

Low resolution structure of the plant HSP90-SGT1 complex with ADP

M. Taube^{1*}, A. Jarmołowski² and M. Kozak^{1,3}

¹Adam Mickiewicz University, Faculty of Physics, Joint Laboratory for SAXS studies, Umultowska 85, 61-614 Poznan, Poland

²Adam Mickiewicz University, Faculty of Biology, Institute of Molecular Biology and Biotechnology, Department of Gene Expression, Umultowska 89, 61-614 Poznan, Poland

³Adam Mickiewicz University, Faculty of Physics, Department of Macromolecular Physics, Umultowska 85, 61-614 Poznan, Poland

Keywords: small angle X-ray scattering, HSP90 protein, chaperones, low resolution structure

*e-mail: mtaube@amu.edu.pl

HSP90 is a protein (MW=90kDa), which belongs to the family of heat shock proteins, molecular chaperons involved in protein folding and maturation [1,2]. This protein is involved in heat shock response and against other stresses but is also engaged in maintaining protein homeostasis during normal growth of the cell. HSP90 protein binds to the partially folded client proteins and interacts with many co-chaperones that modulate HSP90 client folding cycle through inhibition/activation of HSP90 ATPase activity.

Three structural domains (N-terminal - responsible for nucleotide binding and its hydrolysis, middle domain which is mainly involved in the substrate binding and C-terminal domain - responsible for dimerisation) form the HSP90 molecule. In solution HSP90 protein exists in equilibrium between open conformation and closed conformations in the absence and in complex with various nucleotides [3]. 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.

Co-chaperone of HSP90 is SGT1 protein (suppressor

of G2 allele of SKP1). This 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 molecule is composed of three domains: TPR (N-terminal domain) - required for dimerisation, middle CS domain that interacts with HSP90 and RAR1 protein, SGS domain (C-terminal) required for association with R proteins.

The aim of our study was characterization of low resolution structure of the complex between deletion mutants of HSP90 protein or full length HSP90 protein (HSP90-FL with SGT1ΔSGS protein (SGT1 protein with deleted SGS domain) in the presence of ADP. The pure SAXS curves of the complexes were obtained from experimental SAXS data using MCR-ALS algorithm. The modelling of the low resolution structures (bead models) was performed using *ab-initio* modeling approach. HSP90 in complex with SGT1 exists in open conformation so there is no interaction between HSP90 monomers that could disrupt SGT1 binding. We also proved that N-terminal domain is sufficient for the protection of monomeric state of the SGT1ΔSGS protein. The analysis of molecular mass of the HSP90-FL:SGT1ΔSGS complex obtained from SAXS revealed 2:1 stoichiometry. Our results are unexpected because each HSP90 monomer possesses one binding site for CS domain of SGT1 and also SGT1ΔSGS exists as a dimer in solution.

Acknowledgments: This work was supported by the grant (2012/05/N/ST3/03087) from National Science Center.

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