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Protein folding of the SAP domain, a naturally occurring two-helix bundle

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ABSTRACT

The SAP domain from the *Saccharomyces cerevisiae* Tho1 protein is comprised of just two helices and a hydrophobic core and is one of the smallest proteins whose folding has been characterised. Φ-value analysis revealed that Tho1 SAP folds through a transition state where helix 1 is the most extensively formed element of secondary structure and flickering native-like core contacts from Leu35 are also present. The contacts that contribute most to native state stability of Tho1 SAP are not formed in the transition state.

1. Introduction

The principles which govern the folding and unfolding of proteins have fascinated the scientific community for decades [1]. One of the most successful approaches has been to apply chemical transition state theory and treat the folding reaction as a barrier-limited process between two conformational ensembles of proteins populated at equilibrium: the native and denatured state ensembles.

The transition state, the high energy conformation transiently adopted by the polypeptide chain as the protein crosses the barrier between native and denatured ensembles, provides information on the structural mechanism of protein folding. This information cannot be determined by traditional structural techniques, and instead is inferred from kinetics and mutational analysis using the technique of Φ-value analysis [2–7]. The Φ-value of a mutation is defined as the change in stability of the transition state upon making the mutation ($\Delta\Delta G_{\ddagger\text{-}D} = \Delta G^{WT}_{\ddagger\text{-}D} - \Delta G^{mut}_{\ddagger\text{-}D}$) expressed as a fraction of the change in stability of the native state ($\Delta\Delta G_{N\text{-}D} = \Delta G^{WT}_{N\text{-}D} - \Delta G^{mut}_{N\text{-}D}$) for the same mutation: *i.e.* $\Phi = \frac{\Delta\Delta G_{\ddagger\text{-}D}}{\Delta\Delta G_{D\text{-}N}}$. For non-disruptive deletion mutations where reorganisations in native or denatured state structure are not predicted, a value of $\Phi = 1$ indicates that any interactions made by the deleted sidechain or chemical group are as formed in the transition state as they are in the native state. A value of $\Phi = 0$ indicates that the interactions are not present in the transition state, and a fractional value of Φ may indicate partial formation of interactions, complete formation of a subset of multiple interactions, or complete formation of interactions in a fraction of cases (*i.e.* heterogeneity in the ensemble of transition states).

In order to draw general conclusions about the principles governing protein folding and unfolding, it is important to determine detailed folding information (including structural information on the transition state) for a number of model proteins of different sizes, structures and topologies. We have previously presented the folding and unfolding behaviour of the L31W (fluorophore) mutant of the SAP domain from the *Saccharomyces cerevisiae* Thol protein [8] (SAP so-named after the first initial of the three proteins in which it was first identified [9]). Thol SAP is monomeric in solution and folds reversibly in an apparent two-state transition making it ideal for further study. The overall fold comprises just 51 residues, which form two approximately parallel helices separated by an extended loop, and possesses a hydrophobic core of just four residues (Leu13, Leu17, Trp31 and Leu35). Its motif of two parallel helices is quite unusual – model α-helical proteins more frequently

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contain antiparallel or perpendicular helices in a helix-turn-helix arrangement [10–14] – and Tho1 SAP is one of the smallest proteins whose folding has been studied experimentally. It is therefore of interest to study the folding of Tho1 SAP in more detail.

In this paper we have conducted a Φ-value analysis of the Tho1 SAP domain. The Φ-values we obtained were fractional, indicating that Tho1 SAP folds through a transition state with transient formation of a core and flickering elements of helical structure. The best formed element of secondary structure was helix 1. Interestingly, the contacts which contributed most to native state stability were not formed in the transition state. In order to obtain a crude indication of the validity of our results across multiple temperatures, we measured the folding of L31W SAP across the range of 283–323 K. As judged by the change in solvent-accessible surface area upon folding (β_T value and analogous ratio of heat capacities), there are no gross changes in the transition state of Tho1 SAP with temperature.

2. Materials and methods

2.1. Reagents

L31W SAP domain was expressed and purified as detailed previously [8]. Point mutations were generated using Stratagene Quikchange mutagenesis. Mutant proteins were expressed and purified as described for SAP L31W until completion of cleavage of the fusion protein, when tag and target were separated by flowing once more through Ni-charged IMAC resin (GE Healthcare BioSciences, Sweden) before concentration and gel filtration on an S75 column into 50 mM MES pH 6.0, total ionic strength of 500 mM made up to this value using NaCl. A single peak was obtained for all proteins and fractions within this peak pooled.

2.2. Equilibrium denaturation

Far-UV CD spectroscopy (thermal denaturation) and fluorescence emission (chemical denaturation) were carried out as described previously [8].

2.3. Kinetic measurements

We measured relaxation kinetics on the μs–ms timescale using T-jump fluorescence spectroscopy and temperature jumps of 3–5 K on a modified Hi-Tech PTJ-64 (Hi-Tech Ltd., Salisbury, UK) capacitor-discharge T-jump apparatus as previously described [8]. Arrhenius analysis of the plot of microscopic rate constant against temperature was carried out constraining the overall ΔH, ΔS and ΔC_p to their equilibrium values at the thermal midpoint.

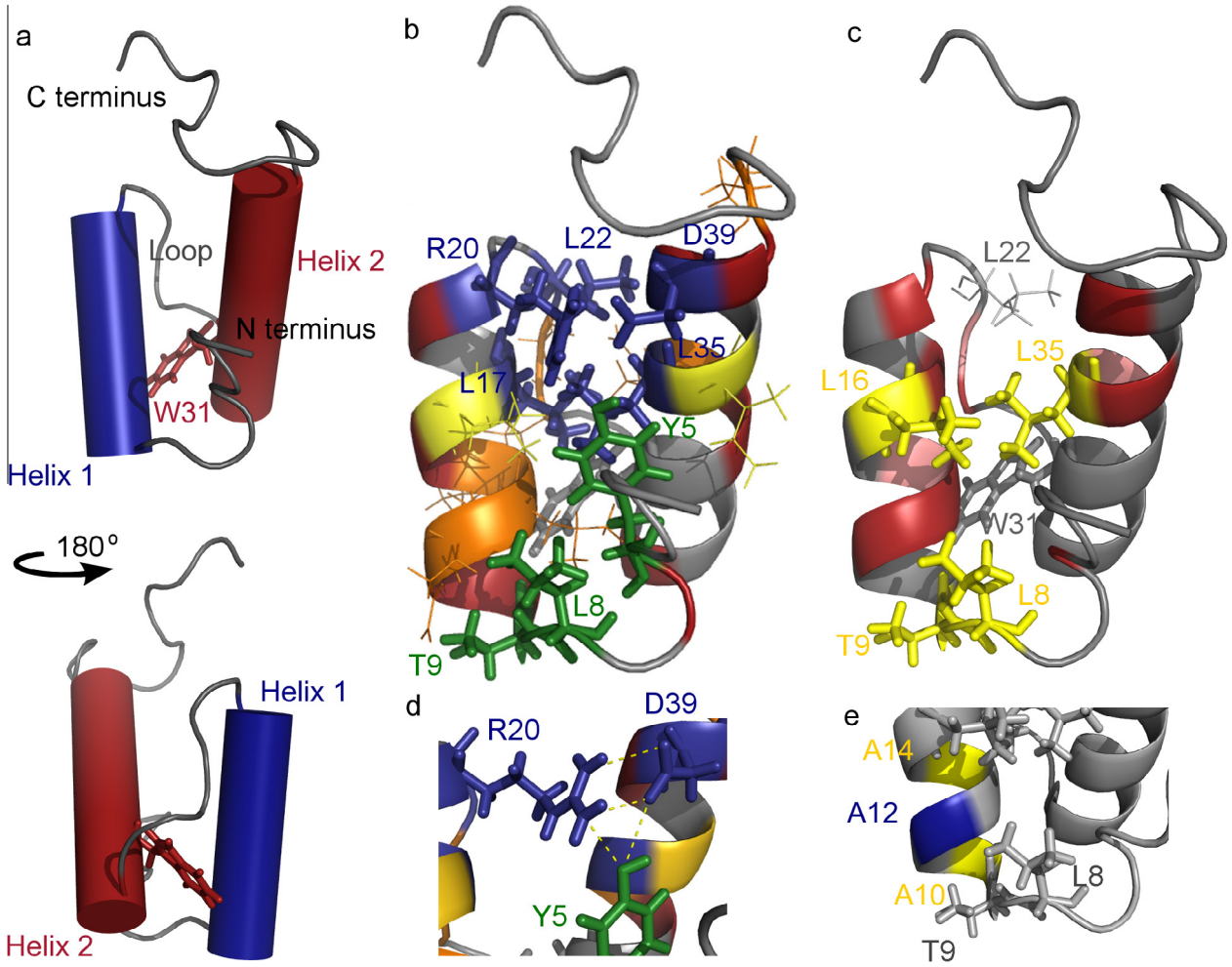

Fig. 1. Structure of L31W and energetic contribution of different residues to stability. (a) Connectivity of L31W. Trp31 is shown in stick representation. (b) Contribution of side chains to native state stability. $-0.5 < \Delta\Delta G_{N\text{-}D} < 0.5$ kcal mol^{-1} (red), $0.5 < \Delta\Delta G_{N\text{-}D} \leqslant 1.0$ kcal mol^{-1} (orange), $1.0 < \Delta\Delta G_{N\text{-}D} \leqslant 1.5$ kcal mol^{-1} (yellow), $1.5 < \Delta\Delta G_{N\text{-}D} \leqslant 2.0$ kcal mol^{-1} (green), $\Delta\Delta G_{N\text{-}D} > 2.0$ kcal mol^{-1} (blue). Trp31 is shown in grey. (c) Contribution of side chains to transition state stability. Φ-Values coloured by $\Phi_f < 0$ (pink), $0 \leqslant \Phi_f < 0.3$ (red), $0.3 \leqslant \Phi_f \leqslant 0.6$ (yellow) and $\Phi_f > 0.6$ (blue). Trp31 and Leu22 shown in grey. (d) Putative hydrogen bonding network stabilising the native state of Tho1 SAP. Proposed hydrogen bonds shown with dashed yellow lines, other colours as for (b). (e) Φ-values for alanine-glycine scanning of helix 1 with A10G, A12G and A14G. Colours as for (c).