Swelling of cowpea chlorotic mottle virus studied by proton nuclear magnetic resonance

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1. INTRODUCTION

Cowpea chlorotic mottle virus (CCMV) is a spherical plantvirus of the group of bromoviruses. An interesting feature of bromoviruses is the increase in hydrodynamic volume when the pH is raised from 5.0–7.5 at low ionic strength (<0.2) [1]. A hysteresis around pH 6.5 is observed for the reversible proton release [2] and the Stokes' radius variation [3] during this pH-dependent swelling. At pH 5.0 the virus is not sensitive to peptidases and RNases. However, degradation of the virus occurs on adding peptidases or RNases at pH 7.5 [4,5]. These effects have been interpreted in terms of rearrangements of the protein and the RNA in the virus [4,5].

In [6], a nuclear magnetic resonance (NMR) study has been presented of CCMV and its protein. It has been shown that the N-terminal region of CCMV protein is the RNA binding part. This N-terminal region is very mobile in the absence of RNA. In the presence of RNA (as in native virus) the N-terminal region is immobilized. Here, we extend our NMR work to the study of the pH-dependent swelling of CCMV. Only a few CH_2 and CH_3 groups of animo acid sidechains are found to be mobile on a timescale of 10^{-7}–10^{-8} s. It is shown that neither the RNA nor the N-terminal protein region are mobile on this timescale at any pH during the titration. From our experiments, it is concluded that the protein–RNA interaction in CCMV is not altered during the pH dependent swelling of the virus.

2. MATERIALS AND METHODS

The virus was purified as in [7]. After purification, the virus was dialyzed against 200 mM KCl, 10 mM $MgCl_2$ and 1 mM sodium phosphate (pH 5.0) H_2O in the virus was substituted by D_2O through 3 cycles of centrifugation and resuspension of the pellets in the above solution made up in D_2O. In D_2O solutions, pH meter readings were taken without correction for the presence of D_2O. The final virus concentration was $\sim$20 mg/ml. The pH of the virus solution was adjusted by adding very slowly 0.1 M solutions of DCl or NaOD.

^{1}H-NMR spectra were recorded with a Bruker WM250 supercon spectrometer: 500 µl samples were measured at 7°C in the quadrature detection mode with D_2O lock and without ^{1}H-decoupling. The acquisition time was 0.41 s with a pulse delay of 0.6 s and 4000 scans were taken. The sensitivity enhancement is 10 Hz. The ppm scale is relative to sodium 2,2-dimethyl 2-silapentane-5 sulfonate (DSS). The vertical scale is corrected for (small) concentration differences. Spectral intensities were determined using a planimeter. The absolute spectral intensity at pH 5.0 was measured using spectra with a spectral width of 25 kHz. The relative spectral intensities were measured from peak areas in difference spectra at 10 kHz spectral width. The estimated error in the absolute spectral intensity is 20%. The error in the comparison of spectral intensities is much smaller.

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3. RESULTS AND DISCUSSIONS

Since CCMV has a large molecular mass ($\sim 4.6 \times 10^6$), the NMR spectral intensity of sharp peaks is a direct measure of the number of mobile nuclei [6,8,9]. Rigid nuclei give rise to broad unresolved resonances. Fig.1 represents the 250 MHz ^{1}H-NMR spectra of CCMV at several pH-values between 5.0 and 8.0. More than 90% of the resonances are contained in a broad unresolved background signal, indicating that most of the protein and the RNA is immobile at the NMR timescale. The 3 sharp peaks in the 0.5–2.5 ppm region correspond to mobile CH_2- and CH_3- groups in amino acid side chains. The rotational correlation time of these groups is $\sim 10^{-8}$ s [6]. Fig.2 shows the total spectral intensity of the sharp peaks as a function of pH. Since the T_1-values are almost equal ($T_1 \sim 0.1$–0.2 s) for all these peaks and independent of pH, an increase in spectral intensity is solely due to an increase in the number of mobile groups. Fig.1J is the spectrum of CCMV after back-titration from pH 8.0 to pH 5.6. This spectrum is identical to the original pH 5.8 spectrum. This proves that the observed increase in mobility is reversible. The pK-value for the titration is 7.1, which is in good agreement with the results obtained in [2].

The percentage mobile protons increases from 2.0 ± 0.5% at pH 5.0 to 8 ± 2% at pH 8.0. This mobility can solely be ascribed to CH_2- and CH_3-groups in amino acid sidechains [11]. The thickness of the protein shell is known to remain constant throughout the titration [4]. The observed increase of mobility seems therefore to result from CH_2- and CH_3-groups of amino acids at the protein-subunit contact faces that gain space to move when the virus protein shell is loosened by the radial expansion of the protein subunits.

RNA would give a strong resonance at 3.8 ppm. From the absence of sharp peaks at this position in our spectra, it is concluded that upon swelling no RNA is released from the virus and that no increase of mobility of RNA occurs. In the absence of RNA, the N-terminal region of CCMV protein gives a strong arginine signal at 3.2 ppm [6]. From the absence of sharp peaks at 3.2 ppm in all our spectra, it is concluded that the immobility of the N-terminal protein region, due to the protein–

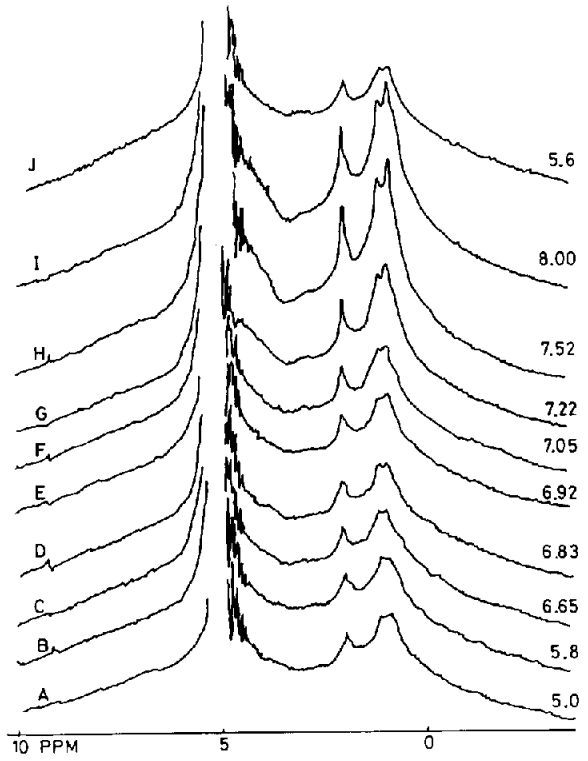

Fig.1. 250 MHz ^{1}H NMR spectra of CCMV at several pH values between 5.0 and 8.0.

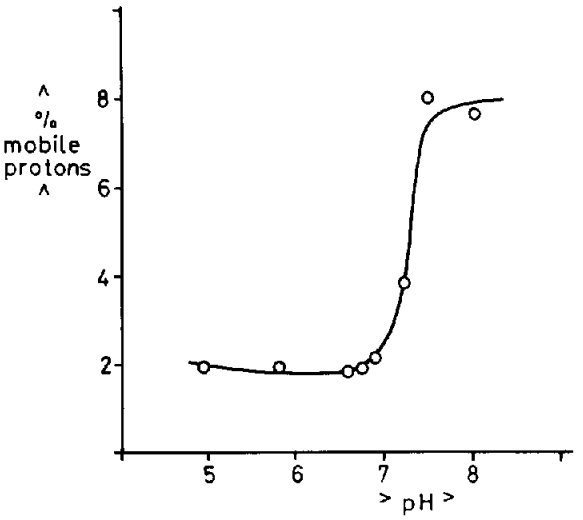

Fig.2. Percentage mobile protons in the 250 MHz ^{1}H NMR spectra of fig.1 as a function of pH.

RNA interaction [6], is not influenced by an increase of pH.

Although no structural information can be derived from our NMR results, they strongly suggest that the interactions between the N-terminal protein region and RNA remain unaltered during titration from pH 5.0–8.0 and back. This is in agreement with work on CCMV [10] and a similar virus BMV [4] where in regions of RNA that interact with the protein were suggested not to reorganize during the swelling. Sensitivity of CCMV to proteases may also result from disclosure of sites hitherto hidden on the subunit contact faces, rather than from a partial unfolding of the protein subunits themselves [4]. This idea is experimentally confirmed by our NMR results, since a partial unfolding of the protein subunits would give rise to NMR signals with a linewidth according to mobility on a timescale much faster than observed in our spectra.

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