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Crystal structures of highly simplified BPTIs provide insights into hydration-driven increase of unfolding enthalpy

Mohammad Monirul Islam[a,b], **Yutaka Kuroda**[a *]

[a]Department of Biotechnology and Life Sciences, Tokyo University of Agriculture and Technology, Tokyo184-8588

[b] Department of Biochemistry and Molecular Biology, University of Chittagong, Chittagong-4331, Bangladesh.

1 Introduction

Sequences encoding natural proteins constitute a tiny fraction of the enormous variety of sequences that can be derived from the combination of 20 amino acids. This variety is a major barrier to the elucidation of how a protein structure is encoded in its sequence. However, artificial sequences encoding functional and stably folded proteins have been designed from a reduced set of amino acids or by specifying a reduced number of sites along the amino acid sequence. This suggests that the sequence determinants for specifying a protein structure is limited, and that the redundant information in a natural protein sequence can be experimentally minimized without compromising its native structure.

2 Experiments

All six BPTI variants were constructed using a pMMHa vector by standard genetic engineering methods. X-ray diffraction data were recorded from single crystals at the Photon Factory, and structures were determined by molecular replacement.

3 Results and Discussion

Here, we report a structural and thermodynamic analysis of six extensively simplified BPTI variants, where 19–24 of its 58 residues are alanines, using differential scanning calorimetry (DSC) and X-ray crystallography.

First, DSC indicated a two-state thermal unfolding, typical of a native protein with densely packed interior. It was surprising that a protein sequence where more than 40% of the residues are alanines can indeed fold into a native-like, well packed structure, which can be solved at high resolution (Fig. 1). Next, comparison with the crystal structures indicted that the enthalpy of stabilization correlated with the increase of water molecules recruited at places left by substituting large side chains (Fig 2). These results provide new insights into the hydration term of unfolding enthalpy and could be rationalized by assuming that the water molecules recruited nearby the alanines are stabilized both by the hydration shells of methyl groups and the formation of H-bonds between water molecules and protein atoms.

Overall, our results confirm and reinforce our previous conclusion that the determinants of a protein are located into the reduced sets of buried amino acids, which significantly simplifies the protein folding problem. Secondly, our report represents the first experimental molecular "visualization" of a hydration-driven enthalpy stabilization, which has been computationally predicted but not demonstrated *per se*.

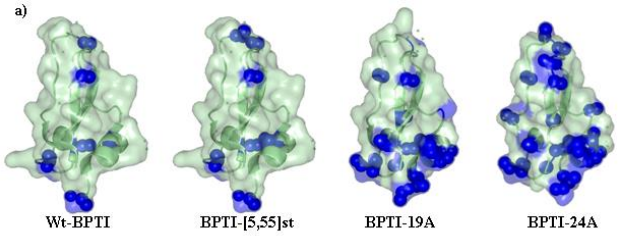

Fig 1: Structures of the simplified BPTI variants. (a) Surface model of wt BPTI, BPTI-[5,55]st, BPTI-19A and BPTI-24A containing, respectively, 6, 8, 19 and 24 alanines out of 58 residues. Alanines are shown as blue spheres (adapted from ref 1).

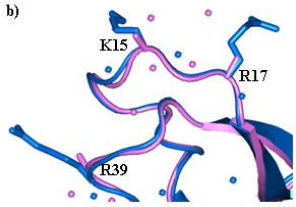

Fig 2: New hydration networks around the K15AR17A (left) site. Wt-BPTI and BPTI-24A are shown with a ribbon model in blue and violet respectively. Spheres represent water molecules around the alanine substitution sites. (adapted from ref 1).

References

1) Crystal structures of highly simplified BPTIs provide insights into hydration-driven increase of unfolding enthalpy. Mohammad M Islam, Masafumi Yohda M, Shun-Ichi Kidokoro, Yutaka Kuroda *Scientific Reports* 7, Article number: 41205. (2017 Mar 7)

2) The coordinates and structure factors of BPTI-21A, BPTI-22Ab, BPTI-23A and BPTI-24A variants are deposited in the Protein Data Bank under the PDB entry codes 5JB4, 5JB5, 5JB6 and 5JB.

*ykuroda@cc.tuat.ac.jp