X-Ray Crystal Structures of Extensively Simplified BPTI Variants Determined Using the KEK Photon Factory Facility

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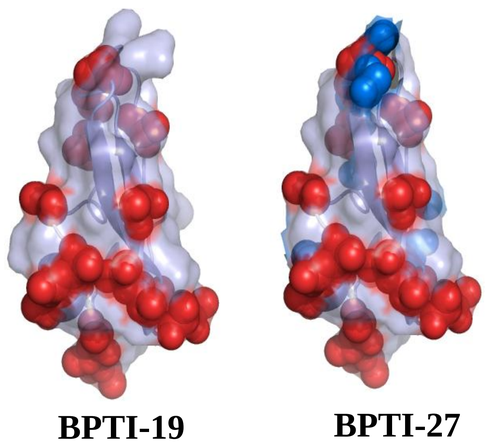
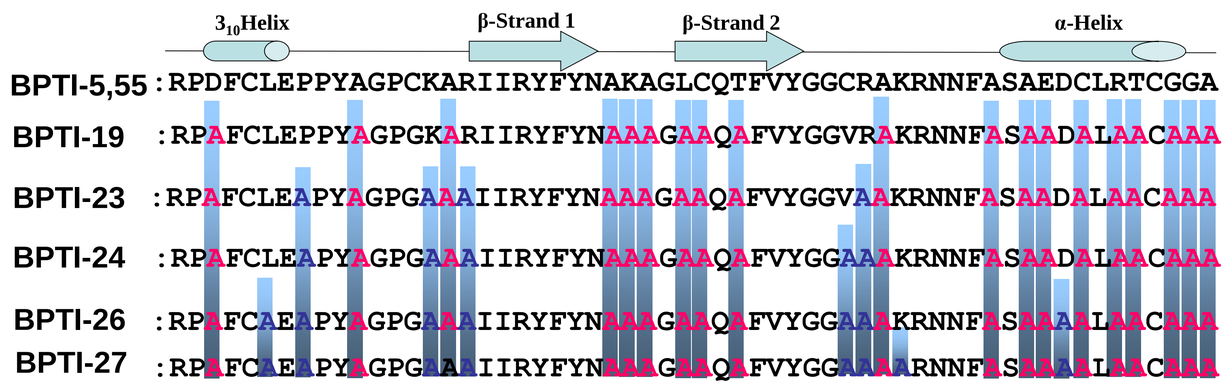
☐ Introduction

Protein stabilization is very difficult to rationalize as it often results from multiple mutations, whose effects are intertwined. Rather, it is worth investigating the 3-D structures of proteins differed by a single and/or a few amino acid substitutions, followed by their thermodynamic studies to elucidate the stabilization mechanism. Here, we report the X-ray crystal structures of several BPTI variants containing 19 to 23 alanines (out of 58 residues) that were determined using the Photon Factory synchrotron radiation source at KEK. All extensively simplified BPTI variants retained almost perfectly the wild-type BPTI structure. However, pair wise RMS deviations at Cα atoms indicated that small local structural fluctuations, found in the wild-type structure (7PTI), were significantly reduced in the simplified BPTI structures. The temperature factors (main chain, side chain and average temperature factors) were also significantly reduced at and/or around the alanine substitution sites. Moreover, new hydration structures (protein water interaction) were observed at/around the substitution sites that could contribute to rigidify the native structure.

☐Methods



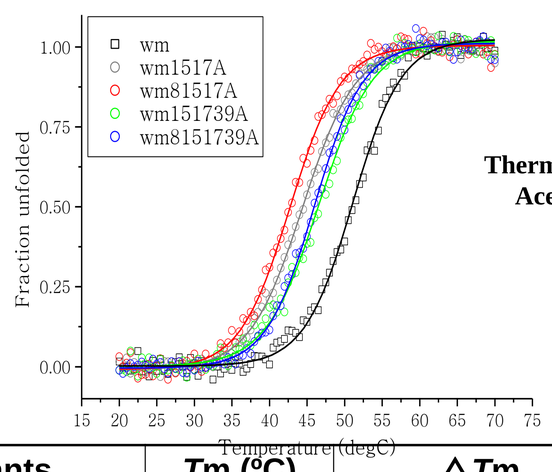
Surface representation of BPTI variants. Alanines are in red while alanines present BPTI-27 but not in BPTI-19 are in blue.



Sequences of BPTI variants. BPTI-[5-55] contains only the [5-55] bond, and four wild type SS bonding cysteines substituted to alanines. BPTI-19 to 27 contains 19 to 27 alanines, respectively

☐Results

☐ Thermal stability by circular dichroism

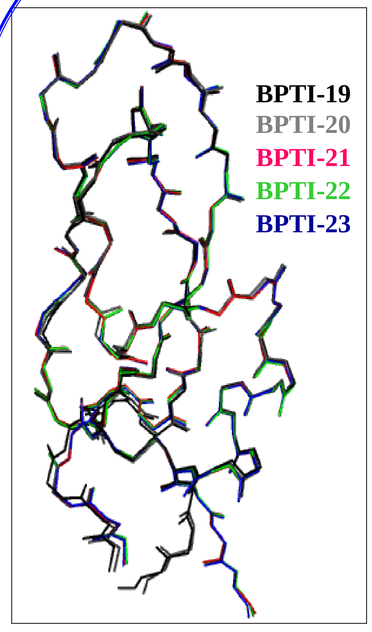


Thermal stabilities measured in 20mM Acetate (pH4.7) at 10 µM protein concentration.

Mutants	*T*m (ºC)	△*T*m (experimental)	△*T*m (estimated)
BPTI-19	**51.35**	**-**	**-**
BPTI-21	**44.33**	**-7.02**	**-4.50**
BPTI-22a	**42.81**	**-8.54**	**-7.00**
BPTI-22b	**46.56**	**-4.79**	**-4.50**
BPTI-23	**46.68**	**-4.67**	**-7.00**

What are the effects of alanine substitutions to the protein structures and stability?

☐ Structure determination: X-ray crystallography

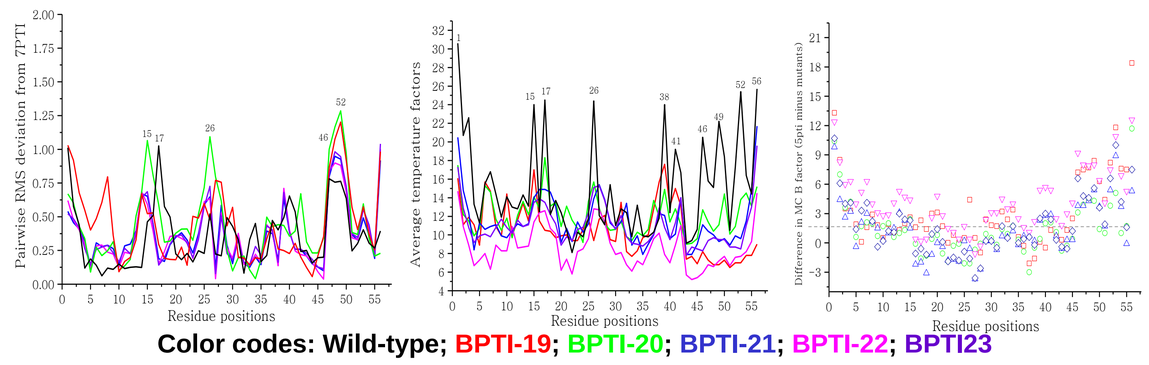


Parameters — **Refinement statistics**

	BPTI-19	BPTI-20	BPTI-21	BPTI-22	BPTI-23
Space group	C2	$P2_12_12_1$	$C2_12_12_1$	$C2_12_12_1$	$C2_12_12_1$
Matthews coefficient	2.15	2.14	2.16	2.18	2.27
Solvent content	43.5%	42%	43.1%	43.1%	45.2%
Reflection used	49171	38836	12300	23855	13898
Max. resolution (Å)	1.00	1.391	1.99	1.60	1.90
Rfactor/Rfree	0.14/0.16	0.156/.20	0.17/0.23	0.18/0.23	0.18/0.21
Ramachandarn plot statistics					
Res in most favored	90.4%	90.7%	89.4%	90.7%	88.0%

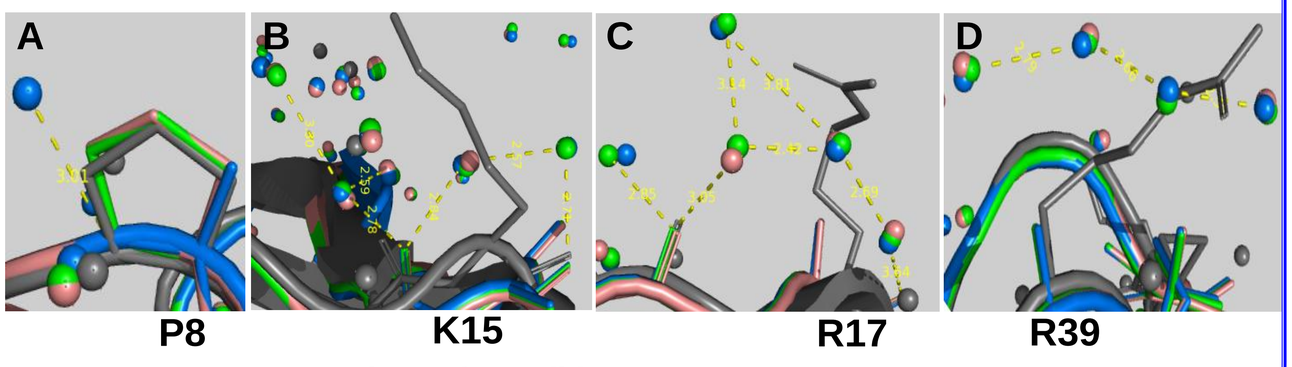
Backbone RMS Deviations at Cα Atoms

Template structures	Simplified BPTIs				
	BPTI-19	BPTI-20	BPTI-21	BPTI-22	BPTI-23
BPTI-[5,55]	0.364	0.484	0.386	0.374	0.378
4PTI	0.420	0.429	0.336	0.312	0.306



Color codes: Wild-type; BPTI-19; BPTI-20; BPTI-21; BPTI-22; BPTI23

Structural fluctuations in wild-type and mutant BPTI structures. Left: Pair wise RMS deviations at Cαatoms from 7PTI; Middle: average temperature factors; and Right: difference in main chain temperature factors (5PTI minus simplified BPTIs).



Color codes: Wild-type; BPTI-21; BPTI-22; BPTI23

New hydration structures at and/or around the alanine substitution sites in the wild-type and mutant BPTI structures. (A) P8A; (B) K15A; (C) R17A; and (D) R39A substitution sites. 7PTI is used as the wild-type BPTI structure.

☐Conclusions

- **All extensively simplified BPTI variants retained almost perfectly the wild-type BPTI structures.**
- **Pair wise RMS deviations at Cα atoms indicated small local structural fluctuations, found in the wild-type, were significantly reduced in the simplified structures.**
- **The temperature factors (main chain, side chain and average) were also significantly reduced at and/or around the alanine substitution sites.**
- **New hydration structures (protein water interactions) were observed at/around the substitution sites that could contribute to rigidify the native structures.**

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