Mobility involved in protein–RNA interaction in spherical plant viruses, studied by nuclear magnetic resonance spectroscopy

G. Vriend, B.J.M. Verduin*, M.A. Hemminga and T.J. Schaafsma

Department of Molecular Physics, Agricultural University, De Dreijen 11, 6703 BC Wageningen, The Netherlands and **Department of Virology, Agricultural University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands*

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1. INTRODUCTION

In a previous paper [1] we have shown that the protein region in cowpea chlorotic mottle virus (CCMV) involved in protein–RNA interaction is very mobile in the absence of RNA. Upon binding RNA, immobilization of this protein region occurred. From a comparison with other systems, e.g. tobacco mosaic virus [2–4], tomato bushy stunt virus [5], southern bean mosaic virus [6], histones [7] and protamines [8] and using protein secondary structure predictions [9] we have suggested a general model for the protein–RNA interaction in simple plant viruses. This model is based on a random coil to α-helix transition for the protein region, interacting with RNA upon binding.

In this paper we extend our work to three other spherical plant viruses. We used brome mosaic virus (BMV), belladonna mottle virus (BdMV) and cowpea mosaic virus (CPMV) since the stability of these three viruses depends to a different extent upon protein–RNA and protein–protein interactions [10]. The results obtained confirm our previous ideas about protein–RNA interaction in simple plant viruses and permit a further refinement of our model.

Correspondence address: G. Vriend, Dept. of Molecular Physics, Agricultural University, De Dreijen 11, 6703 BC Wageningen, The Netherlands

2. MATERIALS AND METHODS

2.1. *Virus preparation*

BMV was purified as described for CCMV [11]. Dimeric coat protein prepared from virus by the $CaCl_2$ method [12] was assembled into empty protein capsids by dialysis against 300 mM NaCl, 1 mM dithiothreitol (DTT) and 50 mM sodium acetate (pH 5.0).

The strain of BdMV used in the present study was obtained from Dr P. Argos and was originally isolated from *Physalis heteraphylla* [13]. Virus was purified from tobacco *Nicotiana glutinosa* as in [14] with the modification as in [15]. The naturally occurring empty protein capsids (BdMV-T) were separated from the bottom component (BdMV-B) by isopycnic centrifugation in 40% (w/w) cesium chloride in 0.05 M potassium phosphate buffer (pH 5.5).

The Sb isolate of CPMV was grown in *Vigna unguiculata* L. var. Blackeye Early Ramshorn and purified as in [16]. The top, middle and bottom components (CPMV-T, CPMV-M and CPMV-B) were separated by centrifugation in a linear 15% to 30% (w/w) zonal sucrose gradient in 0.01 M sodium phosphate buffer at pH 7.0. The gradient was centrifuged for 16 h at 23 000 rev./min and 5°C in an MSE B XV Ti rotor. CPMV was also purified using chloroform–butanol clarification of the leaf homogenate and iso-electric precipitation of the virus in order to avoid contamination of the virus with polyethylene glycol and sucrose.

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2.2. *NMR measurements*

Preparations of BdMV and CPMV were dialyzed against 200 mM KCl, 10 mM $MgCl_2$ and 5 mM sodium phosphate (pH 7.5). BMV was dialyzed against the same buffer at pH 5.0 and its protein against 300 mM KCl, 10 mM $MgCl_2$ and 5 mM sodium phosphate (pH 5.0). H_2O in the solutions was substituted by D_2O through extensive dialysis against the above solutions made up in D_2O. The final concentration of protein or nucleoprotein varied from 5–20 mg/ml. ^{1}H-NMR spectra were recorded with a Bruker WM 250 or a Bruker WM 500 supercon spectrometer. Samples of 500 μl were measured in the quadrature detection mode with D_2O lock and without ^{1}H decoupling. The acquisition time plus pulse delay was 1 s and 4000 scans were taken. The sensitivity enhancement was 5 Hz. The ppm scale was relative to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). The vertical scale was corrected for concentration differences between the components of each virus. The spectral width was 40 ppm.

3. RESULTS

3.1. *BMV*

Figure 1A represents the 250 MHz ^{1}H-NMR spectrum of BMV. The few peaks in the 1.0–2.5 ppm region contain about 2% of the total spectral intensity. These peaks can only be assigned to $-CH_2$ and $-CH_3$ groups in hydrophylic amino acids [17], probably located at the protein surface. The linewidth of these peaks corresponds to an upper limit of the rotational correlation time of $\sim 10^{-8}$ s [1–4]. The spectrum of the BMV empty protein capsids (BMV-PT) is shown in fig.1B. The sharp peaks in the 0.5–4.5 ppm region of this spectrum contain ~15% of the total spectral intensity. The position and relative intensities of these peaks are in good agreement with what is predicted from the primary structure of the 25 amino acid N-terminal arm of BMV protein [17,18]. The linewidth of these peaks corresponds to an upper limit for the rotational correlation time of 10^{-9} s.

3.2. *BdMV*

Figure 1C represents the 500 MHz ^{1}H-NMR spectrum of BdMV-B. Except for one small sharp peak at ~3 ppm which probably arises from a mobile lysine sidechain at the protein surface, no mobility on a timescale faster than 3×10^{-8} s can be observed. The spectrum of BdMV-T is shown in fig.1D. The sharp peaks in the 1–4 ppm region contain ~10% of the total spectral intensity. The linewidth of these peaks corresponds to an upper limit of the rotational correlation time of 3×10^{-9} s. In this case we have carried out experiments at 250 MHz (spectra not shown) and 500 MHz, so that we are able to conclude that the observed linewidth is due to isotropic chemical shift differences [3]. The real rotational correlation time is therefore probably much smaller than the above mentioned upper limit of 3×10^{-9} s.

The observed resonances may be assigned to a

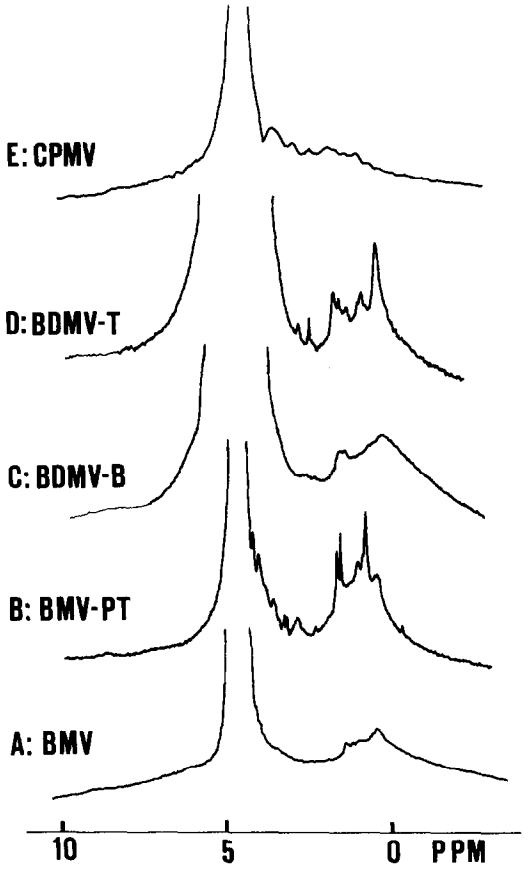

Fig.1. ^{1}H-NMR spectra: (A) BMV at 250 MHz. (B) BMV-PT at 250 MHz. (C) BdMV-B at 500 MHz. (D) BdMV-T at 500 MHz. (E) CPMV combined T, M and B fractions at 250 MHz. Only 10 ppm is shown.

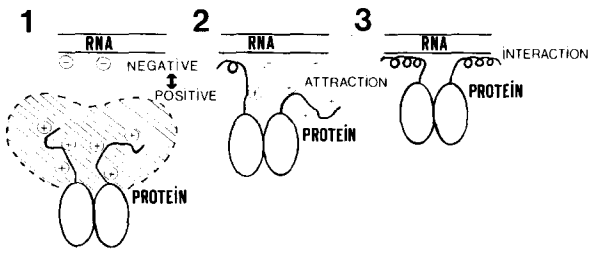

Fig.2. Schematical representation of the protein–RNA interaction model.

few lysine residues, some threonine residues and probably one or two aspartic acid, glutamic acid, glutamine and asparagine residues. No aromatic residues nor cysteine or cystine are present among the mobile amino acids.

3.3. *CPMV*

Figure 1E represents the 250 MHz ^{1}H-NMR spectrum of the unseparated CPMV-T, CPMV-M and CPMV-B components of CPMV purified by the chloroform–butanol method. Since no sharp peaks are observed in this spectrum, it is concluded that neither one of the CPMV components contains internal mobile groups. The spectra of the individual components are not shown since they could not be obtained without large sugar and polyethylene glycol resonances. Except for these resonances, no sharp peaks were observed in the spectra of the individual components.

4. DISCUSSION

In a previous paper we presented a protein–RNA binding study on CCMV. For this virus the 25 N-terminal amino acids were shown to be very mobile in the absence of RNA. In the presence of RNA no mobility was observed. Protein lacking these 25 amino acids was not capable of binding RNA. From the great genetical and morphological resemblance of BMV and CCMV, we expect a similar behaviour for BMV. The spectra in fig.1A and B prove this idea to be correct.

The 25 N-terminal amino acids of BMV protein are almost completely predicted to be in an α-helix conformation [9]. The observed rotational correlation time of 10^{-9} s makes it hard to believe that this protein region is completely α-helical. Therefore, we suggest that the protein region, which binds the RNA adapts a flexible random coil conformation in the absence of RNA. Such a conformation is also found from secondary structure predictions, where the positive charges on the arginine and lysine residues are retained (G. Vriend, unpublished results).

The mobility of the N-terminal protein region is of great importance since it provides a mechanism to enhance the probability of interaction between protein and RNA. The large number of basic residues in this protein region (6 arginine and 3 lysine in CCMV [19]; 7 arginine and 1 lysine in BMV [18]) suggests that interaction between the negative phosphate groups in the RNA and the positive basic amino acids is the primary driving force for nucleoprotein assembly. It has been proven [6,8,2–4,21] or suggested [1,7,9] that many nucleic acid binding proteins provide the best protein–nucleic acid interaction when the protein adapts an α-helix conformation. For this reason we suggest a random coil–α-helix transition for the N-terminal protein region upon binding RNA. Figure 2 is the schematical representation of the model.

The observed spectra of BdMV fit this model very well. The mobile lysine residues in the BdMV-T component provide the positive charges for binding the RNA phosphate groups. Figure 1D proves that immobilization of the mobile protein region occurs upon binding RNA. Interaction between protonated cytosine residues and dicarboxyl acidic acids have been found in turnip yellow mosaic virus (a virus closely related to BdMV) as a secondary mode of protein–RNA interaction [10,21]. The presence of glutamic acid and aspartic acid in the mobile protein region of BdMV suggests that these interactions also take place in BdMV.

Protein–RNA interactions in CPMV strongly differ from those in all other viruses mentioned [10,22]. No internal mobile amino acid residues are observed in any CPMV component, indicating that the assembly process is directed by another mechanism than phosphate–basic amino acid interaction. Interactions between virus protein and genome linked protein have been suggested to be important in the assembly process [22]. The ab-

sence of a mobile RNA binding protein region supports this idea.

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