# Analysis of the role of N-terminal domain as a factor that modulates functions of globular domains of *Ultraspiracle* receptor

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## ABSTRACT

The object of research in this work was a nuclear receptor *Ultraspiracle* from *Helicoverpa armigera* (HaUsp). Usp is an invertebrate nuclear receptor (NR), but it is homologous to mammalian retinoid X receptor (RXR). The main aim of this work was investigation whether N-terminal domain (NTD) is a factor that modulates the structure and thus functions of remaining domains of Usp. Theoretically, NTD may perform this role in two ways. Firstly, what seems obvious, NTD may directly interact with different compounds or coactivators and thus modulate functions of whole NR. Secondly, what is not so obvious, regulatory role of NTD may be the result of its interaction with remaining globular domains.

To accomplish main purpose of this work two expression vectors were prepared. One of them encoded full length HaUsp, and second one encoded HaUsp deprived of NTD (HaUsp_ΔNTD). Then, optimal expression conditions of both proteins in bacterial system were determined and efficient purification procedures were developed. It provided sufficient amount of both proteins characterized with high purity necessary for further analyses.

An introduction to *in vitro* analyses was a complex *in silico* analysis. It allowed for identification of intrinsically disordered regions (IDRs) in HaUsp and determination of their range. Registered with circular dichroism spectroscopy (CD) spectrum of HaUsp was typical spectrum for globular proteins, however further deconvolution indicated also a significant amount of disordered fragments (around 25%). Finally, with hydrogen-deuterium exchange mass spectrometry (HDX-MS), we were able to precisely determine the location of disordered fragment using experimental technique. Considering complex, partially disorder organization of HaUsp we decided to analyze its unfolding process in the presence of chaotropic salt, which is GdmCl. Two independent techniques was utilized – CD spectroscopy and size-exclusion chromatography (SEC) and both of them showed that HaUsp is stable up to 1,5 M GdmCl. Structure destabilization begins from 1,75 M GdmCl. Furthermore, SEC analysis suggested presence of three different conformers in the sample of HaUsp during unfolding process. This may be the result of unfolding beginning with one of two domain.

All obtained results were in good agreement and indicated that HaUsp possesses typical for NRs modular organization and therefore it may serve as good NR model for further analyses.

The influence of NTD on functions of HaUsp was tested by investigation of two main roles of NRs – interaction with specific DNA sequence and oligomerization. Electrophoretic mobility shift assay showed that HaUsp similarly to Usp from *Drosophila melanogaster* (DmUsp) forms monomeric and dimeric complexes with DNA. However, in case of HaUsp this interaction seems

to be weaker than in case of DmUsp. What is more, the presence of NTD significantly affects the interaction with specific DNA sequence changing the pattern of this interaction observed on gel. To investigate the influence of this domain on oligomerization process different, independent techniques were utilized. Sedimentation velocity analytical ultracentrifugation (SV-AUC) experiments showed that both HaUsp and HaUsp_ΔNTD have tendency for concentration dependent, fast and reversible self-association and dimer formation, but in a case of HaUsp formed dimers seemed to be more stable. Sedimentation equilibrium analytical ultracentrifugation (SE-AUC) experiments confirmed previous assumptions that NTD stabilizes dimers formed by full-length HaUsp. Subsequent experiments with size-exclusion chromatography coupled with multi-angle light scattering (SEC-MALS) also indicated concentration dependent dimerization and NTD influence on formation of more stable HaUsp dimers. Finally, experiments with small-angle X-ray scattering (SAXS), allowed for better understanding of NTD role. In this case, concentration dependent dimerization was also observed, but obtained data allowed for creation of models of HaUsp and HaUsp_ΔNTD. Based on them, we suppose that present in full-length HaUsp NTD bends toward ligand binding domain (LBD) and forms so called *scorpion-like* structure. This structure may lead to the interaction of these two domains and in turn it may influence the structure of LBD and surface of dimerization.