Poster Presentation

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Structure-Function Studies of A. thaliana heterotrimeric G-protein subunits

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Heterotrimeric G-proteins, consisting of alpha, beta, and gamma subunits, participate in major signaling pathways in eurkaryotes and mammals, including those in the nervous and sensory systems. G proteins are activated upon stimulation of G-protein coupled receptors and function as molecular switches through nucleotide cycling by the alpha subunit and by their interactions with intracellular effectors. The mechanism of activation and deactivation of the mammalian heterotrimer is well understood through extensive structural and functional studies, and is being used as a basis for predictions for mechanisms involving the plant heterotrimer. Recent evidence, however, indicates significant differences in the two systems [1]. We undertook a structural investigation of the A. thaliana complex in order to gain insights for G-protein activation and the molecular interactions in the G-protein related signaling pathways in plants. The alpha subunit GPA1 was cloned and expressed in P. pastoris and purified with high yield and homogeneity in functional form. Similarly the beta and gamma subunits (AGB1 and AGG2) were cloned and expressed in E. Coli. AGB1 expression resulted in production of unfolded protein whereas AGG2 could be obtained on its own with high yield and purity. Biochemical analyses and biophysical studies using SAXS, CD, DLS, UV-vis spectroscopy reveal structural parameters of GPA1 for comparison with its mammalian counterparts and indicate the propensity of GPA1 to form oligomers in solution. After purification form E. coli AGG2, can be stabilized in dimeric form and has a flexible and extended structure. Structure of the dimer is sensitive to the concentration of salt and reducing agent in the solution.

[1] *D. Urano, J-G. Chen, J. R. Botella, et al., (2013) Open Biology. 3:120186.*

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