BL-5A, BL-17A/2015G128

Analysis and control of protein crystallization using short Solubility Controlling Peptide (SCP) tags that change solubility without affecting structure

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

[a]Department of Biotechnology and Life Sciences, Tokyo University of Agriculture and Technology, Tokyo 184-8588. [b]Department of Biochemistry and Molecular Biology, University of Chittagong, Chittagong-4331, Bangladesh

1 Introduction

The production of high quality crystals is a prerequisite first step for determining high resolution structures of biomolecules. However, the biophysical mechanism of crystallization remains to be fully understood, and the success rate of protein crystallization projects is surprisingly low, even for well folded proteins with moderate sizes.

Here, we report a systematic investigation of the influence of short solubility-controlling peptide tags (SCP) on the structure, and crystallization behavior of our model protein, BPTI-19A, a Bovine Pancreatic Trypsin Inhibitor variant. BPTI-19A was attached to SCP tags made of five residues of a single amino acid type (Acidic, Basic, Polar and Hydrophobic). None of the SCP tags affected the trypsin inhibitory activity, thermodynamic properties, or structure of BPTI-19A, whereas the crystallization properties were clearly influenced by the changes in long-term solubility (*LS*) and precipitation speed generated by the SCP tags [1].

2 Experiments

All SCP tagged BPTI variants were constructed using a pMMHa vector. DNA sequences corresponding to the SCP tags were added to the C-terminus of the BPTI-19A sequence 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

We examined the effects of ten SCP tags on crystallization the behavior of a BPTI variant. The tags did not affect structure, thermodynamics and activities of BPTI. Moreover, eight of the tagged variants crystallized under the same condition and six of them diffracted at high resolution [2]. All variants with long-term solubility (*LS*) between 1-6mg/mL produced crystals that diffracted well, while variants with *LS* <1 and >6mg/mL did not crystallize, produced poorly diffracting crystals, or crystallized under a different condition (Fig.1). The only exception was a glutamine tagged variant, which had *LS* of 5mg/mL but fast aggregation kinetics and produced mere needles that were unsuitable for further analysis.

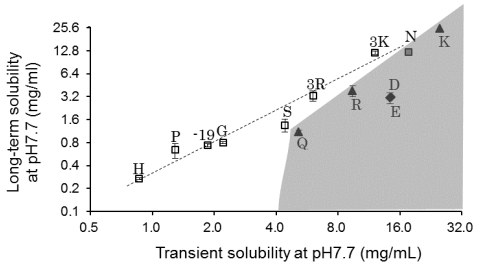

Fig 1: The crystallization properties of the tagged BPTI-19A variants at pH7.7 are displayed on a correlation plot between *LS* versus *TS*. The crystallization behavior is indicated as follows: □: good crystals and structures solved; ♦: large crystals but poor diffraction; ▲: needles (no structure solved). The shaded area indicates the *LS*/*TS* region where BPTI-19A variants had poor crystallization properties, whereas the dotted line indicates an *LS*/*TS* region that yielded good crystals.

Crystal structures indicated that BPTI-19A remained unchanged, and that most tags were largely invisible, indicating high flexibility, without having interactions with nearby residues. In conclusion short peptides, introducing a mere 5-7 residue elongation, could provide a useful technology for tuning protein solubility without affecting its other properties, and hence for overcoming problems associated with excessively low or high solubility, such as in crystallization [3].

References

[1] AM Khan et al, *BBA Proteins and Proteomics*, **2013,**1834(10):2107-15

[2] The coordinates of the tagged BPTI variants are deposited under PDB IDs: 3AUB, 3AUC, 3AUD, 3AUE, 3AUG, 3AUH, and 3WNY

[3] MM Islam, et al, *Crystal Growth & Design*, **2015**, 15 (6), pp 2703–11

E-mail *YK: ykuroda@cc.tuat.ac.jp