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Low resolution solution structure of the HSP90:SGT1 complex from the Small-Angle X-ray Scattering

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Heat Shock Protein 90 kDa (HSP90) is a molecular chaperon that is involved in protein folding and maturation [1,2]. It is conserved across species and in higher organisms exist in multiple isoforms that are differentially regulated or have distinct cellular localization. HSP90 plays important role during heat shock and other stress conditions but is also engaged in maintaining protein homeostasis during normal growth of the cell. In contrast to other heat shock proteins HSP90 binds to the partially folded client proteins and interacts with many co-chaperones that modulate HSP90 cycle through inhibition/activation of its ATPase activity. Moreover some co-chaperones are specific for one class of client proteins like for example kinases or innate immunity receptors. Briefly, co-chaperone cycle of Hsp90 is as follows [2]: partially folded protein is transported to the HSP90 from the HSP40/HSP70 protein complex by the binding to the HOP protein. At the same time HSP90 dimer binds two ATP molecules. Subsequently Peptidyl-Proly Isomerase (PPIase) binds to the HSP90/HOP/ATP/client protein complex and next AHA1 protein displaces HOP from HSP90 complex and stimulates formation of closed N-terminal dimerized conformation of HSP90. After N-terminal dimerisation, p23 binds and displaces from the complex AHA1 and stabilizes the closed conformation. After ATP hydrolysis PPIase, p23 and mature client protein is released.

HSP90 is composed of three structural domains: N-terminal domain (NTD) responsible for nucleotide binding and its hydrolysis, middle domain (MD) that plays role in ATP hydrolysis and is involved in the protein substrate binding and C-terminal domain (CTD) which is responsible for constitutive dimer formation. In solution HSP90 exists in equilibrium between open "apo-like" conformation and closed "nucleotide bound-like" conformations in the absence and in complex with ATP [3]. In addition in electron microscopy experiments, distinct, ADP bound compact conformation could be observed. Conformational equilibrium can be changed by the increased concentration of osmolites and pH. Equilibrium is also species dependent. In bacteria HSP90 homolog exists as a mixture of open and closed states as opposed to the human homolog which exists mainly in open conformation and closed state could be observed in electron microscopy after cross-linking.

Suppressor of G2 allele of SKP1 (SGT1) protein is HSP90 co-chaperone and its also conserved across species. SGT1 protein plays important role in innate immunity in both plants and animals. It is required for proper function and stability of nucleotide binding leucine-rich repeats (NB-LRR) class of cytosolic receptors that recognizes pathogenic molecules inside the cell and triggers immune response [4,5]. Sgt1 consists of three domains N-terminal TPR domain required for dimerisation, middle CS domain that interacts with HSP90 and RAR1, SGS domain required for association with R proteins.

In this study we investigated complex formation between truncated SGT1 protein (without SGS domain) from barley and HSP90 protein from wheat in the presence of ADP. Using MCR-ALS method and series of SAXS experimental data for the SGT1-TPR-CS, HSP90 and complex at various stoichiometries we extracted pure scattering curve for the complex. Unexpectedly, comparison of molecular weight obtained from SAXS revealed presence of putative 2:1 HSP90:SGT1 complex. Our results are unexpected because each HSP90 monomer possesses one binding site for CS domain of SGT1. Moreover HSP90 in complex with SGT1 exists in open conformation so there is no interaction between HSP90 monomers that could disrupt SGT1 binding.

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