FA1-MS11-P01

Biophysical Analyses on Plant Heterotrimeric G-Proteins. Burcu Kaplan Türköz[a], Sedef İskit[a], Zehra Sayers[a]. [a]*Sabanci University, Biological Sciences and Bioengineering, Istanbul-Turkey.*
E-mail: bkaplan@su.sabanciuniv.edu

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 βγ 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 βγ dimer. Purification of β and γ subunits for reconstitution of the recombinant heterotrimer are being investigated.

Keywords: GTP-binding proteins; biophysical analysis; SAXS

FA1-MS11-P02

Purification and Structural Analysis of Durum Wheat Metallothionein Domains. Filiz Collak[a], Filiz Yesilirmak[a], Gizem Dinler[a], Zehra Sayers[a]. [a]*Sabanci University, Faculty of Engineering and Natural Sciences, Orhanli, Tuzla, 34956, Istanbul, Turkey.*
E-mail: filiz@su.sabanciuniv.edu

Metallothioneins (MTs) are ubiqutious 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.

Keywords: metalloproteins; SAXS; structural modelling

FA1-MS11-P03

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

University/Faculty of Engineering and Natural Sciences/Istanbul-Turkey.
E-mail: cerensaygi@su.sabanciuniv.edu

Metallothioneins (MTs) are classified as low molecular weight, cysteine-rich, metal binding proteins. The large number of cysteine (Cys) residues in MTs bind a variety of metals by mercaptide bonds [1]. A novel MT gene (dmt) in Triticum durum was identified and cloned for overexpression in E.coli [2]. T. durum metallothionein (dMT) displays three sequence domains: metal binding N terminus (β domain, 1-19th residues) and C terminus (α domain, 61-75th residues) and a long hinge region (20-60th residues). Cysteines are clustered equally in N and C termini with a "Cys-X-Cys" motif (Cys-motif) and the hinge region possess no Cys residues. dMT was overexpressed in E.coli as a GST (glutathione-S-transferase) fusion protein (GSTdMT). Both GSTdMT and dMT cleaved from GST were purified and characterized by biochemical and biophysical methods. It was shown that GSTdMT binds 4±1 moles of Cd per one mole of protein and has a high tendency to form stable oligomeric structures [3]. The aims of the present work are investigation of the effect of removal of the hinge region connecting the two metal binding domains on the stability of the protein structure, and determination of the effect of Cys-motif modifications on the metal binding capacity and affinity of dMT. Furthermore removal of the hinge region will allow comparison with the structure of mammalian MTs which tend to possess short connecting hinge region. Structural features of all mutants will be investigated using biophysical methods such as gel filtration chromatography, SDS- and native PAGE, dynamic light scattering, atomic absorption spectroscopy and circular dichroism spectrometry. Removal and linking procedure of hinge region are executed by PCR techniques. The "chimeric" dMT is inserted to the vector pEGX4T-2 and BL21 strain E.coli has been transformed with this construct. Cys motifs are modified to produce mutant proteins with CCSCG, GCSCC or CCSCC motifs. Mutations are accomplished by site-directed mutagenesis and the mutant constructs are introduced into the pGEX4T-2 vector for expression in E. coli. Results of mutations on the expression of recombinant proteins and their metal-binding properties will be presented.

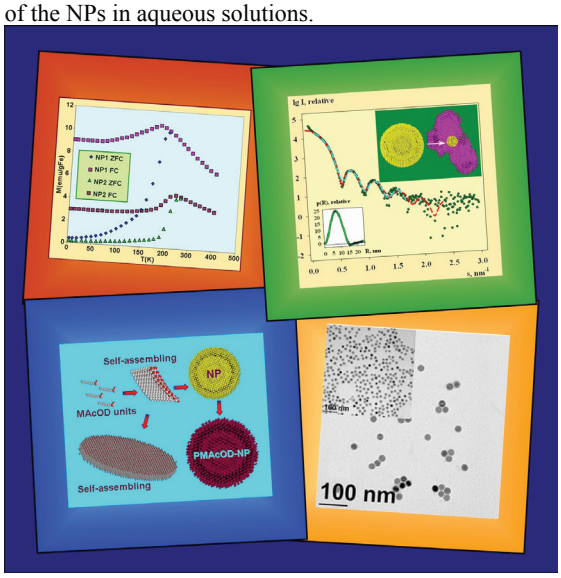
[1] C. Cobbett, P. Goldsbrough, A*nnual Review of Plant Biology*, vol. 53, no. 3, pp. 159-182, **2002**. [2] K. Bilecen, Ü.H. Öztürk, A.D. Duru, T. Sütlü, M. Petoukhov, D.I. Svergun, M.H.J. Koch, U. Sezerman, I. Cakmak and Z. Sayers, *The Journal of Biological Chemistry*, vol. 280, no. 14, pp. 13701-13711, **2005**. [3] F. Dede, G. Dinler, and Z. Sayers, *Proc. of the NATO AdvancedResearch Workshop*, Published by Springer Verlag, Heidelberg, pg 135-146, **2006**.

Keywords: metallothioneins; mutagenesis; cys motifs

FA1-MS11-P04

Structural Study of Metal Nanoparticles, Promising Biotechnological Materials. Eleonora Shtykova[a], Petr Konarev[b], Lyudmila Bronstein[c], Dmitri Svergun[b]. [a]*Institute of Crystallography, Russian Academy of Sciences, Moscow, Russia.* [b]*European Molecular Biology Laboratory Hamburg Outstation, Hamburg, Germany.* [c]*Department of Chemistry, Indiana University, USA.*
E-mail: shtykova@ns.crys.ras.ru

Iron oxide magnetic nanoparticles (NPs) are increasingly popular in life science and medicine because they are easily metabolized or degraded *in vivo*, and can be used as biosensors, bioprobes, in cancer treatment, *etc*. Precondition for such applications is water solubility, which can be achieved by introducing a biocompatible shell on the hydrophobic NP surface. In this work we report structure and properties of iron oxide NPs synthesized by decomposition of iron oleates and encapsulated by different methods. The detailed structure of these particles in aqueous solutions was determined using small angle X-ray scattering (SAXS) providing structural information at resolution from about 1-2 to about 100 nm. The SAXS studies were complemented by several other methods, in particular, X-ray diffraction, transmission electron microscopy, dynamic light scattering, magnetometry etc providing a comprehensive description of the NPs formation and encapsulation processes. Novel SAXS data analysis methods [1-2], in combination with several complementary techniques allowed us to build detailed low resolution three-dimensional structural models of the NPs in aqueous solutions.



This work has been supported, in part, by the NATO Science for Peace Program (grant SfP-981438), NSF grant BES-0322767, MetaCyt funds, the European Union FP6 Infrastructures Program (Design Study SAXIER, RIDS 011934), the grant of Leading Scientific School "Dynamic and Kinematic Scattering of X-Rays and Electrons" and the grant of the Russian Academy of Sciences "Chemistry and Material Science 2008".

[1]Svergun, D. I., **1999**, Biophys. J. 76, 2879. [2] Petoukhov, M.V. & Svergun, D. I., **2005**, Biophys J. 89, 1237.

Keywords: SAXS; biotechnological materials; *ab-initio* structure determination