Investigation of Lti30-membrane interaction mechanism using Fluorescence correlation spectroscopy and molecular dynamics simulations

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Dehydrins are intrinsically disordered proteins, generally expressed in plants as a response to embryogenesis and water-related stress. Studies suggest that these proteins perform the role of membrane stabilization and cell protection. The dehydrin Lti30 is expressed in *Arabidopsis thaliana* which gets upregulated in cold stress conditions. All dehydrins contain at least one copy of the highly conserved K-segment. Lti30 comprises of six K-segments each with two adjacent histidines which are shown to be responsible for the binding of this protein with the membrane. The existing literature suggests that the mode via which Lti30 interacts with the membrane is electrostatics and it is pH dependent. In the current work, Fluorescence correlation spectroscopy and molecular dynamics simulations have been used to investigate the membrane interaction mechanism of Lti30. Confocal-FCS is used to measure the translational - diffusion of individual K-segments with and without flanking histidines in solution. However, this FCS modality is not ideal for membrane-based studies as it is prone to many artefacts given the long measurement durations that are required in membrane based studies. Here, for diffusion studies on the membranes Imaging total internal fluorescence correlation spectroscopy (ITIR-FCS) is used. This multiplexed modality has several advantages, firstly allows measurement of diffusion over the whole region of interest simultaneously thereby, reducing the measurement time. Secondly, this method is a calibration-free method as observation area is defined by the chip of the EMCCD camera. The information provided by this method is still diffraction-limited so for sub-resolution information FCS diffusion laws are used. FCS diffusion laws provide information about the mode of diffusion i.e. free diffusion or transient domain trapping.The effect of Lti30 on the diffusion of lipids in the supported lipid bilayers of various compositions has been studied. Since the net charge of Lti30/K-seg are altered by histidine protonation experiments were performed at four mechanistically important pHs. Subsequently, the diffusion of individual K-segment with and without histidines has been measured. The experimental observations are further complemented with molecular dynamics simulations. Results reveal that the effect of Lti30 on the membrane lipid diffusion is composition and pH dependent. K-segment with histidines and without histidines have distinct diffusion profiles indicating the importance of histidines in the interaction mechanism. There are indications of higher-order protein-protein interactions in Lti30 bound to the membrane. The findings gained here are useful for the understanding of the stress tolerance mechanism of Lti30 and for the dehydrins in general.

References:

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