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## The study of the conformational flexibility and oligomerization state of the N-terminal fragment of the HYL1 protein by the small-angle X-ray scattering and the circular dichroism spectroscopy

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The HYPONASTIC LEAVES 1 (HYL1) protein from *Arabidopsis thaliana* is one of the core components of microRNA biogenesis machinery alongside the SERRATE (SE) and the DICER-LIKE 1 (DCL1) proteins [1]. It was shown to interact with both SE and DCL1 *in vitro* and *in vivo* and to be required for accurate excision of mature miRNAs from the precursors (pri-miRNA). The N-terminal fragment of HYL1, possessing two double-stranded RNA-binding domains (dsRBD1 and dsRBD2) was shown to be sufficient for rescue of the HYL1-deficient phenotype of *Arabidopsis thaliana* plants, restoring the level of miRNA accumulation. The crystal structures of dsRBD1 and dsRBD2 revealed the double-stranded RNA binding surface and dimerization interface, respectively [2]. The dimerization was further confirmed by the *in vivo* and *in vitro* experiments and found crucial for proper selection of the DLC1 cleavage sites within the miRNA precursor. The dsRBD2 is also responsible for interaction with SE and DCL1 proteins.

The in-solution structure of N-terminal fragment containing both dsRBDs connected by the linker of 15 amino acids (residues 15-172, $HYL_{15-172}$) was examined by the circular dichroism (CD) spectroscopy and the small-angle X-ray scattering (SAXS). Since the Kratky plot suggested clear conformational flexibility of the protein, we applied Ensemble Optimization Method (EOM) [3] for the SAXS data analysis. EOM generates pool of models consisting of the rigid bodies connected by linkers of random conformation. In the next step, the software uses the genetic algorithm to select the ensemble of models that fits best to the experimental data. In the modeling we used two pools of models - one generated for monomeric and second one generated for dimeric assembly of $HYL_{15-172}$ - to check the oligomerization state of the protein. We performed EOM analysis using both pools individually and after mixing them both. The best fit ($\chi^2 = 0.951$) was obtained for the ensemble consisting of seven models of the $HYL_{15-172}$, one of which being model for the dimeric $HYL_{15-172}$ with an abundance of ~ 7% and radius of gyration ($R_g$) of ~ 42 Å and the rest being models for the monomeric protein with $R_g$ ranging from 21 to 30 Å, which indicates flexibility. The best $\chi^2$ value obtained from EOM analysis for the ensemble consisting of the monomers only was 0.958 with slight discrepancy of the fit at low scattering angles, prompting the conclusion that a small fraction of the dimer was indeed present in the studied sample. This stays in agreement with previously published data, indicating that the purified full-length HYL1 is monomeric but can be shiftted towards the dimeric state by addition of the dsRNA [2].

The results of EOM analysis were complemented by the CD spectroscopy measurements. Fitting the experimental CD spectrum with the Contin-LL algorithm revealed that almost 40% of the $HYL_{15-172}$ protein is disordered, pointing the low level of the ordered secondary structures and suggesting possible conformational disorder.

These results together indicate that the dsRNA-binding domain of the HYL1 protein is connected with the second domain responsible for the dimerization and protein-protein interactions *via* flexible linker and that $HYL1_{15-172}$ protein has dynamic structure in solution. They also demonstrate the usefulness of the EOM tool in determination of the oligomerization state of protein in the studied sample, as was also shown in the previous works [4]. Hopefully, presented data together with future studies will provide a step towards understanding the miRNA biogenesis in plants on the structural level.

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