Functional Dynamics of Human Profilin I and II: Interaction with Phosphatidylinositol (4, 5) bisphosphate and Poly-L-proline

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Certification

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

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Kannan Krishnan

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Abstract

Profilins are small (~14 kDa) actin binding proteins found in eukaryotes and certain viruses. Profilins are implicated in cytokinesis, membrane trafficking, cell development and cell motility. Apart from actin, profilins also interact with polyphosphoinositides and proline rich domains containing proteins. However, the role of its interaction with Phosphatidylinositol (4, 5) bisphosphate PI(4,5)P_2 is still poorly understood. In this work, the interaction between profilin and PI(4,5)P_2 was investigated using Giant Unilamellar Vesicles (GUV), a model of the cell membrane. Using Number and Brightness analysis, it was demonstrated that, in the absence of profilin, molar ratios of PI(4,5)P_2 above 4% resulted in lipid demixing and cluster formations. Furthermore, adding profilin to GUVs made with 1% PI(4,5)P_2 led to the formation of clusters in the presence of profilin. The diffusion coefficient of PI(4, 5)P_2 measured on GUV membranes showed large variations but the overall diffusion coefficient change, with and without profilin confirmed the cluster formation.

Using LAURDAN GP, we found that 16% of PI(4, 5)P_2 molecules on GUVs formed domains that were 2500 nm^2 in size. When profilin was added, the membranes become more water accessible suggesting differences in the domains in the absence and presence of profilin, however, the domain size remained the same.

Phosphorylation of profilin has been previously reported, however, the interaction of phospho-profilin on model membranes has not been studied. Our results with respect to PI(4, 5)P_2 interaction suggested that profilin phosphorylation does not affect its interaction with PI(4, 5)P_2 on the membrane. Furthermore, the cluster formation on GUV membranes was similar to that of wild-type profilin.

There are four isoforms of profilin present in humans, apart from the spliced forms of profilin-II. The significance of the presence of these isoforms is not clearly understood. We carried out equilibrium unfolding studies on profilin I and profilin IIA, with and without poly-L-proline. Our results revealed the stabilization effects of poly-L-proline on both profilin isoforms. Our data demonstrated that the stabilization effect of poly-L-

proline was greater for profilin-I than profilin-II but that profilin II was intrinsically more stable than profilin I. 1-anilino-8-naphthalene sulphonate fluorescence revealed that there were non-polar binding sites, which are buried in the native state, and become exposed at a low denaturant concentration. These results suggest that profilin binding to proteins containing proline rich domains would stabilize their structure which would have important effects on the dynamics and regulation of profilins function.