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Crystal structure of the backbone-circularized G-CSF

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1 Introduction

Backbone circularization, connecting the N- and C-termini of a polypeptide by a peptide bond, is a powerful approach for stabilizing proteins. Since the alteration of amino acid sequence by backbone circularization (henceforth referred to as "circularization") is much smaller than that introduced by other stabilization strategies (e.g., amino acid substitution), circularization is also much less likely to induce immunogenicity. Therefore, we propose that the method might be especially applicable for the engineering of biopharmaceutical proteins.

We have recently reported the design of circularized granulocyte colony-stimulating factor (G-CSF) [1]. G-CSF is a four-helix bundle cytokine that regulates the development of neutrophils. Recombinant G-CSF has been utilized as a biopharmaceutical.

In this study, we present the crystal structure of a circularized variant of the granulocyte colony-stimulating factor (G-CSF), C163. The variant was created by connecting N- and C- terminal helical segments with a two-residue connector.

2 Experiment

C163 harboring the split intein from *Nostoc punctiforme* was expressed in *Escherichia coli* BL21 (DE3) cells. Circularization of C163 by the split intein occurred in the bacterial cells. Upon cell sonication, the insoluble fraction was collected, washed, resolubilized, and refolded. The refolded sample was purified by using three chromatography steps, i.e., anion-exchange chromatography on HiTrapQ (GE Healthcare), size-exclusion chromatography on Superdex 75 (GE Healthcare), and anion-exchange chromatography on MonoQ (GE Healthcare).

C163 was concentrated to 7 mg/mL and used in crystallization trials. After screening using Crystal Screen (HAMPTON RESEARCH), suitable crystals were obtained in 0.4 M ammonium phosphate buffer.

3 Results and Discussion

We determined the crystal structure of C163 at a 1.65-Å resolution (Figure 1, PDB entry code: 5GW9). The connection between Gly163 and Ser1 was clearly visible in the electron density map, indicating that the structure of a circularized protein was indeed determined. Most parameters for the peptide bond geometry between Gly163 and Ser1 were almost the same as the ideal values from the International Tables Online site hosted by the International Union of Crystallography [2].

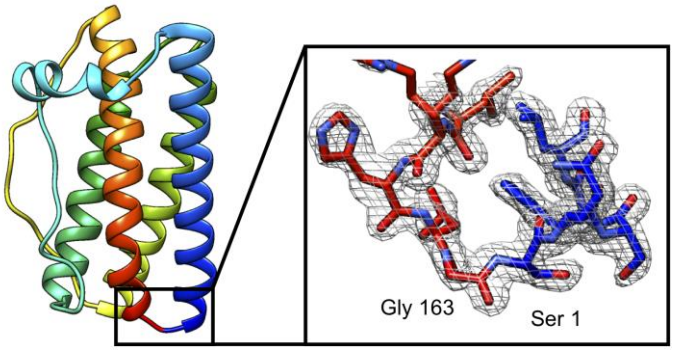

Figure 1 Crystal structure of C163.

To the best of our knowledge, this is the first-ever report of a crystal structure of a backbone-circularized protein. Until now, only one published report exists on the 3D structure of circularized proteins, in which the circularized variants of the N-SH3 domain from protein c-Crk were evaluated by NMR analysis [3]. In that study, the peptide bond between N- and C- termini was created by applying additional constraints, thus our crystal structure allows the first-ever direct observation of the circularized segment.

Our observations will further the development of methodology to stabilize the structure of proteins for biomedical and other applications.

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References

[1] T. Miyafusa *et al*., *ACS Chem. Biol.* **12**, 10 (2017).
[2] R.A. Engh and R. Huber, *International Tables for Crystallography.* Vol. F, Chapter 18.3 (2006).
[3] Schumann et al., *Frontiers in Chemistry*, **3**, 26 (2015)

Research Achievements

1. T. Miyafusa *et al*., *Biochem. Biophys. Res. Commun.,* (2018) Epub ahead of print.

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