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Proteins that switch folds

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Summary

An increasing number of proteins demonstrate the ability to switch between very different fold topologies, expanding their functional utility through new binding interactions. Recent examples of fold switching from naturally occurring and designed systems have a number of common features: (i) The structural transitions require states with diminished stability; (ii) Switching involves flexible regions in one conformer or the other; (iii) A new binding surface is revealed in the alternate fold that can lead to both stabilization of the alternative state and expansion of biological function. Fold switching provides insight into how new folds evolve, but also indicates that an amino acid sequence has more information content than previously thought. A polypeptide chain can encode a stable fold while simultaneously hiding latent propensities for alternative states with novel functions.

Introduction

One of the most basic tenets of biochemistry is that a protein adopts a specific, three dimensional topology under native conditions [1]. Examples continue to accumulate, however, of proteins with ambiguous fold propensity. Changes in protein conformation, including changes from disordered to ordered states [2], occur frequently throughout biology. But some proteins undergo large-scale transitions from one ordered state to another involving major shifts in secondary structure, repacking of the protein core, and exposure of new surfaces.

One of the first examples of such structural rearrangement in proteins was shown for the serpin class of serine protease inhibitors [3]. The irreversible insertion of a loop into the center of a core β -sheet provided a glimpse of just how malleable protein folds can be. Subsequent observations have shown that more than one β -strand can be inserted into another serpin molecule to form a domain-swapped stable dimer [4]. Another early example was the influenza virus hemagglutinin where a pH-induced change leads to an expanded helical structure in going from pre-fusion to post-fusion states [5]. Since that initial discovery other viral fusion proteins have been found to adopt a similar mechanism [6,7]. More recently, a number of studies have highlighted that alternatively folded states can co-exist in equilibrium.

Our purpose here is to discuss the phenomenon of fold switching, to comment on how switching is reconciled with classical views of protein folding, to compare some recent natural examples, and to consider how fold switching works mechanistically. At the outset this phenomenon appears antagonistic to classical ideas about protein folding. Protein folding often can be approximated as a two-state reaction. The folded state is highly populated under native

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conditions with a free energy of folding typically less than -5 kcal/mol. The unfolded state can be approximated by a random coil. These assumptions hold quite well for many proteins when changes in the equilibrium constant for folding are temperature or denaturant-induced. The way a problem is approached can create a certain myopia, however. For example, when proteins do not conform to two-state behavior, we often try to trim off annoying bits to facilitate analysis. This is a widespread practice but may be steering us away from understanding what is really going on and undoubtedly contributes to the idea that fold switching is exotic or outside “normal” folding behavior [8].

Paradoxically, our own interest in conformational switching began a number of years ago when we set out to identify ideal two state proteins for biophysical study. We chose the two binding domains of protein G (G_A and G_B) because they are small, fold without intermediates, and exhibit two-state behavior in calorimetric experiments. Having identified our models, we began mutagenesis studies making another common assumption – mutations affect stability but not two state behavior. Years later we observed the ultimate breakdown of two-state behavior, a mutational path from one fold to another in which the unfolded state is never highly populated [9] (Figure 1).

This result highlights two facts. First, many amino acids in a protein can be mutated without changing the overall fold (and function). This is well known. Second, although two-state assumptions appear to hold through much of the pathway, the protein is silently creeping toward a new destination in fold/function space. Finding mutational pathways which avoid the unfolded state is not trivial, but designers have brought several proteins to the brink [10–15]. It turns out that nature has done the same [16–19].

Naturally occurring protein switches

Lymphotactin

Lymphotactin is one of the most dramatic examples of a protein that undergoes conformational switching. Under physiological conditions it exists in two forms in approximately equal amounts, a monomeric chemokine fold (Ltn10), and a novel dimeric β -sandwich fold (Ltn40) [8,20]. The equilibrium between these two species can be shifted completely from one form to the other by varying salt and temperature conditions. As with our designed G_A/G_B system, there are wholesale changes in structure where many residues that were in the core of Ltn10 are now on the surface of Ltn40. In converting from Ltn10 to Ltn40, the N-terminal loop reorganizes to form a β -strand interaction while the C-terminal α -helix becomes unstructured (Figure 2A). Further, the three β -strands which at first glance appear to be similar between the two structures have in fact undergone changes in register with new H-bonding partners. The structural transition involves generation of a new hydrophobic surface in the Ltn40 subunit that is used to form the dimer interface. It is important to note that Ltn10 is missing a disulfide that is conserved in other chemokines and so the protein is effectively destabilized. When this absent disulfide is engineered into Ltn10, it is locked into the monomeric state and cannot switch to the alternative fold [21]. The monomeric chemokine form acts as an *in vivo* agonist of the G-protein coupled XCR1 receptor while dimeric Ltn40 has been shown to bind glycosaminoglycans. Thus, lymphotactin provides an elegant example of how new functions can be acquired through the ability of a protein to switch folds reversibly. Other examples of β -slipped structures have also been reported. Certain PAS domain mutants can exist in two states that, while very similar in fold, present different residues on the surface of their β -sheets, paving the way to new binding options [22,23]. Changes in β -register have also been shown to convert soluble proteins into higher oligomer amyloids [24,25].

Mad2 spindle checkpoint protein

The mitotic arrest deficiency 2 protein (Mad2) is involved in monitoring the correct attachment of microtubules to kinetochores and exists in two conformations that are quite different [26–29]. Both states have a central core structure that remains unchanged but approximately 60/200 residues, about 50 at the C-terminal end and 10 at the N-terminal end, undergo a large conformational switch between inactive open (O-Mad2) and active closed (C-Mad2) forms (Figure 2B). Switching from a kinetically trapped open state to the more stable closed state is essential so that a latent cdc20-binding site, blocked by the C-terminal β -hairpin in O-Mad2, can be revealed. Conformational change is catalyzed by interaction of O-Mad2 with a C-Mad2/Mad1 complex that is bound to the kinetochore. The C-Mad2 component of this complex binds to its alternatively folded O-Mad2 and catalyzes the structural transition to an active conformer. Interestingly, the conversion is reminiscent of an early model for template driven propagation of prions. What is the mechanism for this fold switch? It has been proposed that C-Mad2 binds to and stabilizes a partially unfolded intermediate state, consisting of the same core structure but with more flexible N- and C-terminal regions, which can then refold and re-equilibrate between the two forms [30].

Chloride intracellular channel 1 (CLIC1) protein

The CLIC1 protein can adopt at least two conformations in solution but neither of these appears to have a direct connection to its ion channel function in membranes [31,32]. Under reducing conditions, CLIC1 is monomeric with an $\alpha/\beta/\alpha$ -sandwich N-domain and a larger α -helical C-domain. Under oxidative conditions, an intramolecular disulfide forms between cysteines 24 and 92 with a concomitant rearrangement of the N-domain into a three α -helical bundle (Figure 2C). This large-scale rearrangement leads to the exposure of a significant hydrophobic surface that forms the interface between helical N-domains in a stable dimer structure. The physiological relevance of the soluble dimer is not clear but it has been proposed that the interfacial hydrophobic surface may facilitate localization to the membrane *in vivo* instead of dimer formation. Further structural transitions are likely to take place in a lipid environment before a channel-competent state is realized. It should be noted that the cysteine residues responsible for disulfide formation are far apart in the reduced $\alpha/\beta/\alpha$ N-domain and so inherent flexibility of this region must play an important role in being able to trap the alternatively folded state. Hydrogen/deuterium exchange indicates that the N-domain is of low stability and dynamic, particularly under conditions of acidic pH that may be relevant near membrane surfaces or in the endosome [33,34].

Conformational switching and the evolution of new folds

The above examples demonstrate that certain amino acid sequences can adopt more than one ordered state and that these folds can be in equilibrium. While such observations are rare, they provide some insight into how new folds and functions may evolve through the process of conformational switching. Earlier work on Arc repressors showed that its C-terminal β -strand could readily transit to α -helical structure with small changes in sequence [35]. More recent examples involving larger segments add further support to the idea that some folds may have resulted from switching an existing structure rather than evolving independently. Within the Cro family of repressors, for example, proteins from two different organisms have 40% sequence identity and show clear homology over the whole sequence [36]. The standard view would be that these polypeptide chains should assume similar three-dimensional structures. However, the x-ray structures of Pfl 6 and Xfaso 1, while retaining similar folds for DNA-binding motifs in the N-terminal part of the sequence, have structurally divergent C-termini. Whereas the C-terminal region is α -helical in monomeric Xfaso 1, the corresponding part of the sequence adopts a β -sheet structure in Pfl 6 which is stabilized through dimerization.

Another example of novel fold evolution through conformational switching comes from structural studies of the transcription factor NusG and its paralog RfaH [37]. NusG has two independent domains separated by a flexible linker. The N-domain contains a solvent accessible hydrophobic surface that is postulated to bind RNA polymerase (RNAP) while the C-domain has a β -barrel structure. RfaH has a similarly structured N-domain, but its C-domain is a two helical coiled-coil where many of the residues that were in the core of the β -barrel now form a hydrophobic surface that interacts tightly with the putative RNAP-binding site of the N-domain. These differences in C-domain structure therefore have important functional consequences. While NusG is a general transcription factor, RfaH has evolved a more specialized regulatory function such that the latent RNAP-binding site is only revealed when additional sequence-specific interactions with DNA take place. Although the sequence identities of the NusG and RfaH C-domains are only around 20%, it is important to note that all of the hydrophobic core residues of the NusG β -barrel are conserved in the RfaH coiled-coil. Indeed, it is possible that the RfaH C-domain could act as a *bonafide* switch from coiled-coil to β -barrel structure in the absence of interdomain interactions [38]. This example displays several of the features of naturally occurring switches that were mentioned earlier such as mutually exclusive cores and latent binding surfaces. Further, it serves as a stark reminder that paralogs may not necessarily have the same three-dimensional structure, despite conservation of “core” residues.

Common features in fold switching

In considering many examples of fold switching, three frequent themes emerge: (i) The structural transitions require states with diminished stability; (ii) Switching involves flexible regions in one conformer or the other; (iii) A new binding surface is revealed in the alternate fold that can lead to both stabilization of the alternative state and expansion of biological function.

Stability is a counterweight to fold switching

If the native state is in an energy well of less than -5 kcal/mol, then by definition the energy of any unfolded/alternative state is unfavorable (> 5 kcal/mol). As the stability of the native state decreases, however, alternative folds become more accessible. This has many biological consequences since losses in stability can occur via a variety of mechanisms (e.g. mutation, proteolysis, reduction of disulfide bonds, or chemical modification). A quick survey of the literature shows that decreases in stability or misfolding frequently lead to ordered non-native states. For example, breaking disulfide bonds in even a small protein like BPTI can result in a compact unfolded state [39], loss of a tightly-bound ligand often yields molten globules [40], and misfolding in *E. coli* can lead to inclusion bodies which are amyloid-like [41]. The nature of a polypeptide dictates that certain interactions will persist in the unfolded state (e.g. steric repulsions, buried hydrophobic surface residues, and main chain H-bonds). All of these forces may create weak propensities for alternative topologies once the stability of the native state diminishes.

Disordered segments in proteins can facilitate structural transitions

Disordered segments in proteins are not necessarily neutral in determining fold propensity. We may be a bit numb to this fact by the common practice of engineering new N- or C- termini on proteins to facilitate purification or trimming off the original ones to facilitate analysis. Nevertheless, the N and C- termini in the G_A/G_B switch play a very prominent role in the fold switch (Figure 3), as do disordered regions in the natural examples described above. A disordered segment may have a fold preference which manifests itself only in a particular structural context. A challenge in understanding the energetic contributions of disordered regions to fold switching is that the term “disordered” is quite vague. Consider one example

in which context determines structure. The prodomain of subtilisin is unfolded in its unbound state as judged by CD, NMR, and calorimetry, and yet it is functional as judged by its binding affinity for subtilisin. The reason for the functionality is that the prodomain has a preferred native conformation that is ~ 2% populated in the unbound state. Thus a folded, functional state of the prodomain is energetically accessible, costing only ~ 2 kcal/mol in the free energy of binding [42,43]. The main point is that two disordered segments may look equally unfolded by standard experimental methods but may not be at all the same in terms of fold propensity.

Exposure of new surfaces can reveal latent binding propensities

Fold switching exposes new surfaces which are pregnant with function. This fact is significant in the evolution of new functionality as discussed in the natural examples above. It is also important because new binding interactions can themselves drive a fold switch because folding and binding are thermodynamically linked. This linkage can result in the formation of multimeric complexes when the monomer self-associates. A common example of this phenomenon is domain swapping [44]. Frequently in domain swapping the monomer unit is destabilized by mutation and disruptions in the hydrophobic core expose new surfaces. These new surfaces can form interfaces between monomers and result in stable multimeric species. Overall secondary structures remain similar but the core is rearranged in new tertiary and quaternary interactions. Binding of a small ligand can also pull a protein into a new conformation as evidenced in engineered metal switches [45–47]. The basic relationship is as follows:

$$A \rightleftharpoons B+L \rightleftharpoons BL$$

where A and B are two alternative folds and L is a ligand which binds exclusively to B. The position of the equilibrium is determined by the relative propensity of A versus B, the affinity of the ligand for B and the concentration of ligand. Instability of A, coupled with ligand binding to fold B, can lead to switching. The latent functionality that exists in alternative folds, while facilitating evolutionary change, also can lead to disease states. Amyloid disease is the classic example [48]. Adventitious ligand binding may stabilize intermediate conformations that catalyze unfolding of the native state. Multimer formation in the final amyloid structure stabilizes the alternative fold.

Conclusions

Although much of the protein universe conforms to the classical view that a protein populates a single, stable native state, a significant portion of proteins are either unstable (and of unknown fold propensity) or are ambiguous in fold propensity [49–51]. This fact has many implications for understanding how proteins evolve, how mutation is related to disease, and how best to annotate function to sequences of unknown structure. The existence of protein sequences at the interface between folds [52,53] also creates serious conceptual challenges for understanding how amino acid sequence determines structure. The information content of a polypeptide chain is greater than expected, encoding a stable native state, while simultaneously hiding latent propensities for alternative states with new functions. In the long term, understanding that this is a general property of polypeptides should help in predicting structure from sequence. In the short term, it remains very challenging to predict what protein folds may be switchable and what alternative states may be accessible.

Acknowledgments

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Figure 1.

A functional protein can switch into a completely different conformation with a different function via a mutational pathway in which neither function nor native structure is completely lost. The G_A98 and G_B98 proteins are only marginally stable, but restoration of close to wild type stability and function in either direction can be attained with only three mutations (e.g. to G_{A88} and G_{B88b}).

Figure 2.

Examples of naturally occurring ordered proteins that can exist in alternatively folded states. The structures of Ltn10, O-Mad2, and CLIC1(reduced) are color-coded as follows: β -strand (cyan), helix (red), and coil (yellow). The structures of the respective alternatively folded states Ltn40, C-Mad2, and CLIC1(oxidized), are shown with an identical per residue color code to illustrate the changes that take place in switching folds. (A) NMR structures of Ltn10 (PDB 1J8I, [54]) and Ltn40 (PDB 2JP1, [8]). A disulfide can be engineered between residues 21 and 59 in Ltn10 to prevent conformational switching. Only one of the subunits is colored in the Ltn40 homodimer. (B) NMR structures of O-Mad2 (PDB 1DUJ; [55]) and C-Mad2 (PDB 1S2H; [26]). Unchanged parts of the structure are in gray. (C) X-ray structures of the reduced (PDB 1K0M, [56]) and oxidized (PDB 1RK4, [31]) forms for the N-domains of CLIC1. The cysteine residues involved in disulfide formation are labeled in both states.

Figure 3. Conformational switching from 3- α G_A to 4 β + α G_B [9,14,15]. Regions of secondary structure in G_B are color coded as follow β -strand (cyan), helix (red), and coil (yellow). The residues corresponding to these regions in the 3- α fold are colored in the same way to illustrate the extent of structural changes.