DISSERTATION ABSTRACT

Recent findings of aberrant protein deposits in a number of diseases have turned protein folding into a research area of considerable interest. From this research it is becoming increasingly understood that many features of such deposits arise from common principles, and the ability to form such aggregates is thus a generic property of all polypeptide chains. Clearly, evolutionary pressure has resulted in polypeptide sequences that swiftly adopt their structure in order to avoid detrimental intermolecular bond formation. However, the folding code inherent to any such foldable polypeptide is yet to be cracked.

Several aspects of what makes β-sheet proteins foldable have been studied. *Pseudomonas aeruginosa* azurin was complemented with the remotely homologous spinach plastocyanin in experiments aimed at addressing the somewhat paradoxical situation of why these proteins both irreversibly and reversibly unfold when denatured with heat. It turns out that a site-specific redox reaction between sulfur and oxygen is the sole culprit in inducing irreversibility on the thermal transitions of both proteins, and this reaction is readily catalyzed by the coordinated copper ion. Because molecular oxygen is implicated in this mechanism, its removal makes the thermal transition reversible for all but the Cu^{2+}-coordinating species. The second part of the paradox, however, touches on a relatively unexplored field in biochemistry, namely why some proteins do not aggregate at high temperature despite a high inherent structural propensity to do so. It is speculated that residual structure in the denatured state may be one of the means by which such detrimental aggregation is avoided.

The residual structure identified during thermal denaturation may hint at a high tendency for hydrophobic collapse under native conditions. Indeed, chemically induced folding studies on apo-azurin revealed a burst-phase intermediate accumulating under increasingly native conditions, and because of its ability to bind 1-anilinonaphtalene-8-sulfonate (ANS) it was ascribed molten-globule like status. Nevertheless, two-state folding behavior was well approximated thus hinting at an evolutionary perfected folding mechanism. This very mechanism was further probed by mutating structurally conserved core residues, where a correlation appears to exist between interactions stabilizing the protein structure and the transition state as manifested in the linearity between folding rate and folding free energy. Hence, the structural determinants common to all β-sandwich proteins not only determine the fold, but also the best way of getting there.

KEYWORDS: azurin, folding, thermal denaturation, cysteine oxidation, molten globule

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