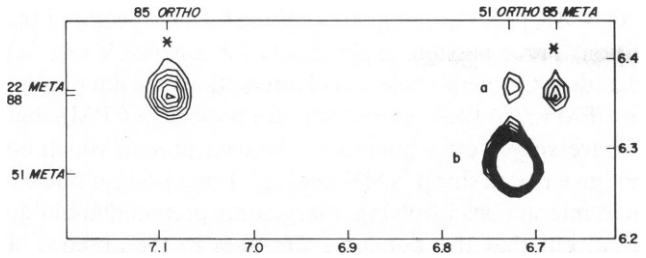

FIGURE 5 Portion of the aromatic region of the two-dimensional pure-phase nuclear Overhauser effect spectrum (13) of the wild-type 1–102 dimeric domain. Cross-peak *a* shows an effect between Tyr22 and Phe51, which are stacked in the interior of the domain. Asterisks indicate effects between Tyr85 and Tyr88. In the crystal structure, Tyr88 comes within 4 Å of Tyr85′. Cross-peak *b* is a very large Overhauser effect between the ortho and meta protons on the same ring, Phe51. 1,024 points were sampled over a sweep width of 5,000 Hz. 96 scans were acquired per t_1 value, and 220 T_1 values were obtained. The mixing time was 200 ms. The data matrix was zero-filled to 1,024 × 1,024. A convolution difference with parameters GM3, EM20, 1.0 was applied in both dimensions. The final data matrix was made symmetric by minimum value.

Under these conditions the Cys88 disulfide-bonded dimer denatures between 56° and 60°C (data not shown). The Cys85 disulfide-bonded dimer denatures between 36° and 40°C (data not shown). Thus, a correct disulfide appears to stabilize the protein, and an incorrect disulfide to destabilize it.

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NUCLEAR MAGNETIC RESONANCE STUDIES OF SPHERICAL PLANT VIRUSES

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We have used nuclear magnetic resonance (NMR) spectroscopy to study protein-nucleic acid interactions and polyamine exchangeability in belladonna mottle virus (BDMV), turnip yellow mosaic virus (TYMV), and cowpea mosaic virus (CPMV). The protein shells of BDMV and TYMV each are composed of 180 copies of a 22,000 d protein; each capsid holds one single-stranded RNA molecule of ~2.0×10^6 d. CPMV, which has a divided genome, consists of two single-stranded RNA molecules packaged separately in identical capsids; the capsid is composed of 60 copies each of two different proteins (22,000 d and 42,000 d). Capsids (devoid of RNA) occur naturally for all the three viruses. The diameters of these virions and their capsids are each ~280 Å. Our results demonstrate that ^{1}H and ^{13}C NMR can be used to observe polyamines (such as spermidine) in a virus particle and to study their effect on the dynamics of capsid groups and overall virus stability. In BDMV and TYMV, a mobile peptide domain, whose NMR signal is easily resolved in spectra of the empty capsids, interacts with the RNA. The capsid of CPMV, by contrast, shows no evidence for such a highly mobile domain.

RESULTS

^{13}C NMR studies of BDMV and its capsid used samples enriched to 7–8% ^{13}C over the natural abundance level of 1.1% (1). Fig. 1 compares the 50 MHz ^{13}C NMR spectra of BDMV capsid and virion. Several sharp peaks are seen in the carboxyl and aliphatic regions of the spectrum of the capsid (Fig. 1 *a*) which are broadened or shifted in the spectrum of the virion (Fig. 1 *b*). When the RNA is absent, as in the naturally occurring capsid or in capsid prepared *in vitro* by chemical treatment, several aliphatic groups give rise to sharp NMR peaks indicative of rapid segmental motion. When the RNA is present, as in the native virion, the sharp peaks are missing. This suggests that aliphatic side chains of the coat protein interact with the RNA. The carbonyl peak at 181.8 ppm in the capsid spectrum (Fig. 1 *a*) is assigned to the C^{δ} of glutamate. The absence of this peak in the virion spectrum (Fig.1 *b*) suggests that one or more carboxylic acid groups interact with the RNA. The resonance may broaden when RNA is present or, as is more likely, may shift upfield into the region of protonated carboxylates. The latter would occur if the pK_a' of the carboxylate is raised above the pH of the solution (5.5) as a result of hydrogen-bond formation with the cytosine base of the RNA. Kaper has proposed a model for such interactions in TYMV (2). The aromatic side chains of tyrosine, phenylalanine, histidine, and tryptophan do not exhibit sharp peaks either in the capsid or virion, suggesting that they are packed tightly in both cases. ^{1}H NMR studies on BDMV, TYMV, and their capsids have yielded similar results (1) which reinforce the conclusions illustrated here based on ^{13}C NMR data.

Contrary to what is seen with the BDMV and TYMV capsids, CPMV capsid does not exhibit additional ^{1}H or ^{13}C NMR peaks over and above those seen in spectra of the virion. Three possible explanations for this result are: (*a*) that direct protein-nucleic acid interactions are not present in CPMV; (*b*) such interactions are present in CPMV but involve very short segments of the coat protein which do not give rise to sharp NMR peaks; (*c*) that protein-nucleic acid interactions involving a large coat protein domain do exist, but that this domain rearranges in the absence of RNA such that it is bound tightly to the rest of the capsid. We are unable to distinguish among these possbilities at present (5).

Several species of plant virus contain varying amounts of polyamines associated with the RNA. Identification of these polyamines in the past has required elaborate chemical procedures combined with thin-layer or gas chromatography and/or mass spectrometry (3, 4). We have shown that NMR spectroscopy provides a sensitive and nondestructive method for detecting polyamines in viruses (1, 5). The ^{1}H NMR peaks near 1.8, 2.14, and 3.14 ppm in Fig. 2 are assigned to virion-associated polyamines. Fig. 2 *a* confirms the presence of polyamine in TYMV (3, 4) and Fig. 2 *c* demonstrates convincingly that polyamine is absent in BDMV (1). We were surprised to find that BDMV has no internal polyamine since it belongs to the same group as TYMV. We attribute the relative instability of BDMV above neutral pH (1) to its lack of polyamine; TYMV, which contains polyamine, is stable up to pH 11.5. Although BDMV lacks polyamine, spermidine can penetrate the capsid, as shown by Fig. 2 *b*. The pH stability of polyamine-treated BDMV is very similar to that of TYMV (1).

CPMV contains ~200 polyamine molecules/virion as could be detected easily by ^{1}H NMR or natural abundance

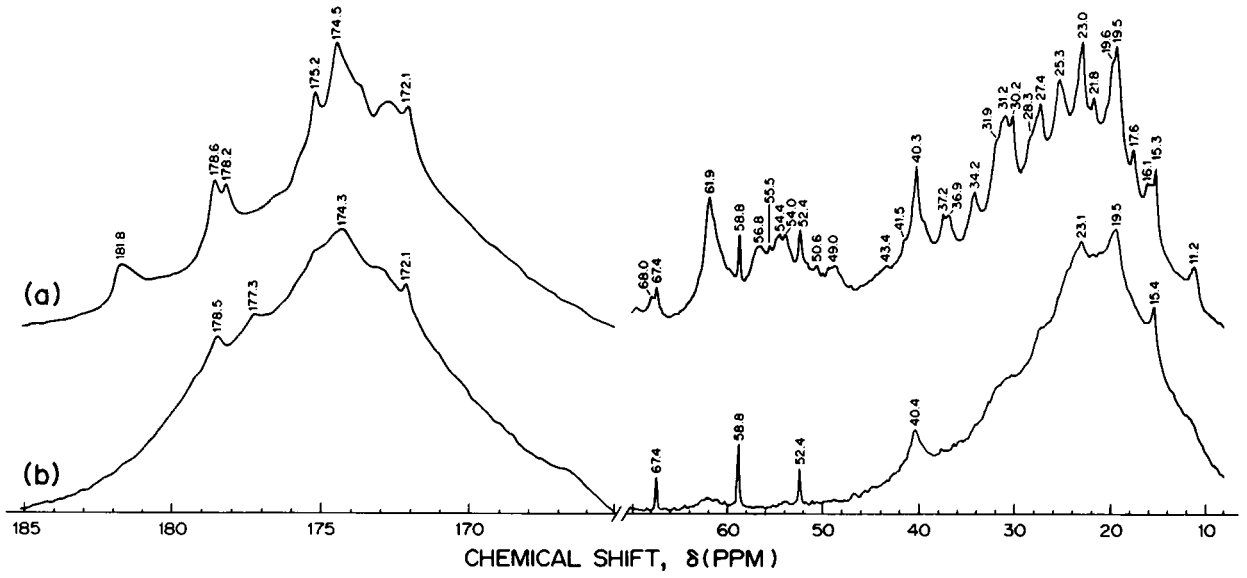

FIGURE 1 50 MHz ^{13}C NMR spectra of (*a*) ^{13}C-enriched BDMV capsid and (*b*) ^{13}C-enriched intact virus. The traces show expansions of the aliphatic and carboxyl spectral regions. Peaks at 52.4, 58.8, and 172.1 ppm are from EDTA, and the peak at 67.4 ppm is from dioxane. All samples were at pH 5.5. (From reference 1.)

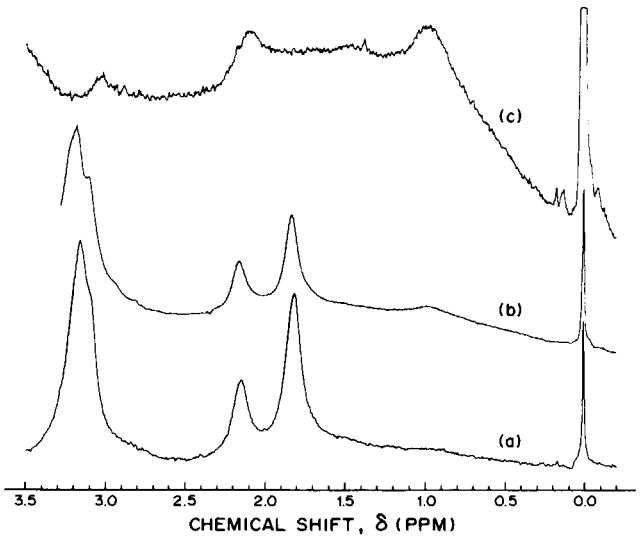

FIGURE 2 470 MHz ^{1}H NMR spectra of (*a*) turnip yellow mosaic virus, (*b*) spermidine-treated belladonna mottle virus, and (*c*) native belladonna mottle virus. All samples were dissolved in D_2O and were at pH 5.5 and 22°C. (R. Virudachalam, J. L. Markley, unpublished results; see reference 1.)

^{13}C NMR (5). A number of investigations have suggested that the virion-associated polyamine is exchangeable for other counterions. We examined this possibility by exposing CPMV samples to 3.6 M CsCl at pH 5.5 and 8.5 and then recording their ^{1}H NMR spectra. The samples dialyzed against CsCl at low pH retained their polyamine, whereas the samples dialyzed against CsCl at high pH lost their polyamine, presumably in exchange for cesium ions. To explain these results one needs to assume that the viral protein coat is impermeable to metal ions or polyamines at acidic pH but becomes porous at alkaline pH. Alternatively, exchange of polyamines for other cations may be facilitated at pH values approaching the pK_a values of the ionizable groups of polyamines, which range from 9 to 11.

In summary, NMR spectroscopy has been found to be a useful technique for studying protein-nucleic acid interactions in viruses and other macromolecular assemblies and for monitoring small molecules associated with them.

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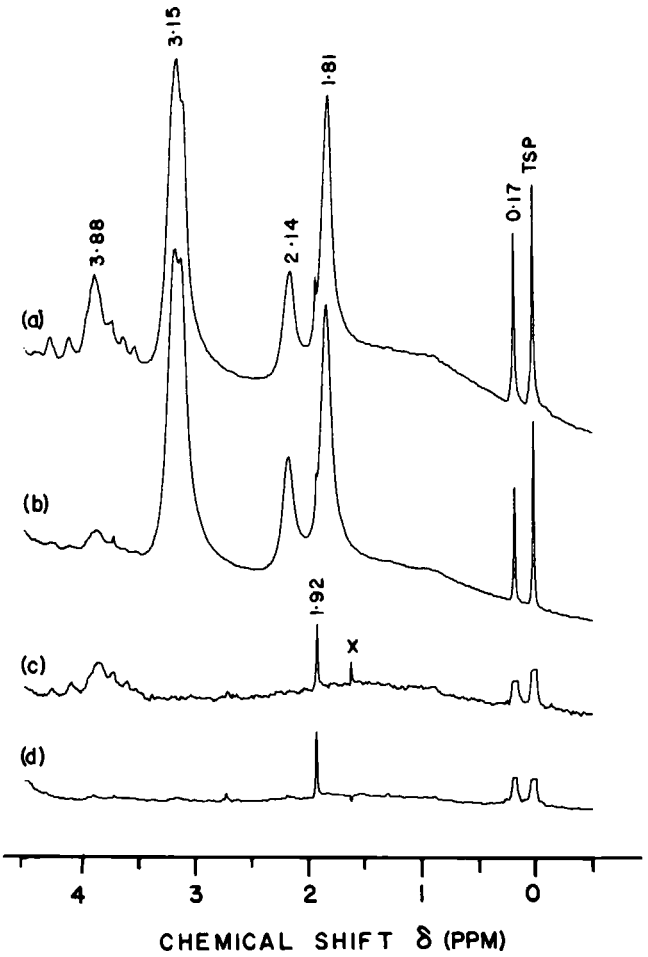

FIGURE 3 470 MHz ^{1}H NMR spectra of cesium chloride-treated cowpea mosaic virus components at pH 7.0 in D_2O, 22°C: (*a*) the CPMV fraction, which contains the smaller RNA; and (*b*) the CPMV fraction, which contains the larger RNA both after a 5-d exposure to 3.6 M CsCl at pH 5.5; *c* and *d* are from the corresponding fractions after a 5-d exposure to 3.6 M CsCl at pH 8.5. (Reproduced from reference 5.)

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