SOLUTION STRUCTURE OF RAR1-GST-TAG FUSION PROTEIN

M. Kozak [1*], M. Taube [1], E. Banachowicz [2], and A. Jarmołowski [3]

[1] *Department of Macromolecular Physics, Faculty of Physics, A. Mickiewicz University, ul. Umultowska 85, 61-614 Poznań, Poland*

[3] *Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, A.Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland*

[2] *Molecular Biophysics Division, Faculty of Physics, A.Mickiewicz University, ul. Umultowska 85, 61-614 Poznań, Poland*

Keywords: small angle X-ray scattering, solution structure, Rar1

**) e-mail: mkozak@amu.edu.pl*

Plants are able to recognize pathogen attack and respond to them by R gene triggered resistance and cell death [1, 2]. Rar1 protein is a common element of signaling pathways in those processes.

Rar1 is a small, monomeric protein (for example Rar1 from *Hordeum vulgare* is characterized by molecular mass of 25.5 kDa and polypeptide chain is build of 232 amino acid residues). Sequence consist of two 60-amino acid zinc binding domains (CHORDs), located on the C- and N-terminus of the protein [1]. In plants a highly conserved motif between those two regions can be found. It is also a zinc binding motif, containing three invariant cysteine and a histidine residues.

At molecular level Rar1 interacts with two important proteins - suppressor of the G2 allele of Skp1 (SGT1) and heat-shock protein 90 (HSP90) via respectively CHORDI and CHORDII domains [2].

The low-resolution structure and conformation of Rar1-Glutathione S-transferase-tag fusion protein from barley in solution has been studied by small angle scattering of synchrotron radiation (SAXS).

SAXS measurements were preformed on the X-33 EMBL beamline at DESY, Hamburg (Germany) using the Pilatus photon counting detector. Protein samples (4.7, 7.5, 15.3 mg/ml) were measured in 50 mM Tris/HCl pH 7.6 using synchrotron radiation (wavelength λ=0.15 nm) at temperature 283 K. The sample-to-detector distance was 1.7 m, corresponding to the scattering vector range form 0.057 to 5.178 nm^{-1} ($s = 4\pi\sin\theta/\lambda$ where 2θ is the scattering angle).

The overall structural parameters characterizing molecule, such as maximum diameter D_{max} and radius of gyration were computed using GNOM. The Rar-1-GST fusion protein forms in solution dimers characterized by R_G= 6.19 nm and D_{max} = 23 nm.

Using experimental SAXS curve also the 3D model has been reconstructed using *ab initio* methods and program DAMMIN [3]. The exemplary low resolution model in solution is presented in Fig. 1.

We also compared the low resolution model of particle with structure predicted by homology-modelling using SWISS-MODEL (www.expasy.ch).

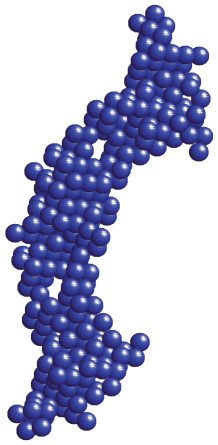

Figure 1. Low-resolution model of Rar1-Glutathione S-transferase-tag fusion protein in solution reproduced by DAMMIN.

Acknowledgements: The data collection was supported by European Community - EMBL Hamburg Outstation, contract number: RII3-CT-2004-506008.

References

[1] K. Shirasu, T. Lahaye, M.W. Tan, F. Zhou, C. Azevedo, "A novel class of eukaryotic zinc-binding proteins is required for disease resistance signaling in barley and development in C. elegans," *Cell* **99** (1999) 355−366.

[2] C.T. Heise, C.S. Le Duff, M. Boter, C. Casais, J.E. Airey, A.P. Leech, B. Amigues, R. Guerois, G.R. Moore, K. Shirasu, C. Kleanthous, "Biochemical characterization of Rar1 cysteine- and histidine-rich domains (CHORDs): A novel class of zinc-dependent protein−protein interaction modules," *Biochem.* **46** (2007) 1612−1623.

[3] D.I. Svergun, "Restoring low resolution structure of biological macromolecules from solution scattering using simulated annealing," *Biophys. J.* **76** (1999) 2879−2886.