Diverse functional manifestations of intrinsic structural disorder in molecular chaperones

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Abstract

IDPs (intrinsically disordered proteins) represent a unique class of proteins which show diverse molecular mechanisms in key biological functions. The aim of the present mini-review is to summarize IDP chaperones that have increasingly been studied in the last few years, by focusing on the role of intrinsic disorder in their molecular mechanism. Disordered regions in both globular and disordered chaperones are often involved directly in chaperone action, either by modulating activity or through direct involvement in substrate identification and binding. They might also be responsible for the subcellular localization of the protein. In outlining the state of the art, we survey known IDP chaperones discussing the following points: (i) globular chaperones that have an experimentally proven functional disordered region(s), (ii) chaperones that are completely disordered along their entire length, and (iii) the possible mechanisms of action of disordered chaperones. Through all of these details, we chart out how far the field has progressed, only to emphasize the long road ahead before the chaperone function can be firmly established as part of the physiological mechanistic arsenal of the emerging group of IDPs.

IDPs (intrinsically disordered proteins) and their functions

IDPs/IDRs (intrinsically disordered regions) form a unique group of proteins of which the functional state is structurally unfolded. These proteins have no well-defined tertiary structure, rather they occur as a structural ensemble of rapidly interconverting alternative conformations, maintained by the highly hydrophilic nature of their polypeptide chain [1,2]. Structural disorder may span from short segments through domains to entire proteins [3]. There is conclusive experimental evidence for the structural disorder of approximately 1400 regions in 600 partially or entirely disordered proteins deposited in the DisProt database [4]. Structural disorder is widespread in eukaryotes; 5–15% of their proteins are fully disordered and approximately 50% of their proteins have at least one long disordered region [5]. The level of structural disorder increases from prokaryotes to eukaryotes [6,7], which can be ascribed to the functional advantages and modes that structural disorder confers. IDPs/IDRs have a loose and highly dynamic structure, which cannot form enzymatic active sites. Structural disorder prevails in proteins involved in signal transduction, regulation of transcription and protein–protein interactions, and it has an elevated level in proteins involved in disease [8,9]. IDPs often bind their partner via short recognition elements [10,11] in a structurally adaptive process termed disorder–order transition or induced folding [12]. Structural disorder confers diverse advantages, such as rapid binding, separation of specificity from binding strength and the ability to carry out distinct functions [2,13,14]. It is becoming clear that chaperone activity relies heavily on these structural–functional attributes.

Chaperone activity of proteins

The term chaperone was introduced for nucleoplasmin, a protein that promotes nucleosome assembly and prevents histone aggregation [15]. The first formal definition was confined to the assistance of folding of proteins into their native state [16]. This activity was later extended also to RNA molecules and to functions of protection of biological molecules against stress-induced unfolding, inactivation and aggregation, and with the support of the protein quality-control machinery by the recognition and subsequent degradation of unfolded molecules [16–18]. Most of our pertinent knowledge comes from studies of chaperones with well-defined three-dimensional structure, such as Hsp (heat-shock protein) 90, GroEL-GroES or Hsp70.

Globular chaperones with structurally disordered segments

Even in the case of these structurally well-characterized chaperones, disordered segments are involved in chaperone activity, as demonstrated for Hsp90 [19,20], GroEL [21,22] and several sHsps (small heat-shock proteins) [23]. Interestingly, the first protein termed a molecular chaperone, Xenopus laevis nucleoplasmin, has an intrinsically disordered

Key words: chaperone, intrinsically disordered protein, intrinsically unstructured protein, protective effect, stress protein.

Abbreviations used: Hsp, heat-shock protein; IDP, intrinsically disordered protein; IDR, intrinsically disordered region; LEA, late embryogenesis abundant; sHsp, small heat-shock protein.

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C-terminal segment which is involved in nucleoplasmin stabilization, chromatin decondensation and the formation of nucleolus-like bodies [15, 24, 25].

Hsp90 has a flexible linker domain that is not in direct contact with the substrate, yet it is essential for chaperone activity. It provides flexibility essential in the ATP cycle, in binding co-chaperone(s), such as Aha1 [26], and also in carrying temperature-switch ability. It was concluded that this region is involved in fine-tuning Hsp90 chaperone activity, as a ‘rheostat’, in a client-, co-chaperone- and perhaps also in an environment-specific manner.

sHsps can be found in virtually all organisms from prokaryotes to humans. They share a common C-terminal \(\alpha\)-crystallin domain and a variable N-terminal part that is disordered in most cases. sHsps act as molecular chaperones in vitro, preventing denatured proteins from irreversible aggregation [27, 28].

Hsp22 is a heat-sensitive sHsp which reversibly unfolds at elevated temperatures and protects model substrates against aggregation [29, 30]. Hsp26, on the other hand, forms an oligomer that is non-functional under physiological conditions, but undergoes structural rearrangements at elevated temperatures that lead to dissociation to the active form [31, 32]. In the dimeric and monomeric forms, the N-terminal domain of Hps26 becomes intrinsically disordered, and available for interaction, which is essential in the chaperone activity and later also in the (re)formation of the oligomeric form upon returning to physiological conditions. Under physiological conditions, 50% of the Hsp22 sequence shows high structural fluctuations; accordingly, it is termed an IDP.

PsHsp18.1 is a chaperone protein in pea that also has a flexible N-terminal domain and a folded \(\alpha\)-crystallin domain. Chemical cross-linking experiments have shown that the flexible N-terminal domain is essential in substrate recognition and binding [33]. Hsp33 is a redox-sensitive chaperone that undergoes massive structural rearrangements upon oxidative stress [34]; however, under physiological redox conditions, it can be found in its inactive conformation. The structural rearrangement is triggered by a C-terminal redox-sensitive domain that consists of a flexible linker and an adjacent redox-sensitive zinc centre. Upon activation, the redox-sensitive domain unfolds due to the formation of disulfide bonds and subsequent release of the \(\text{Zn}^{2+}\) ion. Hsp33 thus turns into a holdase that binds partially unfolded substrates preventing their non-specific aggregation and further unfolding. After the recovery of normal redox conditions, Hsp33 returns to its reduced form and transfers the unfolded substrate to an ATP-dependent foldase for refolding. Its IDR plays an essential role in discriminating between unfolded and partially unfolded species because of its preference for partially unfolded substrates [34].

Another chaperone that undergoes an order–disorder transition upon activation is HdeA [35]. HdeA is one of the smallest chaperones known (9.7 kDa), and it becomes activated at acidic pH and protects bacteria against acidic stress, such as in the mammalian stomach. HdeA is located in the periplasm and has a homodimeric structure in a non-acidic environment. Upon decreasing the pH, the protein rapidly dissociates, triggering the unfolding of the monomers. Disordered monomers possess strong chaperone activity with broad substrate specificity.

San1 is a member of the ubiquitin ligase family in the yeast nucleus. It functions in protein quality control via recognizing misfolded protein molecules followed by their ubiquitination and degradation [36]. Unlike other ubiquitin ligases, San1 does not require chaperone cofactors for substrate identification, because its intrinsically disordered N- and C-terminal domains directly recognize misfolded substrates. Both IDRs contain highly conserved short segments within the flexible domains; these substrate recognition sites provide San1 with an inherent plasticity, allowing it to bind many differently shaped misfolded substrates.

**Fully disordered chaperones**

There are also clear cases when chaperone function is ascribed to fully disordered proteins (Table 1). For example, \(\beta\)-synuclein can prevent the formation of \(\alpha\)-synuclein amyloid, which may be relevant with respect to Parkinson’s disease [37]. \(\alpha\)-Synuclein itself also has chaperone-like activity, being able to protect microbial esterases against heat, low pH and organic solvents [38, 39]. \(\alpha\)-Casein, another fully disordered IDP, can also prevent a range of unrelated proteins/enzymes from aggregation induced by heat or chemicals [40]. This effect may even have applications in food biotechnology [41].

Hsp12 (Saccharomyces cerevisiae) is an outstanding sHsp because it is fully disordered, lacking a folded \(\alpha\)-crystallin domain. Under physiological conditions, Hsp12 is located in the cytoplasm, whereas, in the case of stress, it translocates to the cell membrane and increases membrane stability [41a].

**LEA (late embryogenesis abundant) proteins in plants and animals**

One of the most prominent class of disordered chaperones is the LEA proteins, representing more than 700 members in plants and animals. LEA proteins are subclassified into 12 classes [42] or seven groups [43] by the presence of conserved sequence motifs. The vast majority of LEA proteins are highly charged and fully disordered. Historically, LEA proteins were first identified in plants under water and cold stress conditions; later, some LEA or LEA-like proteins (groups 1 and 3) were also found in prokaryotes [44], invertebrates [45] and even vertebrates [44, 46] that live under harsh environmental conditions. In most cases, their exact function is unknown, whereas their general role in stress-tolerance is supported by a good deal of experimental evidence (for a review, see [47]). Several LEA proteins show chaperone-like activity either alone or in combination with carbohydrates, e.g. trehalose (Table 1).
The chaperone activity of the group 2 LEA proteins ERD10 and ERD14 was directly addressed and demonstrated in a range of assays used to assess chaperone activity of traditional chaperones [48,49]. In maize embryo, 12 LEA proteins from the heat- and acid-soluble proteomes were identified and tested in anti-aggregation assays [50]. The chaperone activities of three candidates were assessed; each showed a protective effect against desiccation, freezing and heat stress under both \textit{in vitro} and \textit{in vivo} conditions.

Prevention of aggregation of model substrates caused by desiccation or freezing was also observed in the case of AavLEA1 (from the anhydrobiotic nematode \textit{Aphelenchus avenae}) and Em (group 1 wheat) [51] and other LEA proteins (see Table 1). The nematode protein can abrogate desiccation-induced aggregation of the water-soluble proteomes in mammalian cells, and of polyglutamine and polyalanine expansion proteins associated with neurodegenerative diseases [52]. To date, more than ten LEA proteins have been shown to have protective effects in heat-, cold- or drought-induced aggregation or deactivation of proteins (Table 1). A recent study of disordered \textit{A. avenae} anhydrin showed a similar protective activity to that AavLEA1, but with an additional exonuclease activity [53].

MtPM25 is an atypical hydrophobic LEA protein that can prevent both freezing- and desiccation-induced aggregation. Furthermore, it also has a unique capacity to resolubilize protein aggregates that formed under such conditions [54].

**How does a disordered chaperone work?**

With respect to the molecular mechanism of disordered chaperones, we have little insight. Intuitively, a disordered polypeptide chain can assist the folding and/or prevent the aggregation of another protein (or RNA) molecule by a variety of, sometimes only hypothetical, mechanisms.

(i) Hydration buffer: the IDP chaperone may act indirectly due to its high capacity to bind hydrate water [55], by shifting the relative chemical activities of the unfolded and folded states in favour of the latter.

(ii) Molecular shield: the IDP chaperone might partially bind to the unfolded substrate and prevent collisions with other misfolded molecules, by entropic exclusions, as suggested in the case of plant dehydrins [56].

(iii) Classical chaperone: the IDP chaperone may be able to recognize the partially unfolded state of the substrate through exposed hydrophobic patches, in a manner similar to that of classical chaperones [34,36] by preventing

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**Table 1 | IDP chaperones**

<table>
<thead>
<tr>
<th>Chaperone</th>
<th>Source organism</th>
<th>Substrate</th>
<th>Activity</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Synuclein</td>
<td>Homo sapiens</td>
<td>α-Synuclein</td>
<td>Inhibition of amyloid formation</td>
<td>[37]</td>
</tr>
<tr>
<td>α-Synuclein</td>
<td>Homo sapiens</td>
<td>Esterases</td>
<td></td>
<td>[38,39]</td>
</tr>
<tr>
<td>α-'/β-Casein</td>
<td>Bos taurus</td>
<td>κ-Casein</td>
<td>Inhibition of amyloid formation</td>
<td>[40,62]</td>
</tr>
<tr>
<td>MAP2</td>
<td>Capra hircus</td>
<td>ADH, insulin</td>
<td>Anti-aggregation</td>
<td>[63]</td>
</tr>
<tr>
<td>ERD10 and ERD14</td>
<td>Arabidopsis thaliana</td>
<td>Various substrates</td>
<td>Anti-aggregation and enzyme protection</td>
<td>[48]</td>
</tr>
<tr>
<td>AAVLEA1</td>
<td>Aphelenchus avenae</td>
<td>LDH, whole-cell protein extract</td>
<td>Enzyme protection during freezing, anti-aggregation effect during desiccation</td>
<td>[52]</td>
</tr>
<tr>
<td>Tubulin</td>
<td>Homo sapiens</td>
<td>ADH, insulin</td>
<td>Anti-aggregation effect</td>
<td>[64]</td>
</tr>
<tr>
<td>26/27 kDa dehydrin</td>
<td>Glycine max</td>
<td>LDH</td>
<td>Enzyme protection during freezing</td>
<td>[65]</td>
</tr>
<tr>
<td>α-Crystallin</td>
<td>Homo sapiens</td>
<td>β-Crystallin, various substrates</td>
<td>Anti-aggregation, folding activity</td>
<td>[23,66]</td>
</tr>
<tr>
<td>Prion protein</td>
<td>Homo sapiens</td>
<td>Nucleic acid</td>
<td>RNA dimerization</td>
<td>[67]</td>
</tr>
<tr>
<td>PSlEAm</td>
<td>Pisum sativum, mitochondria</td>
<td>Fumarase, rhodanese</td>
<td>Enzyme protection against drying</td>
<td>[68]</td>
</tr>
<tr>
<td>Emb564</td>
<td>Trichium spp.</td>
<td>Escherichia coli proteome</td>
<td>Heat, desiccation, freezing</td>
<td>[50]</td>
</tr>
<tr>
<td>Rab17</td>
<td>Trichium spp.</td>
<td>Escherichia coli proteome</td>
<td>Heat, desiccation, freezing</td>
<td>[50]</td>
</tr>
<tr>
<td>Mlg3</td>
<td>Trichium spp.</td>
<td>Escherichia coli proteome</td>
<td>Heat, desiccation, freezing</td>
<td>[50]</td>
</tr>
<tr>
<td>Anhydrin</td>
<td>Aphelenchus avenae</td>
<td>Model substrates</td>
<td>Desiccation</td>
<td>[53]</td>
</tr>
<tr>
<td>Cor15am</td>
<td>Arabidopsis thaliana</td>
<td>LDH, firefly luciferase</td>
<td>Cold-, heat stress, desiccation</td>
<td>[69]</td>
</tr>
<tr>
<td>LjIDP1</td>
<td>Lotus japonicus</td>
<td>CS, LDH</td>
<td>Desiccation, freezing</td>
<td>[70]</td>
</tr>
<tr>
<td>MIPM25</td>
<td>Medicago truncatula</td>
<td>CS, LDH, Escherichia coli proteome</td>
<td>Anti-aggregation, resolubilization of aggregated proteome</td>
<td>[54]</td>
</tr>
<tr>
<td>HIV-1 NCp7</td>
<td>HIV</td>
<td>Nucleic acid</td>
<td>DNA organization, RNA dimerization</td>
<td>[67]</td>
</tr>
</tbody>
</table>
The chaperone function of IDPs may manifest in their anti-aggregation activity, which is combined in some cases with the protection of the active conformation of the substrate. In the absence of chaperones, the substrate is prone to aggregation (b), which is inhibited in the chaperone-bound form (h). Disaggregation activity of IDPs has also been reported recently (i), without any information of the final stage of the substrate. It is still not clear whether or not some IDP chaperones can only prevent aggregation without preserving biological activity (g).

(iv) Entropy transfer: the disordered region of the chaperone may also engage in transient interactions with the misfolded substrate, in which the chaperone becomes locally more ordered and the substrate becomes more disordered [57]. This mechanism was demonstrated for the highly flexible apical domain region facing the internal cavity of GroEL [58] and the C-terminal tail of α-crystallin [59].

The chaperone function of IDPs may manifest in their anti-aggregation activity, which is combined in some cases with the protection of the active conformation of the substrate. It has also been reported that the IDPs can promote the refolding of the unfolded substrate, and that one member has resolubilization effects on protein aggregates (Figure 1). However, in the case of stress proteins, it is still barely known at what stage the IDP chaperone binds to the substrate, or whether unfolding or stress conditions trigger binding to the native protein (for possible mechanisms, see Figure 1).

The flexible characteristics of IDPs make them able to adapt to many various substrates, as mentioned above. This phenomenon has also been demonstrated in the multifunctionality of these proteins, termed moonlighting, expressing their capacity to have various unrelated biological functions [13]. In a recent study, it was shown that some disordered ribosomal proteins have promiscuous chaperone activities, being able to chaperone both RNA (for a review of RNA/ribosomal chaperones, see [60]) and proteins [61]. The promiscuous activity of these and possibly other disordered chaperones has been termed ‘Janus chaperones’ [61], which may epitomize the diverse functional potential of IDPs.

**Conclusion and outlook**

IDP chaperones embody a unique structure–function relationship, because their structural adaptability enables them to interact with structurally diverse substrates. Compared with globular chaperones, where in most cases the chaperone utilizes spatial sequestration/confinement to hold substrates, IDPs can accomplish this feat by direct molecular contact(s). IDP chaperones are also highly resistant to stress-induced structural-functional damage. Lastly, they represent an energetically cheap solution for the cell, because they do not utilize ATP for their function, and neither do they need to fold to obtain their final biologically active conformation.

Nevertheless, our knowledge of unfolded chaperones is still limited. In the last few years, evidence has accumulated on the relevance of structural disorder in globular chaperones, along with mechanistic information on their functional roles. Notwithstanding this progress, the IDP field still lacks such description of the completely unfolded chaperones outlined in the present paper.

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**References**


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