A mobile element on a virus particle surface identified by nuclear magnetic resonance spectroscopy

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The presence of a mobile element in the coat protein of pepper ringspot tobnavirus (PRV) has been established by ^1H n.m.r. spectroscopy; two-dimensional correlation spectroscopy (COSY) measurements show that this element consists of alanine, aspara-

gine, glycine, proline, serine and threonine only. By reference to the amino acid sequence of the coat protein, it is concluded that the mobile element is associated with the C-terminal region and consists of between 11 and 38 residues.

INTRODUCTION

Transmission of viruses from infected to healthy hosts and infection of cells of hosts are thought often to involve specific chemical bonding between virus particles and either the vector species (Harrison and Murant, 1984) or the membranes of host cells (Rossman et al., 1987). These may involve regions of the surfaces of the virus particles that have a degree of motional freedom, in which case identification of the relevant chemical groups should assist greatly our understanding of these processes. In this paper we report the use of solution proton n.m.r. spectroscopy to identify a mobile element on particles of the plant virus, pepper ringspot tobnavirus (PRV), which is transmitted by soil-inhabiting nematodes.

MATERIALS AND METHODS

PRV (previously known as the CAM strain of tobacco rattle virus; Robinson and Harrison, 1985) has rod-shaped particles 21 nm in diameter with lengths of either 197 nm (L) or 52 nm (S) (Harrison and Woods, 1966). The virus particles are helical arrays of approx. 1960 (L particles) or approx. 520 (S particles) protein subunits, each with an M_r of 23 000 (Mayo and Robinson, 1975).

PRV was propagated in and purified from infected *Nicotiana glauca*. The virus was extracted from frozen leaves by trituration in 0.02 M borate buffer (pH 8) and filtration through muslin (Harrison and Robinson, 1981). Particles were isolated from the filtrate by precipitation with polyethylene glycol followed by two cycles of differential centrifugation (Harrison and Robinson, 1981). Final purification was by centrifugation to equilibrium in CsCl solution (Cooper and Mayo, 1972). In the solutions used in the final centrifugation steps, $^2\text{H}_2\text{O}$ was substituted for H_2O .

Conventional one-dimensional and two-dimensional correlation spectroscopy (COSY) ^1H n.m.r. spectra of samples containing 25 mg of virus in 0.5 ml of 0.03 M $^2\text{H}_2\text{O}$ /phosphate buffer (pH 7) were recorded at 297 K on a Bruker AM500 FT-NMR spectrometer. For all spectra, the Bruker Presat program was used for suppression of the water peaks. Additional experimental details are given in the legends to the relevant Figures.

RESULTS AND DISCUSSION

The one-dimensional ^1H n.m.r. spectrum of PRV particles (Figure 1) shows the presence of several peaks that correspond to mobile residues, but it is difficult to interpret unambiguously because of extensive peak overlap; tentative assignments are indicated on the spectrum. The peaks at 2.5 and 2.7 p.p.m. are, however, characteristic of asparagine protons.

In the two-dimensional COSY spectrum (Figure 2), the presence of alanine, asparagine, glycine, proline, serine and threonine was deduced by using the amino acid assignments documented by Wuthrich (1986); this spectrum also indicates that no other amino acid residues were present in this mobile element. Similar results were obtained with several different preparations of the virus. Also, the widths of the peaks in the one-dimensional spectra were significantly greater than those of free amino acids in solution, which suggests that these amino acids are components of the PRV particles and not products released by proteolytic decomposition of the virus particles.

The amino acid sequence of PRV coat protein (Bergh et al., 1985) shows that only in the 38 C-terminal residues are alanine, asparagine, glycine, proline, serine and threonine present without any other intervening amino acids. All six are present in the last 11 residues, where the sequence is:

Ser²¹³-Gly-Ala-Ala-Pro-Thr-Pro-Pro-Pro-Asn-Pro²²³

This suggests that, if the mobile residues detected by n.m.r. correspond to a single element, it must be an amino acid sequence, at or within one residue of the C-terminus, and containing between 11 and 38 amino acids. Recent immunological work with monoclonal antibodies (Legorburu et al., 1993) has identified the C-terminus of tobacco rattle tobnavirus coat protein as a major epitope. Thus the antigenicity may well be associated with the segmental mobility that we have identified; segmental mobility in tobacco mosaic virus coat protein has previously been linked with antigenicity (Westhof et al., 1984).

Tobnaviruses are transmitted by trichodoriid nematodes (Harrison and Robinson, 1978); PRV is thought to be transmitted by *Trichodorus christiei* (Salomao, 1973). The specific binding to the nematode oesophagus (Taylor and Robertson, 1970) probably involves some chemical interaction with external parts of the coat protein, and we speculate that the mobile C-terminal peptide of the coat protein is the structure involved in the binding and/or

Figure 1 500 MHz proton n.m.r. spectrum of PRV

The spectrum was obtained after 256 scans, using a 90° pulse and 16 K data points. The Bruker Presat program was used for water suppression. (N, asparagine. Other tentative assignments are: A, alanine; G, glycine; P, proline; S, serine; T, threonine.)

Figure 2 Two-dimensional phase-sensitive COSY spectrum of PRV

The spectrum was recorded using 512 data points and 512 increments with 48 scans per increment. The Bruker Presat program was used for water suppression and data were processed by using sine bell enhancement (A, alanine; G, glycine; N, asparagine; P, proline; S, serine; T, threonine).

release of virus particles during vector transmission. The surfaces of some virus particles have been shown to be involved in interactions with a variety of vector organisms (Harrison and Murrant, 1984) and, at least during infection of cells by particles of animal viruses, virus particle surfaces are also known to be involved in interactions with receptors on host cells (Rossman et al., 1987). The non-invasive solution n.m.r. technique thus represents a novel approach to the detection of candidate elements for involvement in these crucial steps in the viral life cycle.

Conclusions

Using a combination of one-dimensional and two-dimensional n.m.r. procedures, the amino acid composition of the mobile element in PRV particles is shown to correspond to that of the C-terminal extremity of the coat protein. This solution n.m.r. approach is effective because the limited mobility of the rigid rod-shaped framework (Harrison and Robinson, 1986) of the virus particles results in resonances that are too broad to detect. Consequently, n.m.r. spectroscopy could prove to be a valuable tool for investigating the mechanisms involved in virus transmission.

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