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Hypothesis

The unfolding power of protein dielectricity

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A hypothesis is proposed on a potential role of protein dielectricity as an unfolding factor in protein-protein interactions. It is suggested that large protein complexes and aggregation seeds can unfold target proteins by virtue of their effect on the dielectric properties of water at the protein-solvent interface. Here, similar to the effect of membrane surfaces, protein surface can cause decrease in the local dielectric constant of solvent and thereby induce structural changes in a target protein approaching this surface. Some potential implementations of this hypothetical mechanism are also discussed.

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Introduction

Conditional structure of a protein molecule. Structure (or lack thereof) of a protein molecule is determined by the peculiarities of its amino acid sequence and by the way of how a given polypeptide chain interacts with its environment. In other words, proteins are conditionally structured (or disordered), since their structures are dramatically dependent on the environmental conditions, at which proteins are placed during the experiment. For example, folding of some highly charged polypeptides (as exemplified by some halophilic proteins) requires high salt concentrations. Proteins from acidophilic/alkalophilic organism are able to sustain conditions with extreme pH values, whereas acidification or alkalization of solvent typically denatures mesophilic proteins. Barophilic (also known as piezophilic) protein are folded and functional at high pressures; i.e., under conditions where their corresponding organisms thrive (e.g., deep sea), but globular mesophilic proteins can be denatures by high pressure. Correct folding of many thermophilic globular proteins into their functional structures often occurs at high temperatures; i.e., under conditions favoring denaturation and unfolding of a typical meshophilic or psychrophiles proteins. Similarly, some globular proteins of psychrophilic (or cryophilic) origin "feel" comfortable at sub-zero temperatures, whereas globular proteins isolated from thermophiles and mesophyles would undergo cold denaturation under the same conditions. All this indicates that evolution shapes amino acid sequences of globular proteins to arm them with a unique possibility to be correctly folded, and therefore functional, at conditions which are physiological for a given organism.

If we now move to consideration of a folding behavior of proteins in a given organism (let us consider a typical mesophile for simplicity), we would see a similar picture, namely, environmental conditions that favor formation of a functional structure in 1 protein often could cause denaturation or unfolding of another protein. Soluble globular proteins are known to be able to spontaneously fold into unique 3D-structure being placed into aqueous environment, but chances for a transmembrane protein to gain functional folded state in aqueous environment are minimal. On the other hand, correct folding of a transmembrane protein requires the presence of lipids, but the membrane surface can modify structure and even

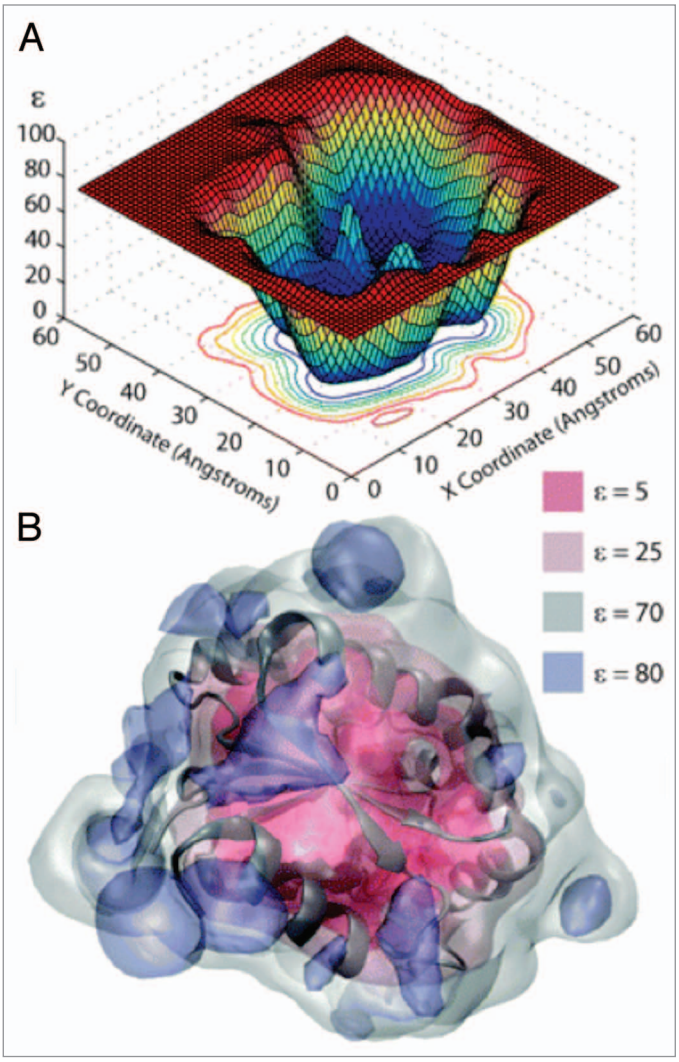

Figure 1. The spatially-varying dielectric function for adenylate kinase (PDB ID: 1AKY). (**A**) The effective scalar dielectric constant on a horizontal plane through the geometric center of the protein. (**B**) Dielectric contours around the 1AKY structure, showing surfaces of ε = 5, 25, 70, and 80. Regions inside the blue globules have dielectric constants larger than that of water. Figure is adapted from reference 80.

unfold some initially folded globular proteins. Also, proteins acting inside of some organelles (e.g., lysosomes) are characterized by the ability to be active at moderately acidic conditions that are known to destabilize or denature of many proteins.

Structural consequences and structural prerequisites of protein function. One also should keep in mind that folded structures ordered proteins preserve significant amount of flexibility and often this flexibility is of functional importance. In fact, functions of many ordered proteins are known to rely on induced fit. In the case of enzymes, the original paradigm was that the active site is continually reshaped by interactions with the substrate. This process continues until the substrate is completely bound, and the amino acid residues which make up the active site are molded into the precise positions that enable the enzyme to perform its catalytic function.[1] Some ordered proteins, including enzymes, are subject to allosteric regulation, where binding of the effector molecules to the specific allosteric sites, which are different and physically distinct from the protein's active site, modulate protein function by inducing specific conformational changes.[2,3] On the other hand, the structure of many ordered proteins, for example non-enzymes that simply mediate interactions, can remain unchanged during their function.

And then we have intrinsically disordered proteins (IDPs) that would stay preferentially disordered under the conditions favoring folding of globular proteins. However, structural ensembles of IDPs are characterized by extreme sensitivity to the environmental conditions.[4,5] Also, the binding of some IDPs to specific partners involves a disorder-to-order transition, as a result of which IDPs adopt more structured conformations.[6-15] However, many IDPs can remain predominantly disordered in the bound state outside the binding interface.[16-19] Such mode of interaction is known as "the flanking fuzziness" in contrast to "the random fuzziness" when the IDP remains entirely disordered in the bound state.[19,20] Furthermore, activities of other IDPs do not directly involve coupled binding and folding, but rather are dependent on the flexibility, pliability, and plasticity of the backbone. These are so-called entropic chain activities, as they rely entirely on an extended random-coil conformation of a polypeptide that maintains motion and dynamic flexibility while carrying out function.[21]

Therefore, biologically active proteins can either have or be devoid of unique 3D structures and structures of proteins can either change or remain unchanged during function. The function-related structural changes range from local partial folding to complete folding, and from allosteric transitions to induced fit adjustments in IDPs and ordered proteins, respectively.

Protein Functions via Conditional Unfolding

Generally, the most common outcome of function-related structural changes is the overall increase in the amount of ordered structure. However, functions of some ordered proteins rely on the decrease in the amount of their ordered structure; i.e., these functions require local or even global functional unfolding of a unique protein structure.[4,5] The most important features of such function-requested structural changes are their induced nature and transient character.[4,5] In other words, the function-related changes in these so-called conditionally disordered proteins[22] are

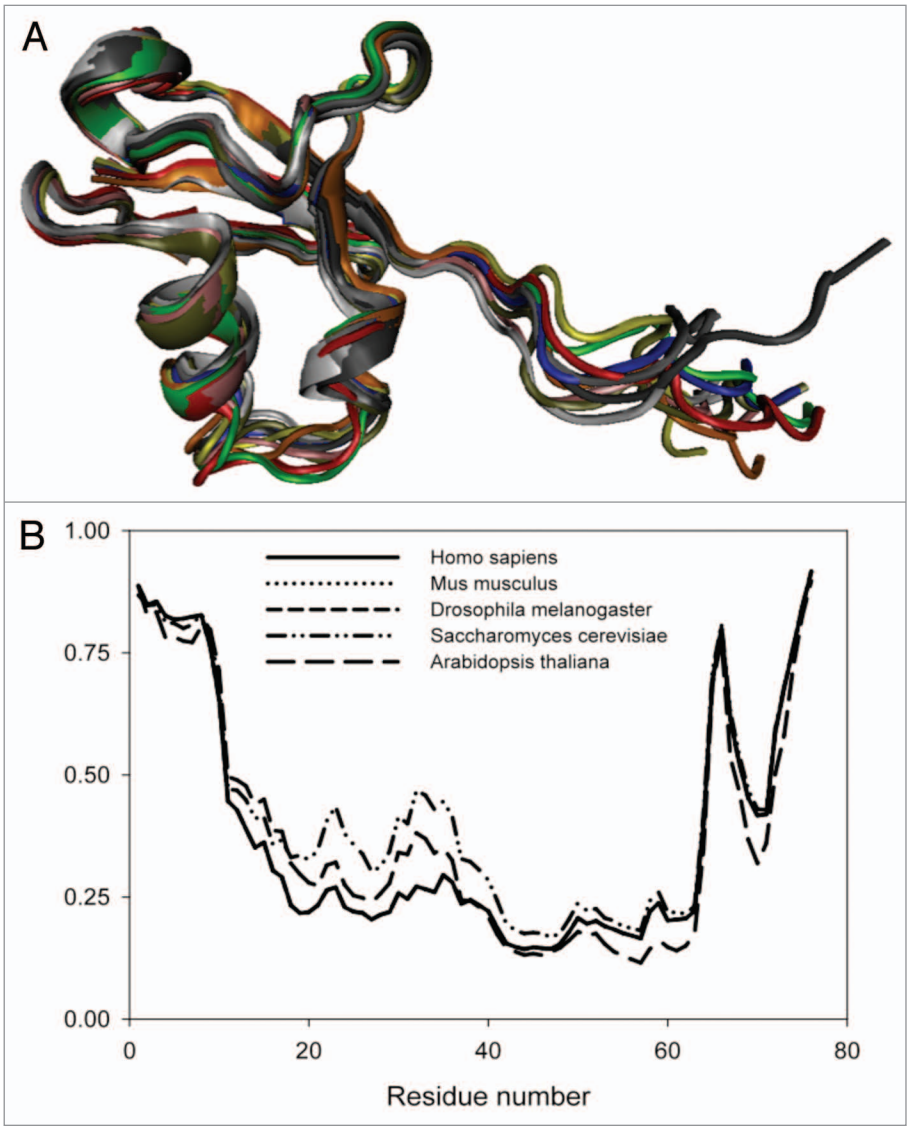

Figure 2. Evaluation of intrinsic disorder in ubiquitin. (**A**) NMR solution structure of ubiquitin from *Saccharomyces cerevisiae* (PDB ID: 1ZW7). Ten representative members of the conformational ensemble are shown by ribbons of different color. This image was generated using the VMD software (http://www.ks.uiuc.edu/Research/vmd/).[92] (**B**) Intrinsic disorder prediction in several members of the ubiquitin family. Note that disorder plots for proteins from human, mice, and fly are completely overlapped. Disorder probability was evaluated by PONDR-FIT algorithm.[93]

induced by transient alterations in their environment or by transient modification of their structures. They are reversed as soon as the environment is restored or the modification is removed.[4,5]

The mentioned proteins with functions relying on the induced or transient disorder need specific means for awakening of their dormant disorder for function. Since, to become functional, these transiently or conditionally disordered proteins require complete or partial unfolding of their originally ordered and completely folded structures, the important question is then what factors in a living cell can force protein to unfold. Among a long list of potential environmental factors that are used by nature to awake such dormant functional disorder are protein-protein interactions.[4,5] In other words, instead of well-known and widely recognized binding mechanism where interaction between two or more proteins is expected to be accompanied by their mutual adjustment and folding, some protein-protein interactions are in fact associated with at least partial unfolding of 1 of the partners. One of the illustrative examples of proteins whose functional dormant disorder is awakened by interaction with binding partners are the partial unfolding of BCL-xL upon binding of its interaction partner PUMA by a mechanism described as entropic compensation, where an intrinsically disordered PUMA extensively folds upon binding to BCL-xL, which undergoes binding-induced partial unfolding of its α-helices 2 and 3.[23] Among other examples of proteins promoting local unfolding of their binding partners are numerous unfoldases, such as ATP-dependent proteases (e.g., proteasomes in eukaryotes and proteasome analogs such as the ClpAP, ClpXP, HslUV, Lon and FtsH proteases in prokaryotes) and the mitochondrial import machinery.[24] A crucial functional step of the ATP-dependent proteases is the active unfolding of their protein substrates, which was demonstrated for ClpAP,[25] ClpXP,[26] FtsH,[26] Lon,[27] the archaebacterial proteasome-regulatory ATPase complex PAN,[28] and the eukaryotic proteasome.[29] Also, several ATP-dependent molecular chaperones were shown to act as unfoldases and assist the refolding process by unfolding of misfolded proteins that are kinetically trapped in local conformational energy minimum.[30]

Molecular Mechanisms of Functional Unfolding Induced by Active Unfoldases

The important mechanistic questions are why binding of some proteins induces their unfolding and what physico-chemical forces can promote such transient unfolding of originally folded structures. In some cases the answer can be obtained from the structural analysis. For example, in the mentioned BCL-xL/PUMA story, NMR spectroscopy and X-ray analysis revealed that the BH3 domain of PUMA folds into an α-helix upon binding within a hydrophobic groove on the surface of BCL-xL and that a π-stacking interaction between W71 at the N-terminus of PUMA's α-helix and H113 of BCL-xL locally distorts the BCL-xL structure, leading to the destabilization of α-helices 2 and 3.[23] Another interesting example is provided by the mitochondrial import machinery complex

that includes translocases in the outer and inner mitochondrial membranes termed 'TOM proteins' and 'TIM proteins', respectively, as well as several other proteins in the matrix. Complexes of TOM and TIM proteins associate to form a contiguous translocation channel that connects the cytosol to the mitochondrial matrix.[31] Mitochondria actively unfold substrates by unraveling them from the targeting signal.[32] During import, most mitochondrial proteins are unfolded by the electrical potential across the mitochondrial inner membrane acting directly on positive charges in the targeting sequences.[33,34] Similarly, it is believed that the flagellar type III protein export apparatus-driven translocation of the majority of the flagellar components across the cytoplasmic membrane is driven by the one-dimensional Brownian motion biased by the proton motive force (i.e., the work per unit charge required to move a proton).[35] As far as ATP-dependent proteases are concerned, it is likely that these proteases unfold their substrates mechanically by pulling the polypeptide chain into their channel.[24] Finally, ATP-dependent chaperones also might unfold their clients mechanically. For example, GroEL binds non-native proteins by means of a ring of hydrophobic residues that line the entrance to the central cavity of its heptameric ring.[36,37] When GroEL binds ATP and the GroES co-chaperonin, massive structural changes double the GroEL cavity volume and occlude its hydrophobic binding surface.[38,39] In fact, before binding ATP and GroES, GroEL's binding sites are located 25 Å from each other, whereas upon the addition of ATP and GroES, the apical domain of each GroEL subunit twists upward and outward so that the binding sites move apart to a position 33 Å from one another. As a result, neighboring binding sites move apart by 8 Å and non-neighboring sites by larger increments, up to 20 Å. These large-scale movements provide the means for the mechanical unfolding of the misfolded substrate protein which, as it is tethered to these sites, will be forcibly stretched and partially unfolded.[40,41]

Passive Unfoldases

ATP-dependent chaperones, ATP-dependent proteases, mitochondrial import machinery and the flagellar protein export apparatus are all active unfoldases. These typically very large and complex protein machines comprise mostly of rather well-folded subunits and use some well-understood energy sources for unfolding of their partners. However, in addition to these active unfoldases, some protein ensembles can act as passive unfoldases. The examples of these are oligomeric forms of small heat shock proteins and other intrinsically disordered chaperones[42-45] and, surprisingly, multimeric misfolded protein ensembles that act as seeds promoting pathogenic protein aggregation. Intriguingly, induced disorder in some parts of the protein resulting from structuring other parts of the protein were reported for SOD1 in the absence of metal ligands, suggesting that the non-local entropy transduction can be observed wherein energetically favorable processes that result in local enhancement of structure may increase entropy non-locally.[46,47]

Several general paradigms originate from the intensive research on protein misfolding and pathologenic aggregation. These paradigms are:

(1) For aggregation to occur, the involved protein should undergo noticeable structural transformations, the nature of which depends on the nature of the causing protein; i.e., extended IDPs should undergo partial folding to gain more ordered and therefore more aggregation prone structure, whereas ordered protein should unfold to be able to gain properties consistent with aggregation. It should be mentioned though that not all aggregation processes are pathogenic and that aggregation can occur without unfolding/major conformational changes of the protein (native aggregation). The best examples of such native aggregation are salting out in protein purification and protein crystallization.

(2) Under normal physiological conditions, the pathogenic aggregation is typically a slow process that can be described using the nucleation-polymerization model. The time-limiting stage of this process is the formation of critical aggregation-prone nuclei which is typically preceded by the unfolding-misfolding-oligomerization of the causing protein.

(3) Aggregation can be dramatically speeded up by adding seeds; i.e., preformed aggregated species that are able to convert native proteins into misfolded and aggregation-prone entities. Once again, one should keep in mind that seeding does not necessarily go through induced conformational change (like those described for the amyloid fibril formation). In protein crystallization, for example, seeding is often used to promote "native" aggregation; i.e., formation of protein crystals.

Looking at points above, the immediate question is how seeds of pathogenic aggregation (these dynamic complexes of rather disordered species) do their trick and accelerate protein aggregation. The existing models utilize the notion that seeds act as templates that force native protein to gain amyloidogenic conformation. Although these models can give a reasonable explanation for the template-based propagation of aggregation of IDPs, where seeds promote partial folding, there is no simple mechanistic explanation of why and how these seeds would act as unfoldases. In other words, it is not clear what chemical or physical force is engaged in inducing rather global unfolding of an ordered protein, which is known to be a crucial prerequisite for aggregation to occur.[48] Of course, one can argue that seeds affect the conformational equilibrium of a target protein by shifting it to more disordered conformation(s). However, this hypothesis, although very likely to be correct, once again does not explain how this "magic" is done and what the major driving force is used by the aggregation seeds for their unfolding action.

Membrane Surfaces as Protein Denaturing Factors

This situation reminds me of my old times in the Laboratory of Protein Physics headed then by Professor Oleg Ptitsyn. There, more than 20 years ago, we were working on an intriguing hypothesis that a universal protein folding intermediate known as molten globule might have crucial biological functions.[49-51] This hypothesis was based on the recognition that the highly dynamic nature of the molten globular polypeptide chain "is almost ideal for a protein which has to adapt itself

to different external conditions (like the conditions in a living cell), maintaining a memory on its overall architecture."[51] At that time, it was believed that in many cases folding intermediates in a cell are the folding kinetic intermediates trapped in the under-folded state by chaperones just after the biosynthesis and before the protein will have a chance to completely fold.[51] Another source of the partially folded protein species inside a cell was associated with mutations that prevented proteins from complete folding. However, both of these mechanisms did not explain the functionality of other proteins or protein domains, which normally exist in a well-ordered state but have to undergo significant unfolding to fulfill their functions. The 2 illustrative examples of such proteins known at that time were pore-forming domains of some toxins,[52] and protein-carriers of large non-polar ligands.[51] How these proteins can denature at usually neutral pH, high ionic strength, and ambient temperatures (i.e., at normal physiological conditions) was not clear.

In other words, at that time we were challenged by a puzzle which is very similar to one prompted now by the mysterious unfolding power of the aggregation seeds, namely we were trying to understand which cellular factors can cause originally folded protein to return to its partially folded intermediate; i.e., to partially unfold under normal physiological conditions. Since both types of functionally unfoldable proteins we were looking at (pore-forming domains of toxins and proteins transporting large non-polar ligands) were proteins interacting with (or at least found in the vicinity of) the biological membranes, the 2 membrane surface-associated potentially denaturing factors were proposed, namely local decrease in the pH and the local decrease in the dielectric constant near the membrane.[50,51] Here, the negative electrostatic potential of the membrane surface can attract protons from the solution, resulting in a noticeable local decrease in pH on the membrane surface and formation of a pronounced pH gradient in its nearest surroundings.[53,54] Typically, this local decrease in pH does not exceed 2 units in salt-free solutions.[54] Therefore, such moderate "acidification" of the local environment is insufficient for pH-induced denaturation of the majority of globular proteins (which often require extremely acidic conditions (pH 2.0–3.0) to become unfolded) and thus cannot be considered as a sole denaturing factor of the membrane surface. On the other hand, it is known from classical electrodynamics that the effective dielectric constant (ε) of water at the water-hydrophobic medium interface is significantly lower than in the bulk water.[55] Such a local decrease in the dielectric constant near the membrane surface was proposed to serve as an additional denaturing factor of the membrane.[50,51] In agreement with this hypothesis, it has been shown that the denaturation of a globular protein, β-lactoglobulin, and the formation of the molten globule-like intermediate in a mixture of water with simple alcohols directly correlated with the decrease in the dielectric constant of the media, a conclusion based on the fact that the structure-modulating effects of different alcohols were described by a single "master" curve, when the dielectric constant of the media was used as a measure of the alcohol content in the mixture.[56] Curiously, in another study of the effect of simple alcohols on β-lactoglobulin it was shown that independent of the alcohol used (methanol, ethanol, or 2-propanol) the midpoints of the structural transformation from a predominantly β-structure into a predominantly α-helical species occurred around the dielectric constant ε of ~60, whereas the decrease in ε to ~50 led to the dissociation of the retinol/β-lactoglobulin complex.[57] Both structural and functional changes were completely reversible and when the dielectric constant of the medium is raised back to ε of ~80, both the refolding of β-lactoglobulin into a β-structure and the re-association of the retinol/β-lactoglobulin complex were detected.[57]

Hypothesis: Unfolding Power of Protein Dielectricity

It is tempting to hypothesize that the unfolding power of disordered aggregation seeds resides in the protein dielectricity. In fact, the interior of a typical well-folded globular protein is known to be characterized by the dielectric constant ε ranging from 2.0 to ~4.0.[58] This value is consistent with the static dielectric constants measured for the dry protein and peptide powders,[59-62] and agrees well with the protein interior dielectric constant predictions by a variety of theoretical calculations based on normal mode analysis and on molecular dynamics simulations.[63-66] Even if the mentioned value of the dielectric constant ε of ~4.0 is at the lower limits, and if the actual dielectricity of the protein interior can be characterized by the higher ε values ranging from 2 to 40 (ref.[67]) due to the polarization contributions from the configurational freedom of polar side chains,[64,68] or because of the proton fluctuations in titratable groups,[69-71] or due to some solvent intrusion into the protein interior,[67] these dielectric constant values of protein interior are still much lower than the dielectric constant of bulk water (78.5 at 25°C).

This means that there is a dielectric interface between a protein and a solvent and therefore there is a chance that this low protein dielectricity can act on water at the water-hydrophobic medium interface making its dielectric constant significantly lower than that of the bulk water. One should keep in mind that a lower dielectricity at the protein-solvent interface actually means lower polarizability of water molecules originating from their non-random orientation at the surface of the protein. Therefore, this concept is not that different from hydrophobicity concept, which also acts on lowering orientational freedom of water molecules. In line with this hypothesis, the anomalous properties of water at or near the protein surface were reported in several studies, based on which it was concluded that the dielectric relaxation of aqueous solutions of micelles, proteins, and many complex systems possesses an anomalous dispersion at frequencies intermediate between those corresponding to the rotational motion of bulk water and that of the organized assembly or macromolecule.[72,73] For example, based on the umbrella sampling method of free energy calculation applied to the analysis of the hydration water layer of chicken villin head piece (HP-36), it was shown that the interfacial water molecules from the hydration layer at the surface of a protein are separated from the bulk solvent by a noticeable free energy barriers preventing

water molecules from free escape from the protein hydration layer, which is manifested by the presence of clusters of slow, quasi-bound (but transient) water molecules on the protein surface.[74]

Therefore, similar to lipid micelles, large disordered conglomerates of aggregated proteins that constitute aggregation-promoting seeds can potentially decrease the effective dielectric constant of the solvent at the water-aggregate interface and thereby induce partial unfolding of a folded target protein approaching this interface. Obviously, the efficiency of such protein dielectricity-based unfoldase activity of the aggregation promoting seeds should be dependent on the seed's size, with very small oligomers being expected to be less effective than larger seeds in their ability to induce local unfolding of target proteins. The magnitude of the spatial spread of this phenomenon is not clear at the moment, and this question requires special studies. However, as with the dielectric constant lowering effects of the membrane surface, it is expected that there is a rather sharp gradient between the high dielectric constant of the bulk solvent and the lowered effective dielectricity at the protein-solvent interface. In other words, it is expected that the proposed phenomenon of decreased dielectricity does not spread too far from the protein-water interface, suggesting that a target protein should be in close proximity to, if not in a direct contact with, the seed to fill its unfolding power.

Potential Implementations of the Hypothesis

Dielectricity driven unfolding and aggregation. There are a few interesting considerations related to the proposed unfolding capability of protein dielectricity. It was recognized long ago that hydrophobic interactions (in addition to b-structure propensity, aromatic content and charge) play an important role in protein aggregation.[75-77] In fact, hydrophobicity is taken as one of the most crucial inputs in computational tools developed for prediction of polypeptide aggregation propensity.[77-79] Therefore, protein dielectricity-induced unfolding might work in conjunction with hydrophobicity-driven self-assembly to generate mechanistic grounds for the chain reaction type propagation of protein aggregation, where, once formed, a disordered seed attracts some originally folded protein molecules, promotes their unfolding, thereby initiating a new level of aggregation leading to the aggregate growth or to the generation of new seeds.

This brings an important question of how specific is the dieletricity-driven unfolding activity. Although the functions of ordered active unfoldase (i.e., the unfolding activities of ATP-dependent chaperones, ATP-dependent proteases, mitochondrial import machinery, the flagellar protein export apparatus, and other active unfolding machines) are obviously rather specific, the dieletricity-driven unfolding activity seems to be entirely non-specific, since it could potentially affect any protein next to any other. However, the specificity of the pathological aggregation, where the proteinaceous deposits accumulating during the development of different diseases are enriched in specific proteins (e.g., Lewy bodies and Lewy neurites in Parkinson disease contain mostly α-synuclein, whereas helical paired filaments and senile plaques of Alzheimer disease are preferentially comprised of fibrous forms of tau protein and Aβ peptide, respectively) argues that there is some specificity in the unfolding action of the pathogenic aggregation seeds.

Hot spots of the dielectricity-induced unfolding. It is important to remember that the dielectric properties are heterogeneously distributed within a given protein molecule, and unlike a homogeneous liquid whose dielectric constant does not vary throughout its volume, the dielectric properties of a biomolecule varies from site to site depending on the local molecular structure.[80] As a result, the heterogeneous protein dielectric theory predicts the presence of regions with relative permittivity comparable to or exceeding that of water on the surface of the protein. **Figure 1** represents an example of a dielectric map calculated for adenylate kinase and illustrates the point made above that protein might contain regions with dielectric constants higher than that of bulk water.[80] A heterogeneous distribution of dielectricity within the protein globule is also evident, given the varying degree of side-chain and backbone fluctuations.[80]

This idea is in line with the concept of the overall spatiotemporal heterogeneity of protein structure according to which some ordered proteins might have unfoldons, i.e., regions that have to undergo order-to-disorder transition in order to make protein active, whereas all IDPs might contain foldons, inducible foldons, semi-foldons and non-foldons; i.e., regions characterized by the very diversified response to their environments.[4] The existence of such structural heterogeneity defines the peculiarities of a given folded protein response to changes in the dielectric constant of its environment, with the regions characterized by the higher dielectric constants than their neighbors being first candidates for the disordered dielectricity-induced unfolding.

Dielectricity-mediated unfolding power of active unfoldases. It is tempting to hypothesize that protein dielectricity-driven decrease in local dielectric constant of a solvent near the protein surface might play a role in function of ordered active unfoldases (such as ATP-dependent chaperones, ATP-dependent proteases, mitochondrial import machinery, flagellar protein export apparatus, etc.). In fact, the majority of these machines are very large protein complexes, which, due to their large size, are able to affect dielectric properties of water at the water-protein interface, making its dielectric constant significantly lower than that of the bulk water. This decreased dielectricity of water at the water-protein interface can serve as an additional destabilizing factor that makes target proteins more susceptible to action of active unfoldases. In other words, proteasome and its prokaryotic analogs, ATP-dependent chaperones, mitochondrial import machinery, flagellar protein export apparatus, and other active unfoldases can use their large size to partially unfold (or at least to destabilize) their target proteins by affecting the solvent dielectric properties. Again, heterogeneous distribution of dielectricity within a target protein defines the existence of some hot sp.ots (i.e., regions with relatively high dielectric constant), which will be melted first due to the unfolding power of dielectricity of their giant partners.

Dielectricity-mediated unfolding and polyubiquitination/multiubiquitination. Ubiquitination, multiubiquitination, and polyubiquitination are important post-translational modifications of proteins that control numerous aspects of protein function, such as degradation, protein-protein interaction, targeting/localization, complex assembly, modulation of function or stability.[81] Multiple lysine residue of one target protein can be used for the ubiquitin attachment resulting in protein multiubiquitination.[82,83] Furthermore, lysines of the substrate-conjugated ubiquitin can be further ubiquitinated leading to the substrate polyubiquitination.[81,83] Both, multiubiquitination and polyubiquitination can generate diverse substrate-ubiquitin structures, defining different fates of target proteins.[84,85]

Since the C-terminal region of ubiquitin is characterized by high conformational flexibility (see **Fig. 2**), each ubiquitin-ubiquitin linker in the polyubiquitin chain is highly flexible.[86] As a result, the polyubiquitin chains are known to possess noticeable flexibility too. For example, Lys48-linked di- and tetra-ubiquitin chains under physiological conditions are in equilibrium between a predominantly "closed" conformation (where the hydrophobic patches of individual ubiquitin molecules are sequestered at the ubiquitin/ubiquitin interface) and one or more open conformations.[86-88] Similarly, Lys11-linked polyubiquitin chains were shown to adopt compact conformation in solution,[89] whereas Lys63-linked chains predominantly adopt an extended structure exposing the hydrophobic patches and making them readily available for interactions with receptors.[90,91] The facts that the Lys48- and Lys11-linked polyubiquitin chains are preferentially in the "closed" (compact) conformation and that these types of polyubiquitination serve as "kiss of death" that targets the designated protein to proteasomal degradation suggest that polyubiquitination might play a dual role in protein degradation. Here, besides serving as a recognizable signal, the compact polyubiquitin chain covalently attached to the target protein can prepare said protein for degradation via its partial unfolding induced by the decreased dielectricity at the polyubiquitin surface.

Conclusions

It is hypothesized here that some large proteinaceous ensembles (e.g., ordered active unfoldases, such as ATP-dependent chaperones, ATP-dependent proteases, mitochondrial import machinery, flagellar protein export apparatus, etc., or compact polyubiquitin chains, or disordered oligomeric species, such as pathologic aggregation seeds, intrinsically disordered oligomeric chaperones, etc.) can induce partial or complete unfolding of other proteins by virtue of their effect on local dielectricity of solvent at the protein-water interface. It is expected that this unfolding capability is of rather short range, being the most powerful in the close proximity to the protein-solvent interface. This hypothesis has multiple implementations in both native and pathological actions of large protein complexes. Some of the potential ways to experimentally verify this hypothesis include careful analysis of the water dynamics at the protein-solvent interface, an analysis of dielectric properties of water in concentrated solutions of large protein complexes, structural characterization of the early stages of seed-activated protein aggregation (e.g., by NMR spectroscopy, where structural properties of the labeled target protein are studied in the presence of the non-labeled aggregation seeds), etc.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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