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Structural analysis of temperature stabilization mechanisms using BPTI variants

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1 Introduction

Enhancing protein stability is an important aspect of protein engineering, attracting considerable interest both from academic and pharmaceutical/biotechnological perspectives. However, most examples of significant stabilization result from a subtle interplay between the effects of multiple mutations, which is difficult to rationalize, because the effects are intertwined.

Analysis of proteins stabilized by one or two mutations may provide direct insight into stabilization mechanisms, since all effects necessarily result from these mutations. Here, we use BPTI-[5,55], which is a small globular 58 residue protein and is widely used as a model system in protein folding and biophysical studies. In order to shed light into stabilization mechanisms of proteins, we analyzed the thermodynamics and high resolution structure of nine BPTI-[5,55] variants with amino acid substitution at positions 14 and 38[1].

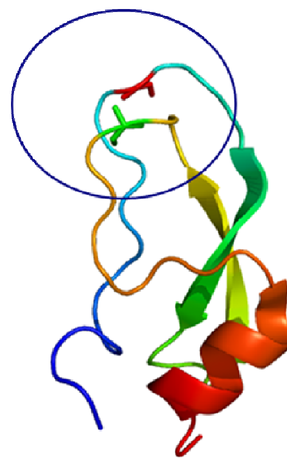
2 Experiments

All BPTI variants were over-expressed in E-coli using the pMMHA expression vector, purified by reverse phase HPLC, and lyophilized. The melting temperatures were measured by monitoring the temperature dependence of the secondary structure content using a Jasco J820 CD spectrophotometer. The X-ray diffraction data were recorded from single crystals at the Photon Factory (Tsukuba, Japan), and the structures were determined by molecular replacement.

3 Results and Discussion

We measured the melting temperatures of nine BPTI-[5,55] variants with G, A, and V at residues 14 and 38 (Table 1). We observed a remarkable twelve-degree thermal stability increase introduced by the A14G substitution, which appeared to be enthalpy driven. High resolution structure of BPTI-[5,55], determined at 1.2 Å resolution, indicated that the enthalpy stabilization is attributable to three structural factors (Fig.1, [3]). First, the A14G mutation displaced the 14-17 loop toward the 36-39 loop by up to 0.7Å improving the local packing, and therefore potentially contributing to enthalpy stabilization through an increase in van der Waals interactions. Second, it removed a potential side-chain steric clash between residues 14 and 36. Last, the A14G mutation also allowed the formation of additional hydrogen bonds between the protein and water molecules in the vicinity of the substitution sites. In contrast, the A38V substitution stabilized BPTI-[5,55] by 4 degree, in terms of entropy, probably by restricting the conformational freedom of the polypeptide chain in the denatured state.

The present observations is one of the first unambiguous analyses of the subtle interplay, taking place at the sub-Angstrom level, between the side-chain and backbone structures producing a major effect on protein stability. They suggest that computational and experimental tools that optimize packing densities by assessing both local backbone deformations and side-chain clashes would have a far-reaching impact on biotechnological industries.



Ribbon model of BPTI-[5,55]. Residues 14 and 38 that were systematically mutated to G, A, V are encircled.

R14/R38	T_m(K)
A/G	310.8
A/A	312.5
A/V	316.7
V/G	313.1
V/A	316.2
V/V	324.4
G/G	322.6
G/A	325.6
G/V	326.9

Table 1: Melting temperatures of 9 variants with the indicated residues at position 14 and 38.

References

[1] Islam, M.M., et al., *PROTEINS,* **77**(4): 962 -970 (2009).

[2] Islam, M.M., et al., *Proc Natl Acad Sci U S A*, **105**(40):15334-9 (2008).

[3] The coordinates of BPTI-[5,55] A14GA38V have been deposited in the Protein Data Bank under the PDB entry code 2ZVX.