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Protein Interaction, Association and Fibrillation

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Abstract

A protein can fold efficiently with high fidelity if on average native contacts survive longer than non-native ones. If native contacts survive long enough to obtain a certain level of probability that other native contacts form before the first interacting unit dissociates this provides the folding process with directionality towards the native state and no particular pathway is needed. Interactions among hydrophobic residues are by far more important than electrostatic interactions in protein assembly, folding and stability. Proteins may under certain conditions and as a function of time give up their native folded state and form amyloid fibrils – a process that is involved in a number of human diseases. The fibrillation process can be perturbed by the presence of foreign surfaces, for example nanoparticles of different surface character.

Introduction

Protein folding and protein folds reflect the large number of non-covalent interactions that form under the very substantial constraints imposed by the covalent chain. Due to steric overlap, roughly ninety percent of the combinations of backbone torsion angles phi and psi are inaccessible. Nevertheless, the accessible ~10% of the Ramachandran map allows for a remarkable variation in protein folds through combinations of extended or helical segments

and loops. In this review we will provide examples of studies aiming at understanding the role of fundamental intermolecular interactions in protein folding, binding and the role of foreign surfaces in protein fibrillation.

Protein Folding through Kinetic Discrimination

Many proteins fold rapidly and spontaneously to the native state. This has puzzled investigators for decades as the process would not be completed within the lifetime of the universe if the protein was deemed to random search through all possible conformations, the Levinthal paradox [1]. Yet, proteins fold on a μs – ms time scale [2 – 5] implying a high degree of directionality of the process. Levinthal interpreted this as an evidence for pathways that direct the search [1] and spontaneous folding is often taken as an evidence that there are one or a few obligatory intermediate structures that the chain must adopt on its way from unfolded to the native state. However, for many proteins no intermediates have been detected and they are classified as two-state folders. The "new view" and funnel model invokes parallel routes for ensembles of proteins [6, 7]. By Monte Carlo simulations we resolved the Levinthal paradox and showed that a protein can fold efficiently with high fidelity if on average native contacts survive longer than non-native ones (Fig. 1) [8]. An important consequence of this finding is that no pathway needs to be specified. Instead, kinetic discrimination among formed contacts is a sufficient criterion for rapid folding to the native state. Successful folding requires that native contacts survive long enough to obtain a certain level of probability that other native contacts form before the first interacting unit dissociates. A modest degree of cooperativity among the native contacts shifts the required ratio of dissociation rates into a realistic regime and makes folding a stochastic process with a nucleation step [8].

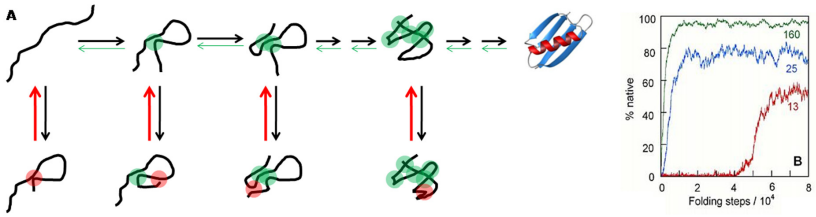

Figure 1. Protein folding through kinetic discrimination. Monte Carlo simulations were performed with the condition that native contacts (green) on average survive longer than non-native ones (red) [8]. (**A**) Successful folding occurs if native contacts survive long enough to obtain a certain level of probability that other native contacts form before the first interacting unit dissociates. (**B**) Results of simulation with different ratios of the average life time for dissociation of native *vs* non-native contacts. The actual life-rime of each contact was picked randomly from an exponentially decaying function with native or non-native lifetime. A modest degree of cooperativity was used among the native contacts so that contacts formed next to preexisting contact get prolonged life time [8].

Protein Reconstitution Studies of the Role of Fundamental Interactions in Protein Assembly

The stability of a protein towards denaturation is often high enough that the protein can tolerate one or more interruptions in the polypeptide chain. After separation, the protein fragments may reassemble spontaneously to regain a complex with a structure and function highly similar to that of the intact native protein [9]. Successful reconstitution has been observed for a large number of proteins including thioredoxin, cytochrome c, ribonuclease, dihydrofolate reductase, calbindins D_{9k} and D_{28k}, troponin C, calmodulin and Trp repressor [10 – 20]. The protein reconstitution process leads to assembly of the fold from more than one chain segment using native contacts in a reaction akin to intramolecular folding.

By the same reasoning, the folding of intact proteins can also be thought of as an association reaction, but one in which the binding partners are imprisoned in the same chain. The analogy between folding and reconstitution has prompted investigators to study the reconstitution reaction as an alternative way to gain insight into protein folding and factors favoring the native states of protein [21 – 27]. In a recent review we made an attempt to summarize the insights gained from reconstitution and folding studies into the molecular factors governing these two processes [28].

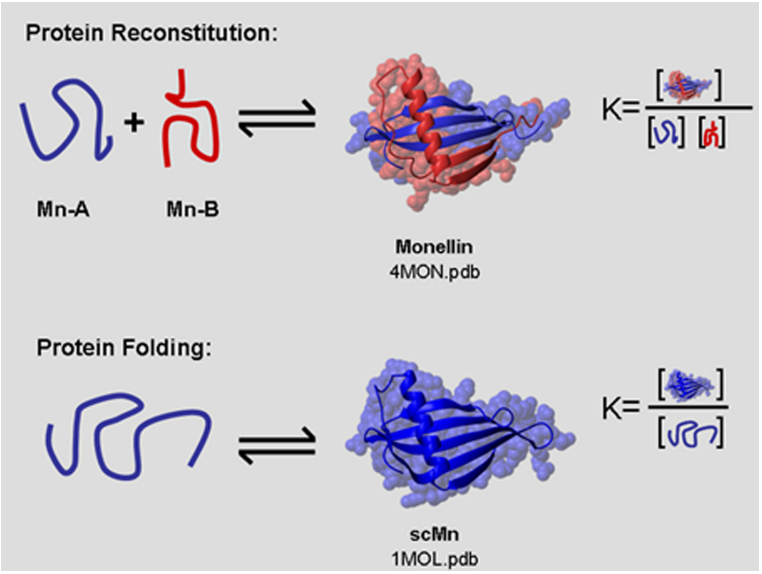

Figure 2. Protein reconstitution and intramolecular folding. The two equilibria for protein reconstitution from two fragments (top) and protein folding of a single covalent chain (bottom) are illustrated for the protein monellin. The equilibrium constants for the two folding processes reflect the difference in their molecularity, with consequent need to define the standard state for the intermolecular reconstitution reaction.

While folding is unimolecular process, protein reconstitution is a bimolecular or higher order process (Figure 2). The fraction of fragments associating as a reconstituted complex will like for all binding reactions be governed by the total concentration of fragments and the equilibrium constant for complex formation, while for the intact protein the fraction of folded protein will be independent of protein concentration (within reasonable limits avoiding aberrant aggregation).

Thereby the reconstitution reactions gain a very significant practical advantage by bringing protein folding under the control of mass action. We have utilized this feature to develop direct experimental approaches to studies of contributions of individual residues to protein assembly (Figure 3; [18, 19, 29, 30]). Our approaches can be used under physiologically relevant solution conditions, and at the conditions under which the protein is maximally stable. This is not possible in traditional protein stability studies which require perturbation of the equilibrium away from the folded state using harsh conditions.

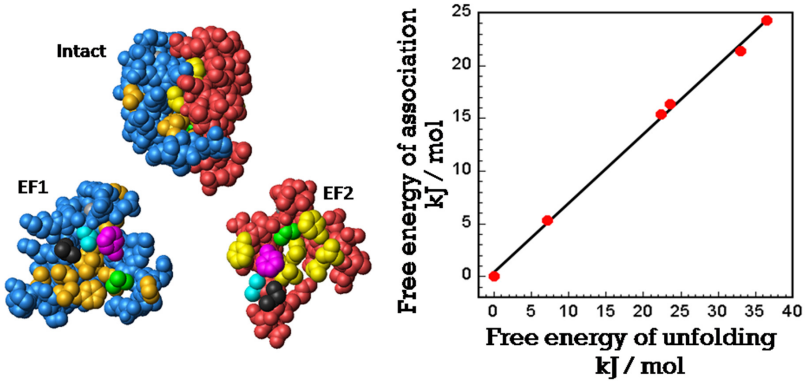

Figure 3. Role of hydrophobic residues in protein assembly and stability. CPK model of intact calbindin D9k (4icb.pdb) and two fragments representing its two EF-hands, EF1 and EF2 (left). The fragments are cut out of the intact structure file, slided apart and rotated 90 degrees towards the viewer to reveal the interaction surface. Hydrophilic backbone and side-chains are colored blue and red, while hydrophobic groups are colored yellow or gold. In this study, the hydrophobic residues with side-chains colored cyan, magenta, green and black were mutated to alter the hydrophobicity and the effects on free energy of fragment association was measured and correlated to the free energy of unfolding of the corresponding intact proteins carrying the same substitutions (right).

The main conclusion from our protein reconstitution studies [18, 19, 29, 30] is that interactions involving the hydrophobic core residues are by far more important for folding and assembly of the protein structure compared to interactions involving charged residues. This settles a long-standing debate as to which physical interactions are most relevant in determining protein tertiary structure.

Three-dimensional domain swapping is a process in which part of the tertiary fold of one chain is replaced by the corresponding part of another chain [17, 31, 32]. Three-dimensional domain swapping may lead to formation of a dimer [33 – 37], or higher multimeric assembly [38 – 40] in which the native fold of the protein is repeated two or more times through association of subdomains from separate chains. Most of the original native contacts are reformed in a reconstituted protein or a domain-swapped oligomer. Swapping thus has in common with fragment reconstitution that the tertiary fold begins in one chain but is completed using segments that originate from another chain. Domain-swapping has been reviewed recently by Liu and Eisenberg [32], and also by Håkansson and Linse [17] who pointed out its relationship to reconstitution. Numerous proteins are found to undergo both processes using similar chain segments.

Protein Fibrillation – the Influence of Nanoparticles

The native fold of a protein represents a free energy minimum that is strongly sequence dependent. In contrast, amyloid fibrils seem to represent an alternative free energy minimum that has very wide tolerance for protein sequence. These amyloid fibrils have a characteristic cross-beta structure [41] regardless of the native fold or sequence of the parent protein. Fibril formation is documented for so many proteins that it may well be universal, and it has been proposed to reflect universal properties of proteins [42].

Currently, about 30 different proteins and peptides are known to cause human amyloid disease [for reviews see refs. 43 – 47]. These diseases involve self assembly of soluble proteins into large insoluble fibrils through nucleation-dependent assembly, often *via* the formation of oligomeric structures that possess toxic properties [48, 49]. It has been shown that surfaces presented by lipid bilayers, collagen fibres, polysaccharides, and other liquid-air, liquid-solid or liquid-liquid interfaces can have specific and significant effects in promoting amyloid formation [50 – 55]. These observations suggest that interactions with different surfaces could promote protein self-assembly into amyloid fibrils and enhance protein conformational changes associated with other protein misfolding diseases.

While the molecular events behind the processes leading from native to fibrillar states remain elusive, accumulated data from many studies suggest that fibrillation involves a number of intermediate oligomeric states of different association numbers and structures [46]. The use of agents that interfere with these processes and/or allow for the isolation of intermediate species may help elucidate the molecular mechanism of fibril formation. Such strategies have also therapeutic potential for the treatment of neurodegenerative diseases.

We have recently identified co-polymeric nanoparticles as agents that accelerate the fibrillation of β2-microglobulin, β2m. Specifically, we found that the presence of nanoparticles leads to a shortening of the lag phase for nucleation of the fibrillation process. The likely

role of the nanoparticles in this process is binding of β2m to the particles, thereby increasing the local concentration and the likelihood of formation of a critical nucleus for fibrillation [56].

These studies are part of an extensive search for potential hazards with nanoparticles. Our premise has been that it is not the nanoparticles *per se* that constitutes the biological risk factor but the nanoparticle with its corona of associated proteins [57]. Nanoparticles in a biological fluid are invariantly coated with proteins and we have developed methodology for mapping which these proteins are and also to study equilibrium affinities and exchange rates for protein-nanoparticle interactions [58 – 60].

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