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Poster Presentation

Potato virus A genome-linked protein is a natively unfolded protein

Kimmo Rantalainen* and Kristiina Mäkinen

Address: Department of Applied Chemistry and Microbiology, University of Helsinki, Helsinki, Finland
* Corresponding author

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Background

The target of the study is *Potato virus A* (PVA, genus *Potyviridae*) and its genome- linked protein (VPg). Most of the PVA proteins are multifunctional, interacting with each other and with host proteins. Many of the functions are still unclear and some completely unknown. VPg is a 23 kDa protein interacting for example with viral polymerase. It has NTP-binding and nuclear localization signals overlapping with each other in the N-terminal end of the protein. Growing evidence shows that genome-linked proteins belong to a class of natively unfolded proteins [1]. Descriptive for this class is regions without fixed structure in the correctly folded and ready-made protein. Interactions between the unfolded region and its natural substrate usually launches folding but structural changes can be regulated also by other reactions such as phosphorylation.

Results

Bioinformatic analysis of PVA VPg was carried out using several different softwares all predicting partly unfolded nature for VPg. Prediction presented in Figure 1A was obtained using PONDR® software http://www.pondr.com and VSL1 algorithm.

CD spectroscopy was used to get general view of VPg structure. Far-UV spectra shows typical characteristics for unstructured protein (see Figure 1B), namely low elliptic-

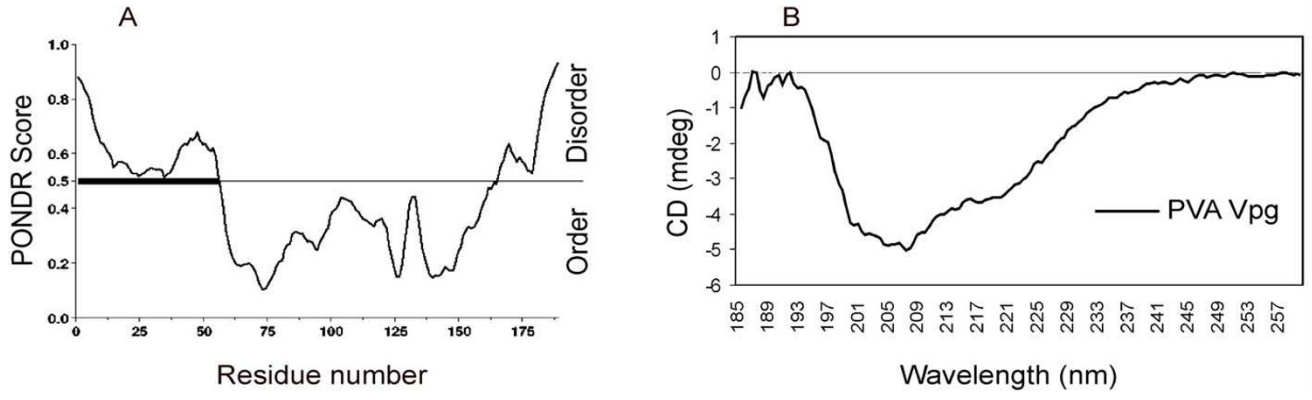

Figure 1
Prediction and experimental evidence of natively unstructured nature of *Potato virus A* VPg *A*, Prediction of unfolded regions in PVA VPg based on amino acid sequence. Region from N' -terminal to Asn56 is here predicted to be unfolded using PONDR® software. *B*, Far-UV spectra of PVA VPg.

ity at 222 nm and strong negative ellipticity near 200. Negative minimum around 208 nm suggests that considerable amount of α-helixes is also present.

Elution profile of size exclusion chromatography gives evidence for either dimeric or unstructured status of VPg (data not shown). Peak corresponding to the smallest protein had approximated size of 49 kDa when calculated size of VPg monomer is 23 kDa. Bulk of the soluble VPg came out in two peaks both corresponding to sizes over 200 kDa indicating VPg's tendency to oligomerize.

Conclusion

Consistently with the predictions our experimental data so far supports the natively unfolded structure fo PVA VPg. In addition, CD spectral data supports the prediction that VPg probably has some stabile structural elements as well. Since VPg is a multifunctional protein, the partly unfolded nature putatively gives possibilities to regulate the VPg function during the different stages of infection. For example, structural stabilization launched by PVA polymerase NIb or nucleatidylation of VPg [2] might be the key regulatory events leading to initiation of replication. However, the possible initiators of structural stabilization at the unfolded region remains to be solved.

References

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