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Intrinsically disordered N-terminal domain of the *Helicoverpa armigera* Ultraspiracle stabilizes the dimeric form via a *scorpion-like* structure

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ABSTRACT

Nuclear receptors (NRs) are a family of ligand-dependent transcription factors activated by lipophilic compounds. NRs share a common structure comprising three domains: a variable N-terminal domain (NTD), a highly conserved globular DNA-binding domain and a ligand-binding domain. There are numerous papers describing the molecular details of the latter two globular domains. However, very little is known about the structure-function relationship of the NTD, especially as an intrinsically disordered fragment of NRs that may influence the molecular properties and, in turn, the function of globular domains. Here, we investigated whether and how an intrinsically disordered NTD consisting of 58 amino acid residues affects the functions of the globular domains of the Ultraspiracle protein from *Helicoverpa armigera* (HaUsp). The role of the NTD was examined for two well-known and easily testable NR functions, i.e., interactions with specific DNA sequences and dimerization. Electrophoretic mobility shift assays showed that the intrinsically disordered NTD influences the interaction of HaUsp with specific DNA sequences, apparently by destabilization of HaUsp-DNA complexes. On the other hand, multi-angle light scattering and sedimentation velocity analytical ultracentrifugation revealed that the NTD acts as a structural element that stabilizes HaUsp homodimers. Molecular models based on small-angle X-ray scattering indicate that the intrinsically disordered NTD may exert its effects on the tested HaUsp functions by forming an unexpected *scorpion-like* structure, in which the NTD bends towards the ligand-binding domain in each subunit of the HaUsp homodimer. This structure may be crucial for specific NTD-dependent regulation of the functions of globular domains in NRs.

1. Introduction

Nuclear receptors (NRs) are a family of transcription factors that bind and respond to certain small lipophilic compounds, e.g., steroid hormones. Despite the many differences in the amino acid sequences of different NRs, the overall molecular structures of NRs are largely similar. Most of characterized NRs share a common structural organization consisting of at least four distinct functional domains [1]. There are two highly conserved well-folded domains of well-known structures as well as variable, often disordered domains of different lengths and amino acid compositions. The intrinsically disordered domains seem to be dominant regulators of NR activation and activity in general. The first evolutionarily conserved domain is a DNA-binding domain (DBD) [2]. This domain contains two zinc-finger modules that allow the DBD to bind to specific response elements (REs) [3]. The second conserved domain is a ligand-binding domain (LBD), which is responsible not only for recognition of specific ligands but also for interactions with modulators, such as coactivators and corepressors, and this domain participates in homo- and heterodimerization of NRs [4]. The overall LBD fold comprises up to 12 α helices and often one β turn [5]. As has been shown for the RXR-α LBD, three of the central helices form a ligand-binding pocket (LBP), whereas the last helix is mobile. After the ligand

Abbreviations: NRs, nuclear receptors; NTD, N-terminal domain; DBD, DNA-binding domain; LBD, ligand-binding domain; EcR, ecdysone receptor; Usp, Ultraspiracle; IDR, intrinsically disordered region; HaUsp, Usp from *Helicoverpa armigera*; HaUsp_ΔNTD, Usp from *Helicoverpa armigera* lacking the NTD; CD, circular dichroism; HDX-MS, hydrogen-deuterium exchange monitored by mass spectrometry; EMSA, electrophoretic mobility shift assay; SV-AUC, sedimentation velocity analytical ultracentrifugation; SE-AUC, sedimentation equilibrium analytical ultracentrifugation; SEC-MALS, size-exclusion chromatography with multi-angle light scattering; SAXS, small-angle X-ray scattering

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binds in the LBP, the movement of the last helix leads to the formation of activation function 2 (AF-2), which in turn provokes the dissociation of corepressors and association of coactivators [5]. An intrinsically disordered region (IDR) D is localized between the DBDs and LBDs. This region serves as a flexible hinge connecting these two folded domains, and in some NRs, this region contains a nuclear localization signal [4]. One of the most puzzling domains of NRs, in addition to the F domain, is the N-terminal domain (NTD). There are numerous papers describing the molecular properties of the DBD and LBD [2,5–8]. However, very little is known about the structure-function relationship of the NTD. The structures of NTDs are often predicted to be highly disordered [9,10], and in general, substantial differences in the sequences and lengths of the polypeptide chains of various NRs and various isoforms of the same NRs are characteristic features of NTDs [4,11]. The variability in the length and structural flexibility of the NTD are important for temporal interactions with either NR coregulators or multi-protein assemblies [12]. NTDs are known to interact with the C termini of NRs when the NTD possesses an FxxLF motif, which is similar to the LxxLL motif of coactivators interacting with AF-2 [13]. However, an in-depth study of the mineralocorticoid receptor (MR) revealed that the NTD of this receptor does not contain an FxxLF or LxxLL motif, and yet, N/C interaction is observed in the presence of a ligand [14]. As a result of alternative splicing, different isoforms of NRs are generated, which are often characterized by different spatial and temporal distributions within various cells. For example, alternative splicing gives rise to several isoforms of the glucocorticoid receptor (GR), which are characterized by different activities in a cell-dependent manner [15]. However, these studies were conducted on vertebrate NRs; when we consider insects, which are usually less complex organisms and are therefore studied as model organisms, the scope of knowledge regarding NTDs in these organisms is even smaller. This lack of knowledge might be due to the lengths of invertebrate NTDs, which are usually shorter and are therefore often difficult to study. Nevertheless, some observations have been made, for example, complex analyzes of ecdysone receptor (EcR) isoforms revealed that they have different NTDs, resulting in different spatial and temporal distributions of these isoforms within various tissues, which in turn leads to different developmental functions. More precisely, it has been suggested that the NTD of isoform A is a weaker transactivator than that of isoform B1 of the *Drosophila melanogaster* EcR [16]. A study of the isolated NTD of the Ultraspiracle (Usp) NR from *Aedes aegypti* clearly showed that this domain is intrinsically disordered, and this study also suggested an important role for the NTD in the dimerization process of this receptor [17].

Usp is a member of the NR family and is homologous to the mammalian retinoid X receptor (RXR) [18]. Usp is involved in the regulation of essential developmental processes, such as metamorphosis or molting, in arthropods [19]. Similar to RXR, which interacts with a variety of other proteins, the interaction of Usp with different proteins has also been demonstrated, e.g., with hormone receptor 38 (HR38) or the Seven-up protein [20,21]. However, the most prominent partner of Usp is EcR. In response to high concentration of 20-hydroxyecdysone (20E), these two NRs form a functional heterodimeric complex and activate the transcription of crucial genes involved in metamorphosis [22]. Various isoforms of Usp differ mainly in the NTD sequence, which often leads to specific differences in the functions of these isoforms, as was shown for the Usp from *Bombyx mori* or *A. aegypti* [23,24]. It seems to be essential to understand the role of NTD as a factor that defines in a specific way the structures of Usp isoforms, leading to differences in the functions of the isoforms. Theoretically, this role may be implemented in two ways. First, in what seems to be an obvious manner, the NTD might exhibit extrinsic functionality, for example, by directly interacting with regulatory proteins. Second, in a less obvious manner, intrinsically disordered NTDs may influence the molecular properties and in turn the functions of the remaining Usp domains that possess well-defined, stable tertiary structures. We decided to experimentally investigate this second possibility by testing the potential role of the NTD in Usp dimer formation and in the interaction of Usp with specific REs. We chose to carry out our experiments using Usp isoform 1 from *Helicoverpa armigera* (HaUsp) (ACD74808.1), which along with *D. melanogaster, A. aegypti* and *B. mori* serves as a good model to study arthropod NRs. First, we obtained homogenous samples of full-length HaUsp and HaUsp lacking the NTD (HaUsp_ΔNTD), and subsequently, we investigated the dimerization capability of both forms. Numerous analyses with techniques such as analytical ultracentrifugation (AUC), size-exclusion chromatography coupled with multi-angle light scattering (SEC-MALS) or small-angle X-ray scattering (SAXS) have shown undisputedly that an intrinsically disordered NTD is indispensable for effective dimerization of HaUsp. Using electrophoretic mobility shift assay (EMSA), we also showed that the protein-DNA interaction patterns of full-length HaUsp and HaUsp_ΔNTD differ significantly. Moreover, the specific complexes formed by HaUsp seem to be less stable than the complexes formed by HaUsp_ΔNTD under the conditions used for EMSA. Thus, the intrinsically disordered NTD, composed of 58 residues, significantly influences the DNA-binding properties of HaUsp. Molecular models reconstructed from SAXS data indicate that HaUsp dimers are most likely stabilized by *scorpion-like* structures of NTDs, which bend towards the respective LBDs. This proximity of NTDs and LBDs in *scorpion-like* structures may result in the interaction of these domains, possibly leading to rearrangement of the LBD structure and in turn to the ability of full-length HaUsp to form dimers with increased stability. Apparently, in the *scorpion-like* structure, some changes occur also in the DBD, which in turn results in effects on the interaction mode of the protein with specific DNA sequences. Thus, the intrinsically disordered NTD is an indispensable element that controls the molecular properties of HaUsp.

2. Materials and methods

2.1. Buffer compositions

All buffers were prepared at room temperature. The lysis buffer contained 20 mM Na_2HPO_4 and 150 mM NaCl (pH 7.0). Buffer A contained 50 mM Na_2HPO_4, 300 mM NaCl, 5 mM imidazole, 5% (v/v) glycerol, and 1 mM βmercaptoethanol (pH 7.0). Buffer B contained 50 mM Na_2HPO_4, 300 mM NaCl, 200 mM imidazole, 5% (v/v) glycerol, and 1 mM βmercaptoethanol (pH 7.0). Buffer C contained 50 mM Na_2HPO_4 and 150 mM NaCl (pH 7.0). Buffer D contained 50 mM Na_2HPO_4, 100 mM NaCl, 10% (v/v) glycerol, 0.5 mg/ml ovalbumin, and 1 mM β-mercaptoethanol (pH 7.8).

2.2. Construction of expression vectors

A cDNA clone encoding the full-length HaUsp (isoform 1) (EU526832.1) was obtained from GeneArt Life Technologies. The ordered sequence was previously optimized for *Escherichia coli* and flanked with restriction sites: *Bam*HI at the beginning and *Hind*III at the end. The obtained plasmid containing the DNA sequence was double-digested with *Bam*HI and *Hind*III and ligated into the corresponding sites of the pQE-80 l (Qiagen) vector. The same cDNA was used to obtain HaUsp lacking the 58 amino acids of the NTD (HaUsp_ΔNTD). First, by PCR, the correct insert was obtained. The reaction was prepared according to the manufacturer's instructions for Phusion polymerase (Thermo Fisher Scientific), and the primers are listed in Table 1. Then, the PCR product was double-digested with *Bam*HI and *Hind*III and ligated into the corresponding sites of the pQE-80 l vector. Sequences of the purified constructs were verified by DNA sequencing.

2.3. Expression and purification procedures

For efficient expression, 200 ml of LB medium containing antibiotics (35 μg/ml chloramphenicol and 50 μg/ml carbenicillin) was inoculated