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Biophysical Analyses on Plant Heterotrimeric G-Proteins. Burcu Kaplan Türköz^a, Sedef İskit^a, Zehra Sayers^a. *^aSabancı University, Biological Sciences and Bioengineering, Istanbul-Turkey.*
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Heterotrimeric G proteins; composed of α , β and γ subunits are important signaling molecules found in eukaryotic organisms. The plant heterotrimer is known to be involved in signaling pathways directing cell and plant growth, development and differentiation, ion channel regulation and drought tolerance and biotic stress resistance. Availability of high resolution structural data led to a comprehensive understanding of the mechanism of signaling in mammalian systems. The α subunits have posttranslational lipid modifications which, upon receptor activation, allow them to attach to the plasma membrane and interact with the hydrophobic regions of the receptor. Following receptor activation heterotrimer dissociation or loosening occurs and the α subunit and the $\beta\gamma$ dimer interact with downstream effector molecules to transmit the signal. The α subunit can bind and hydrolyze GTP and this enzymatic activity serves as an on/off switch for the heterotrimeric signaling cycle. There is a lack of direct biophysical and structural data for the plant heterotrimer. We cloned and expressed *Arabidopsis thaliana* α (GPA1) using *Pichia pastoris* and β (AGB1) and γ (AGG) subunits using *E. coli*. GPA1 was isolated in the presence of detergent using his-tag affinity chromatography with a yield of 20 mg / L culture and the protein was further purified by either anion exchange or gel filtration chromatography. Purified proteins were analyzed by several biophysical methods. Anion exchange resulted in separation of two biophysically different forms of GPA1; one in oligomeric but stable form, the other being monomeric but prone to both aggregation and degradation. Gel filtration column purified GPA1, on the other hand, appeared to be homogeneous with a molecular mass higher than that expected from the monomer. NMR analysis showed that this protein was purified together with detergent/lipid micelles. It was shown that all three forms of GPA1 had comparable GTP binding and hydrolysis activity. CD measurements indicated helical secondary structure elements resembling that observed in the native proteins. Attempts to collect SAXS data from the anion exchange purified forms of GPA1 were not successful. SAXS measurements from the gel filtration purified protein were consistent with the presence of protein-micelle complexes (PMC) and the molecular mass was estimated to be ~2.5 fold of GPA1. Mass spectrometry analyses verified the presence of lipid modifications on recombinant GPA1. Interaction of the monomeric form of GPA1 with partially purified β and γ subunits was demonstrated by PAGE analysis. This interaction appeared to reverse the aggregation observed after storage. These results show that the biophysical properties of the oligomeric form and the PMC form of GPA1 are similar and correspond to a stable state which may resemble the membrane-bound form of native GPA1. These studies highlight the tendency of GPA1 to form complexes. It appears that meaningful studies directed to

develop an understanding of the signaling mechanism in plants would require additionally the presence of the $\beta\gamma$ dimer. Purification of β and γ subunits for reconstitution of the recombinant heterotrimer are being investigated.

Keywords: GTP-binding proteins; biophysical analysis; SAXS

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Purification and Structural Analysis of Durum Wheat Metallothionein Domains. Filiz Collak^a, Filiz Yesilirmak^a, Gizem Dinler^a, Zehra Sayers^a. *^aSabancı University, Faculty of Engineering and Natural Sciences, Orhanli, Tuzla, 34956, Istanbul, Turkey.*
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Metallothioneins (MTs) are ubiquitous low molecular weight and cysteine (Cys) rich proteins which have the ability to bind Group 11 and 12 metals. They are classified in one super-family according to the distribution of Cys motifs in their sequences. Contrary to the extensive amount of information on mammalian MTs, structure- function investigations on plant MTs is limited in the literature. Type I plant MTs, similar to mammalian counterparts, have the Cys motifs clustered in the N- and C-termini constituting the β - and α -domains, respectively. The two domains are connected by a long (42 amino acids) hinge region whose structural and functional properties are unclear. A *mt* gene in Cd resistant durum wheat coding for a Type I MT (dMT) was identified and the recombinant protein (dMT) was overexpressed in *E. coli* as GST fusion (GSTdMT) [1]. In the present study, for detailed structural investigations; GST-fusion constructs of β -hinge, α -hinge and the hinge domains of dMT were overexpressed in *E. coli*. Proteins were purified and were characterized by size exclusion chromatography, SDS- and native-PAGE, limited trypsinolysis, inductively coupled plasma optical emission spectroscopy (ICP-OES), UV-vis absorption spectroscopy, dynamic light scattering (DLS) and small-angle solution X-ray scattering (SAXS). Studies of the isolated domains indicate, similar to mammalian case, distinct metal-binding properties for the β -hinge and α -hinge domains. The combination of SAXS results with biochemical data indicated extended structures for the dMT domains and supports the dumbbell model previously proposed for durum wheat MT [1].

[1]. Bilecen, K., Ozturk, U.H., Duru, A.D., Sutlu, T., Petoukhov, M.V., Svergun, D.I., Koch M.H., Sezerman, U.O., Cakmak, I., Sayers, Z. Triticum durum metallothionein. Isolation of the gene and structural characterization of the protein using solution scattering and molecular modeling. *J Biol Chem.* **2005**, 280(14):13701-11.

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Structural Studies on Mutant Wheat Metallothioneins. Ceren Saygi^a, Anil Akturk^a, Mert Aydın^a, Filiz Yesilirmak^a, Zehra Sayers^a. *^aSabancı*