Research Focus

Nano-dynamics: engineering allostery via tandem duplication and turn energetics

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In a recent article Matthews and coworkers describe a phage T4 lysozyme mutant that incorporates a tandem duplication of an α-helix. Only one repeat can occupy the equivalent wild-type location, however, the structure can be induced to switch to the alternative repeat by modifications to an adjacent turn. The results highlight the interplay between the stabilizing forces of adjacent secondary structures and provide a mechanism for allostery over long distances.

In a recent interview [1] Sir Alan Fersht outlined the most important questions of protein folding that face scientists at this time. Although the ultimate goal is still the ability to predict *de novo* protein folding and unfolding pathways, structure, stability, function and activity from primary structure, progress towards this goal will probably be accomplished by focusing on parts of the problem that appear soluble in the foreseeable future. Key areas for study include *de novo* design and evolution and protein misfolding as well as protein dynamics and allostery. A recent paper by Matthews and coworkers [2] is notable both for its originality and for the relevance of the results to several of the key areas of the protein-folding problem.

Conjoined (helical) twins

In an earlier study by this group, the region comprising residue positions 40–50 in phage T4 lysozyme was duplicated in the primary structure [3]. In the wild-type protein this region adopts an α-helical secondary structure that packs against the N-terminal domain of this bi-lobed enzyme. The helix in question is preceded, and followed, by turn secondary structure (a classification of polypeptide conformation that results in a 180° change in direction). The earlier study showed that a tandem duplication of this helix is accommodated by structural reorganization of the N-terminus turn region and retention of the C-terminus turn structure, and the location of the original helical secondary structure is occupied by the duplicated primary structure of residues 40–50. These results suggest that the C-terminus turn has lower structural free energy than the N-terminus turn. To test this hypothesis, the tandem 40–50 lysozyme mutant was further modified substituting a poly-Gly sequence at the C-terminus region (i.e. residue positions 51–56, using the numbering scheme of the original primary structure). This type of mutation should result in the elimination of key side-chain interactions stabilizing this turn structure. In response to the destabilization of the C-terminus turn, the tandem 40–50 repeat is now accommodated by structural reorganization of the C-terminus turn region and retention of the N-terminus turn structure, and the location of the original helical secondary structure is occupied by the first instance of the 40–50 primary structure (Figure 1).

Battle of the turns

The selection of the specific repeat to adopt the position of the wild-type helix appears to be determined by the comparative energetics of the N- and C-terminus turn regions. Although such modulation was accomplished by Matthews and coworkers by combination Gly substitutions (each of which contributes to the elimination of side-chain interactions that can stabilize turn secondary structure), this modulation could conceivably be achieved by turn regions in which stability is determined by interactions with environmental factors. Thus, turns stabilized by electrostatic interactions might be modulated by alterations in pH that titrate such groups. Similarly, turns stabilized by binding of metal ions might be modulated by changes in the concentration of these ions.

The results of these studies identify a design principle by which a defined conformational change might be engineered and regulated to create the basis of allostery. The generation and transmission of structural changes associated with allostery are of major interest to researchers in the field of protein folding and engineering because the dynamic motions of proteins in relation to their overall 3D structure are key to their function. In this regard, the structural change in the tandem-repeat T4 lysozyme mutant is considered an extreme example of a type of helical shift that is observed in the allostery of aspartate receptors described by Yu and Koshland [4], and a mechanism by which structural changes can be propagated over long distances.

The evolution of allostery

Six of the ten fundamental protein superfolds [αβ plait, triose phosphate isomerase (TIM) barrel, β-trefoil, 'jelly

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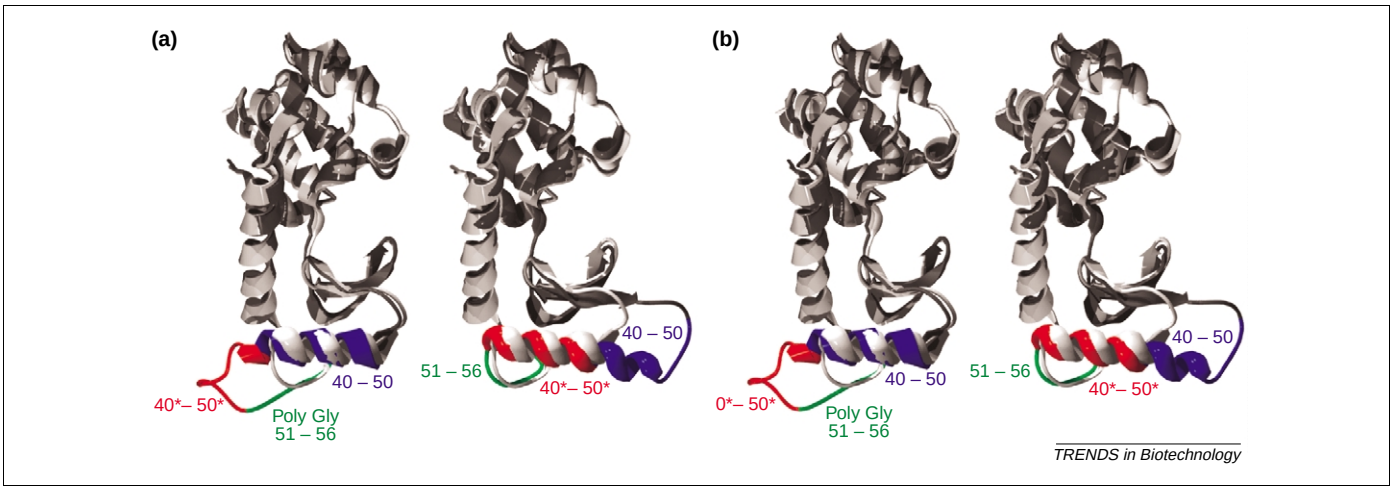

Figure 1. Relaxed stereo diagram of the T4 lysozyme mutant with duplicated region 40–50 (a), and the same mutation with Gly substitutions introduced in region 51–56 (numbering scheme of the wild-type protein) (b). The first instance of the region 40–50 in each mutant is shown in blue and the second instance in red (and indicated by asterisk). The location of the turn structure on the C-terminus side of the helix is indicated in green. Also shown is the wild type structure (light gray) overlaid onto each mutant.

roll', IG-like and 'up-down' superfolds] exhibit a pronounced structural symmetry [5] and many proteins seem to have evolved as the result of gene duplication or fusion events [6–12]. Hence the way a protein responds to a duplicated region is an essential aspect of protein evolution. In this context, an interesting property of symmetric protein superfolds is that although the symmetry might be pronounced at the level of the tertiary structure, it is often largely absent at the level of the primary structure. The constraints that primary structure symmetry could impose on protein folding and stability remain to be elucidated, although they are probably a key factor in the principles of *de novo* protein synthesis [13].

The results of the tandem duplication in phage T4 lysozyme highlight the critical interplay between the energetics of adjacent regions of secondary structure, in particular the stability of turn secondary structure adjacent to duplicated regions. The importance of turn secondary structure in protein structure, stability and folding has been highlighted by the studies of Eisenberg and coworkers. For instance, mutations in turn secondary structure can result in domain swapping and assembly of oligomeric structures [14]. A simple question to ask is whether the structure of a turn is dictated by the stability of the adjacent secondary structure, or vice versa. Despite the importance of understanding the energetics associated with turn regions, there is far less known about this type of secondary structure in comparison with α-helices or β-sheets. In recent work [15], conflicting data concerning the relationship between structure and energetics within specific types of turn structures (i.e. those within the L-α region of the Ramachandran plot) has been elucidated.

In conclusion, there is a common thread between structural duplication, turn stability, allostery, symmetry, evolution and *de novo* protein design. The recent report by Matthews and coworkers illustrates these relationships with astonishing structural dynamics. Understanding the nature of the energetics of turn secondary structure is an important immediate goal for advancing our understanding of the different aspects of the protein folding problem.

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0167-7799/$ - see front matter
doi:10.1016/j.tibtech.2003.10.010