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Solution structure of the plant HSP90-SGT1 complex with ADP

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Heat Shock Protein 90 kDa (HSP90) protein is a molecular chaperone that assists the folding of the client proteins [1]. HSP90 takes part in the last step of this process using ATP to catalyze transition of the partially folded substrate to the stable folded state. It was also shown that HSP90 can stabilize proteins in inactive/active metastable conformation in the absence of particular stimuli for example binding partners or small molecule ligands. To the most important client proteins belongs: oncogenic kinases like BRAF and v-SRC, myosin heavy chain, telomerase reverse transcriptase and ligand binding domain of steroid receptors. Blocking of HSP90 activity in the cell is one of the most promising approach to inhibits tumor growth and several small molecule inhibitors are currently in the clinical studies.

HSP90 protein consists of three domains: N-terminal ATPase domain, middle substrate binding domain and C-terminal domain that is responsible for the dimerization [1]. All three domains of the HSP90 protein participates in protein-protein interactions. From structural studies it's known that upon binding to the ATP molecule HSP90 change conformation from open (with non-interacting N-terminal domains) to the closed conformation (with additional dimerization of the N-terminal domains) [2]. In solution HSP90 protein exists in the equilibrium between open and closed conformation.

Several proteins, called co-chaperons, binds to HSP90 protein and modulates its function by either inhibiting its ATPase activity: like p23 and HOP protein or by activating its enzymatic activity like AHA1 protein. Other proteins are responsible for recruiting special class of client proteins: for example Cdc37 protein is required for the recruiting of kinases to the HSP90 complex. In addition some of those proteins stabilizes one of the conformations of HSP90 protein. For example p23 protein stabilizes closed conformation of HSP90 in the complex with ATP.

In plants and mammals HSP90 protein complex is involved in the innate immunity by the stabilizing NB-LRR (nucleotide binding leucine rich repeats receptors) receptors that recognizes pathogenic molecules or results of its action within the cell and triggers immune response against pathogenic agents [2]. For this function HSP90 protein requires two proteins: SGT1 (suppressor of G2 allele of skp1) and RAR1 (required for MLA12 resistance 1). SGT1 protein consists of three domains: N-terminal TPR domain that is required for dimerization (in plants and fungi), central CS domain that is responsible for the interaction with N-terminal domain of HSP90 and C-terminal SGS domain that is thought to be involved in the NB-LRR protein binding. RAR1 protein consist of two zinc finger domains called CHORDs and in metazoans also CS domain at the C-terminus. Both proteins have dynamic structure with well folded domains behaving as rigid bodies and dynamic unstructured regions between them. Although structure of the core of the HSP90-SGT1-RAR1 protein complex is known there is little information about the structure of the full length complex.

In this work we studied low resolution structure of the plant HSP90-SGT1 complex with ADP in solution using small angle X-ray scattering technique. Using MCR-ALS analysis and *ab-initio* modeling we found that in the complex with the HSP90, SGT1ΔSGS protein exists as a monomer and binds to the HSP90 protein with the 1:2 stoichiometry. This is unexpected because HSP90 protein has two potential binding sites for SGT1 protein and TPR domain which is responsible for dimerization doesn't interact with HSP90 protein. By the studying of the HSP90 C-terminal truncation variant in the complex with the SGT1ΔSGS protein we showed that dimerization of HSP90 is not required for the dissociation of SGT1ΔSGS protein dimer. We also showed that binding of the CS domain alone doesn't change conformation of the HSP90ΔC protein. In the complex with SGT1ΔSGS protein and ADP molecule HSP90 protein adopts open conformation. Because HSP90 protein interacts with many partners at different steps in client folding proper stoichiometry of the functional HSP90 complex is important for its function. Asymmetric binding of its partners may be one of the ways to prevent improper assembly of the HSP90 complex.

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