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# Characterization of the N-terminal segment used by the barley yellow dwarf virus movement protein to promote interaction with the nuclear membrane of host plant cells

*Sarah Rachel Dennison* [a], *Frederick Harris* [b], *Klaus Brandenburg* [c], *David Andrew Phoenix* [a,*]

[a] *Faculty of Science and Technology, University of Central Lancashire, Preston PR1 2HE, UK*
[b] *Department of Forensic and Investigative Science, University of Central Lancashire, Preston PR1 2HE, UK*
[c] *Division of Biophysics, Forschungzentrum Borstel D-2061, Germany*

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ABSTRACT

The barley yellow dwarf virus movement protein (BYDV-MP) requires its N-terminal sequence to promote the transport of viral RNA into the nuclear compartment of host plant cells. Here, graphical analysis predicts that this sequence would form a membrane interactive amphiphilic α-helix. Confirming this prediction, NT1, a peptide homologue of the BYDV-MP N-terminal sequence, was found to be α-helical (65%) in the presence of vesicles mimics of the nuclear membrane. The peptide increased the fluidity of these nuclear membrane mimics (rise in wavenumber of *circa* 0.5–1.0 $cm^{-1}$) and induced surface pressure changes of 2 mN $m^{-1}$ in lipid monolayers with corresponding compositions. Taken with isotherm analysis these results suggest that BYDV-MP forms an N-terminal amphiphilic α-helix, which partitions into the nuclear membrane primarily through thermodynamically stable associations with the membrane lipid headgroup region. We speculate that these associations may play a role in targeting of the nuclear membrane by BYDM-MP.



## 1. Introduction

Barley yellow dwarf virus (BYDV) is the causal agent of a yellow dwarf disease, which is epiphytotic of barley, wheat and oats with infection of these cereals by the virus leading to stunted growth and severe yield losses. This makes BYDV one of the most significant viral disease agents of small grain cereals worldwide and a better understanding of its often poorly understood plant infection mechanisms is of clear agronomic importance [21].

BYDV was first recognized as a new virus in 1951 [30] and is currently taken as the type member of the Luteoviridae family [26]. The virus possesses a single stranded RNA genome [23,24] but in contrast to most other plant RNA viruses [10], infection by BYDV appears to involve a nuclear phase. A number of studies have demonstrated the presence of BYDV genomic RNA, coat protein (CP) and movement protein (MP) in the nucleus of infected oat cells [6,27,28]. Electron microscopy [27] showed that filaments containing both viral RNA and MP were present in the cytoplasm, nucleus and nuclear pores of these infected oat cells for example. It is well established that plant virus MPs promote the intercellular and intracellular spread of the viral genome [2,7,13,20,25,29] but the role of these proteins in promoting the trafficking of BYDV genomic RNA into the nuclear compartment is far from clear. Most recently, some insight into this role was provided by a study on the ability of BYDV-MP to interact with the nuclear envelope (NE) of insect cells [22]. It was found that the protein was able to target the

* *Corresponding author at*: Deans Office, Faculty of Science and Technology, University of Central Lancashire, Preston PR1 2HE, UK. Tel.: +44 1772 893481; fax: +44 1772 892903.
E-mail address: daphoenix@uclan.ac.uk (D.A. Phoenix).
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NE even though lacking classical monopartite (PKKKRKV) [15] or bipartite (KRPAATKKAGQAKKKK) [33] nuclear localization signals (NLSs). It also found that the association of BYDV-MP with the NE caused protrusions to emanate from its surface but that a lipid interactive N-terminal segment of the protein appeared to be required for efficient production of these protrusions [22]. It was speculated by these latter authors that the function of this N-terminal segment was to destabilize membranes, thereby assisting the entry of BYDV into the nuclear compartment. In the present study, we have used theoretical analyses, Fourier transform infrared spectroscopy (FTIR) and monolayer studies to investigate the ability of the BYDV-MP N-terminal segment to form membrane interactive secondary structure and to interact with the nuclear membrane. Our data strongly suggest that BYDV-MP forms an N-terminal amphiphilic α-helix, which partitions into the nuclear membrane primarily through thermodynamically stable associations with the membrane lipid headgroup region. While our results do not support the suggestion that the BYDV-MP N-terminal segment destabilizes the nuclear membrane, we speculate that it may play a role in targeting this membrane.

## 2. Materials and methods

### 2.1. Materials

A peptide homologue (NT1) of residues 4–16 (GEQGALAQFGEWL) of the BYDV-MP N-terminal region [22] was supplied by Pepsyn (UK), synthesized by solid state synthesis and purified by HPLC to purity greater than 99%. Buffers and solutions for monolayer experiments were prepared from Milli-Q water. Dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylserine (DMPS), dimyristoyl phosphatidylphinositol (DMPI) dimyristoyl phosphatidylethanolamine (DMPE) and sphingomyelin were supplied by Alexis (UK). All other reagents were purchased from Sigma (UK).

### 2.2. Graphical analyses

Graphical representation of the putative BYDV-MP N-terminal α-helix was obtained using Winpep software [14], which provides a two-dimensional axial projection taken perpendicular to the α-helical long-axis and assuming an amino acid residue periodicity of 100°.

### 2.3. Preparation of lipid vesicles

Nuclear membrane lipid mix, which possessed a lipid composition representative of the nuclear membrane, was formed from a chloroform solution of PC, PE, PI, PS and sphingomyelin in the molar ratio 62:23:8:5:2 [19]. This lipid mix was then used to prepare vesicles according to Keller et al. [18]. Essentially, the lipid/chloroform mixtures were dried with nitrogen gas and hydrated with 10 mM aqueous *N*-[2-hydroxyethylpiperazine]-*N*′-[2-ethanesulphonic acid] (HEPES) at pH 7.5 to give final total lipid concentration of 50 mM. The resulting cloudy suspensions were sonicated at 4 °C with a Soniprep 150 sonicator (amplitude 10 μm) until clear suspensions resulted (30 cycles of 30 s), which were then centrifuged (15 min, 3000 × *g*, 4 °C) and the supernatant retained.

### 2.4. FTIR analyses of NT1 conformational behavior in presence of lipid

To give a final peptide concentration of 1 mM, NT1 was solubilized in 10 mM aqueous HEPES (pH 7.5) or suspensions of vesicles, which were formed from nuclear membrane lipid mix, prepared as described above. Samples of solubilized peptide were spread on a $CaF_2$ crystal, and the free excess water was evaporated at room temperature. The single band components of the BYDV-MP amide I vibrational band (predominantly C═O stretch) was monitored using an FTIR '5-DX' spectrometer (Nicolet Instruments, Madison, WI, USA) and for each sample, absorbance spectra produced. These spectra were analyzed and for those with strong absorption bands, the evaluation of the band parameters (peak position, band width and intensity) was performed with the original spectra, if necessary after the subtraction of strong water bands. In the case of spectra with weak absorption bands, resolution enhancement techniques such as Fourier self-deconvolution [17] were applied after baseline subtraction with the parameters: bandwidth, 22–28 $cm^{-1}$, resolution enhancement factor, 1.2–1.4 and Gauss/Lorentz ratio of 0.55. In the case of overlapping bands, curve fitting was applied using a modified version of the CURFIT procedure written by D. Moffat (National Research Council, Ottowa, Canada). An estimation of the number of band components was obtained from deconvolution of the spectra; curve fitting was then applied within the original spectra after the subtraction of baselines resulting from neighboring bands. Similar to the deconvolution technique, the band shapes of the single components are superpositions of Gaussian and Lorentzian band shapes. Best fits were obtained by assuming a Gauss fraction of 0.55–0.6. The CURFIT procedure measures the peak areas of single band components and after statistical evaluation, determines the relative percentages of primary structure involved in secondary structure formation with α-helical structure occurring in the region of 1650–1655 $cm^{-1}$ and β-sheet structures in the region of 1625–1640 $cm^{-1}$.

### 2.5. FTIR analysis of NT1 effects on lipid phase transition properties

To give a final peptide concentration of 1 mM, NT1 was solubilized in suspensions of vesicles, which were formed from nuclear membrane lipid mix, prepared as described above. As controls, vesicles formed from this lipid mix alone were prepared. These samples were then subjected to automatic temperature scans with a heating rate of 3 °C $(5\ min)^{-1}$ and within the temperature range 0–60 °C. For every 3 °C interval, 50 interferograms were accumulated, apodized, Fourier transformed and converted to absorbance spectra [5]. These spectra monitored changes in the $\beta \leftrightarrow \alpha$ acyl chain melting behavior of phospholipids with these changes determined as shifts in the peak position of the symmetric stretching vibration of the methylene groups, $\nu_s(CH_2)$, which is known to be a sensitive marker of lipid order. The peak