman hypochromism on the intensity of the Cyt 1258-cm^{-1} line (Chou & Thomas, 1977). Thus, the greater intensity that we observe at pH 5.0 may actually represent the combined effects of protonation per se and decreased stacking of Cyt^+ residues at pH 5.0. It is not possible to ferret out the separate contributions of each to the observed spectral intensity: protonation of Cyt residues of poly(rC) is known to lead directly to unstacking of the bases (Chou & Thomas, 1977). Nevertheless, the fact that the peaks in the difference spectrum of Figure 3c are roughly 50% greater than the same peaks of Figure 1c indicates that the perturbations to the structure of BDMV RNA within the capsid are correspondingly greater than those of naked RNA at the same pH.

(*4*) *Effect of Assembly of RNA and Capsid upon the Raman Spectra.* Raman difference spectroscopy is employed in Figure 4 to compare the spectra of BDMV RNA in protein-free and encapsidated states, for both pH 5.0 (Figure 4a) and pH 8.0 (Figure 4b) solutions. In each case the spectrum of encapsidated RNA (computed by subtraction of the spectrum of capsid from the spectrum of virus) is subtracted from the observed spectrum of protein-free RNA. For the first difference spectrum (virus minus capsid), the intensities are normalized to the 1450-cm^{-1} band (Thomas et al., 1983); for the second difference (protein-free RNA minus encapsidated RNA), the normalization standard is the 1575-cm^{-1} line (Prescott et al., 1984).

Figure 4a reveals that the changes to the Raman spectrum of RNA from encapsidation at pH 5.0 are small but significant. The following features of the second difference spectrum are noteworthy: (i) Lines at 1379, 1481, and 1568 cm^{-1} due to adenine, and to a lesser extent to guanine residues, are altered by encapsidation. The intensity changes are in the direction of less stacking of Ade residues for encapsidated RNA. (ii) Lines at 1255 and 1299 cm^{-1} due to cytosine are altered by encapsidation. These intensity changes are in the direction favoring greater protonation of Cyt upon encapsidation of RNA. (iii) The intensity ratio I_{809}/I_{1097} is slightly diminished, indicating a small loss (6 ± 5%) of ordered A-type structure of RNA upon encapsidation. (iv) Raman lines sensitive to protein conformation (amide I and amide III) do not appear in the second difference spectrum, consistent with the foregoing result (section 2) that the subunit secondary

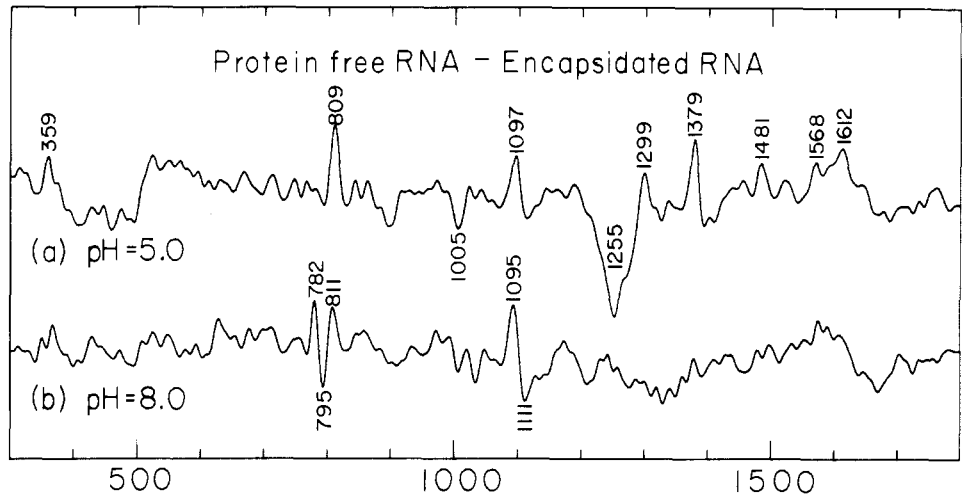

FIGURE 4: (a) Second difference spectrum which compares the Raman lines of protein-free and encapsidated BDMV RNA at pH 5.0, computed as follows: subtrahend = spectrum of BDMV minus spectrum of capsid; minuend = observed spectrum of protein-free BDMV RNA. (b) The second difference spectrum which compares the Raman lines of protein-free and encapsidated BDMV RNA at pH 8.0, computed as in (a) except for pH 8.0 data.

structure is not appreciably altered by encapsidation of RNA. However, the negative peak in the second difference spectrum at 1005 and the positive peak at 1612 cm^{-1} indicate small changes in the environments of phenylalanine and tyrosine, respectively, with encapsidation of RNA. The different intensities of these lines in the virus may result from altered stacking interactions when RNA is present.

The second difference spectrum of BDMV RNA at pH 8.0 is shown in Figure 4b. The characteristics of altered base protonations (items i and ii) are not evident from the pH 8.0 data. Here, the only significant peaks are those corresponding to the RNA phosphate groups (item iii), indicating that interactions of the phosphates are different in the protein-free and encapsidated states of RNA, even though the effects of protonation of RNA base residues and base–protein interactions have been eliminated.

The difference spectra of both parts a and b of Figure 4 contain no peaks that can be assigned to altered states of acidic side chains (aspartic and glutamic acids) or other aliphatic amino acid residues. In this respect BDMV differs significantly from CCMV (Verduin et al., 1984).

Conclusions

The subunit of BDMV is relatively rich in β-sheet structure and relatively poor in α-helix structure. The average secondary structure of capsid protein is not altered by the removal of RNA from the capsid or by the attenuation of RNA–protein interactions in the native virion (pH 5.0) upon increasing the solution pH to 8.0. It is not known whether the increase of pH causes swelling of the BDMV virion, as is the case for CCMV, TBSV, and other RNA plant viruses.

The RNA genome contains a highly ordered secondary structure within the capsid, though a small (~5%) increase in the amount of C3′-endo/anti nucleoside conformers occurs upon removal of RNA from the capsid. This indicates that the average conformation of the single-stranded RNA backbone is only slightly affected by the protein shell, when compared with protein-free aqueous RNA. The phosphodiester and phosphate groups of encapsidated RNA appear to undergo no change in structure or environment with pH increase from 5.0 to 8.0.

Nevertheless, Raman spectra indicate significant changes in structure of the molecular constituents of BDMV when the virus particle is disassembled into RNA and capsid and when the pH of solution of the virion is increased from 5.0 to 8.0. It is clear that substantial protonation of Cyt and Ade residues occurs in encapsidated BDMV RNA at pH 5.0 and that the extent of base protonations is reduced either by an increase of the pH to 8.0 for encapsidated RNA or by removal of RNA from the capsid at pH 5.0. The protonated cytosines of encapsidated RNA may be involved in specific interactions with subunit side chains or with neutral cytosines. We find no evidence for interaction of acidic amino acid side chains of the BDMV subunit with protonated RNA bases, as has been proposed for TYMV (Kaper, 1975). We estimate that up to 10% of the Asp and Glu residues could be involved in such interactions and still remain undetected because of the relative weakness of the characteristic Raman lines that serve as markers for the CO_2^- and COOH groups (Verduin et al., 1984).

Neutralization of Cyt^+ residues by titration to pH 8.0 while maintaining adequate Ca^{2+} ions produces a Raman spectrum virtually indistinguishable from a sum of spectra of RNA and capsid at the same experimental conditions. This indicates little or no protein–RNA interactions at pH 8.0 in the presence of Ca^{2+}. Yet, at these conditions the phosphate groups of encapsiated RNA differ from those of protein-free RNA, presumably because of Ca^{2+}-mediated binding of phosphates to the capsid surface or to one another.

Registry No. Ade, 73-24-5; Cyt, 71-30-7; Ca, 7440-70-2; hydrogen ion, 12408-02-5.

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Analysis of Translational Fidelity of Ribosomes with Protamine Messenger RNA as a Template†

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ABSTRACT: A novel method was developed to estimate the translational fidelity of mammalian ribosomes in vitro with protamine mRNA of rainbow trout as template. Protamines are mixtures of basic proteins consisting of only seven types of amino acids (Arg, Ile, Val, Ser, Pro, Ala, and Gly), arginine (codon, A*G*R and C*G*N) being abundant. Taking advantage of the absence of lysine (codon, A*A*G) in the proteins, we determined the misincorporation of this amino acid into protamines in a cell-free translation system consisting of mouse liver ribosomes, protamine mRNA, [^{3}H]lysine, [^{14}C]arginine, and seven unlabeled amino acids: Ile, Val, Ser, Pro, Ala, Gly, and Met. After the reaction, translation products were analyzed by either sucrose gradient centrifugation or polyacrylamide gel electrophoresis. In the former method, radioactive protamines are mostly found on monosomes, but not on polysomes, probably because of the basic nature of the proteins. The error frequency was calculated from the molar ratio of [^{3}H]lysine to [^{14}C]arginine incorporated into protamines with an appropriate correction. The frequency was found to be 0.0006–0.002. This method enabled us to determine the frequency of misrecognition of purine bases at the second position of arginine codons in mRNA.

In all organisms, it is important to maintain high fidelity of transfer of genetic information. Transcription and translation of genetic messages must be highly accurate for synthesis of functional proteins for cellular activities. Therefore, for an understanding of fundamental biological processes, it is essential to have information on the mechanism of accurate translation of mRNA, i.e., the fidelity of protein synthesis (Kurland, 1979; Yarus, 1979).

In the early 1960's, when decoding of the genetic code was being studied intensively, it was realized that the translational fidelity of ribosomes is not perfect in a cell-free translation system derived from *Escherichia coli* and *Thermophilus* (Gorini, 1974). The frequency of mistranslation in vitro was on the order of 10^{-2} with synthetic polynucleotides such as poly(U) as template. Moreover, in some proteins, such as ovalbumin, hemoglobins, and flagellin, the incorporation of amino acids that are not expected to be present was found to occur at a frequency of 10^{-3}–10^{-4}, a value that is believed to be comparable to that in vivo (Loftfield, 1963; Loftfield & Vanderjagt, 1972; Edelmann & Gallant, 1977).

The discrepancy between the error frequencies in vitro and in vivo might be because synthetic polynucleotides without the specific sequences present in natural mRNAs were used for in vitro studies. Alternatively, there may be specific error-reducing mechanisms in vivo (Lake, 1981). For many years, synthetic messenger RNA has been used in studies on the error frequency of translation in vitro. It seems preferable to use natural mRNAs for this purpose, but no suitable systems with natural mRNA are available for studies on translational fidelity in vitro. One reason for this is that most proteins contain all 20 types of amino acids, making it difficult to distinguish incorrect incorporation of amino acids, if any, from correct incorporation. However, if a natural mRNA that does not code for 1 or more of the 20 amino acids were used, it should be easy to measure incorporation of incorrect radioactive amino acids. mRNAs of this type that could be used for this purpose

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