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Solution structures of the artificial disulfide bond introduced mutants of Staphylococcal nuclease

Hiroki SAWADA[1], Shingo KATO[1], Hironari KAMIKUBO[1], Mikio KATAOKA*[1]
[1]Graduate School of Materials Science, Nara Institute of Science and Technology
Ikoma, Nara, 630-0192, Japan.

Introduction

Non-local interaction among residues separated on a primary sequence is presumed to act an important role in tertiary structure formation. The non-local interaction is represented by hydrophobic interaction, in which several hydrophobic residues collapse into the interior of the protein to segregate these residues from bulk water. Consequently, the site-specific point mutations cannot sufficiently influence non-local interactions.

We previously reported that W140 on the C-terminal region of Staphylococcal nuclease (SNase) is indispensable for the native tertiary structure formation [1]. W140 is surrounded by hydrophobic residues on the C-terminal region to form a small hydrophobic cluster. In addition, the cluster comes to contact with the residues on the N-terminal region [2]. From these facts, we assumed that W140 is engaged in the non-local interaction between the N-terminal and the C-terminal regions of SNase responsible for the formation of the native structure. In this study, to confirm the presumed non-local interaction responsible for the tertiary structure of SNase, we introduced an artificial non-local interaction into the W140 lacking mutant of SNase via a double-cysteine mutation, and examined the influence of this artificial non-local interaction by a disulfide bond on the tertiary structure formation.

Materials and Methods

The double-cysteine mutant (Y54C/I139C) was purified using buffer solutions containing 2 mM DTT, in order to avoid oxidation of the SH group. The protein was oxidized in the air-purged buffer in the absence of DTT in order to form the disulfide bond. Disulfide bond formation was verified by using non-reducing SDS-PAGE. The SAXS measurements were performed using a solution X-ray scattering apparatus installed at Photon Factory BL-10C. The measurements were carried out at 20°C.

Results and Discussion

The solution structures of the oxidized and reduced forms of the double-cysteine mutants were examined by SAXS measurements. Figure 1 shows the scattering profiles of the reduced and oxidized forms of the double cysteine mutant and wild type in the form of a Kratky plot. The Kratky plot of the reduced form does not show any clear peak, but a broad bump with a monotonously increasing tail, indicating that the reduced form take a denatured structure. On the other hand, the oxidized form shows clear peak and the profile is well superimposed on that of wild type.

These results clearly show that the structure of the W140 lacking mutant can recuperate from the denatured structure with the aid of an artificial non-local interaction between the N-terminal and the C-terminal sub-domains. We can conclude that the native non-local interaction between the N-terminal and the C-terminal regions can be replaced by the artificial disulfide bond, and W140 participates in the non-local interaction responsible for the tertiary structure formation. These results support our model that the C-terminal hydrophobic cluster plays an essential role in a non-local interaction that is indispensable for the tertiary structural formation.

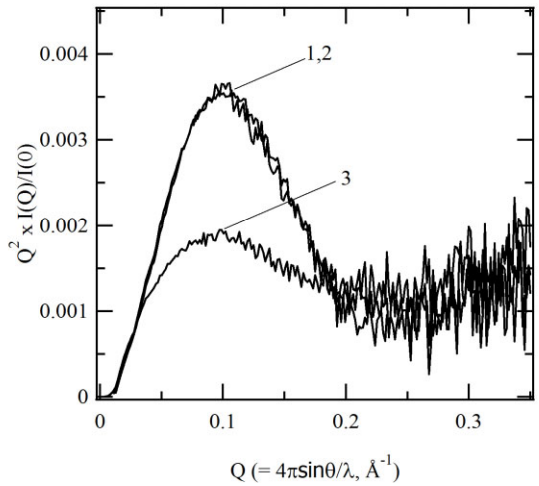

Figure 1. SAXS profiles in the form of Kratky plots of the SNase mutants under the physiological condition. Curve 1, wild type; 2, Δ140-149-SS(+); 3, Δ140-149-SS(-).

References

[1] S. Hirano et al., Proteins 49, 255-265 (2002).
[2] S. Hirano et al., Proteins 58, 271-277 (2005).

* kataoka@ms.naist.jp