Rational thermostabilization of a three-state protein and testing of the role of a native basin intermediate

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Rational stabilization of proteins against thermal denaturation may facilitate their use in biotechnological and medical applications and provides an opportunity to test our understanding of protein energetics. Specially challenging are proteins not showing two-state unfolding equilibria. Long-chain flavodoxins (non covalent complexes between apoflavodoxin and FMN) are electron transfer proteins involved in essential bacterial reactions. Apoflavodoxin thermal unfolding is characterized by accumulation of an evolutionary conserved partly unfolded intermediate whose structure has been determined for the Anabaena protein. The intermediate belongs to the native basin as it is significantly populated at room temperature. Relevant thermostabilization of three-state proteins, such as this one, can only be achieved by specific intervention in the unstable subdomain. Following this simple rule we have designed and combined stabilizing mutations to produce a thermostable apoflavodoxin variant ($\Delta T_m = 32$ °C) with cooperative two-state equilibrium behaviour, thus lacking the equilibrium intermediate of the WT protein.

General reasoning has attributed important roles in protein binding and catalysis to native basin intermediates, but proof is sometimes elusive. Comparison of WT and thermostable apoflavodoxin variant allows to assess the functional importance of this particular intermediate. Our analyses indicate that it only exerts minor influences in folding, FMN binding, protein-protein interaction, electron transfer rates and overall tridimensional structure, which argues for a lack of biological relevance of this evolutionary conserved intermediate and raises some concern for unsubstantiated attributions of adaptative value to similar features in other proteins.