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# The substitution effects at 140th and 141st position of Staphylococcal nuclease studied by solution X-ray scattering

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## Introduction

Staphylococcal nuclease (SNase) is a model protein for protein folding. Studies on the mutant that lacks 13 amino acids from the carboxyl terminus (Δ137-149) demonstrate that the mutant takes a compact denatured structure under a physiological condition but has enzymatic activity. [1][2] These findings indicate that the C-terminal region must be involved in the essential interaction to keep the native structure.

In the last issue of the PF report, we reported the solution structures of the systematic deletion mutants of SNase from C-terminus. We suggested that Trp140 and Ser 141 are important for the stability of the native conformation. [3]

In order to analyze the importance of Ser141 and Trp140, we examined the effect of the substitution of these residues. We constructed 3 deletion and substitution mutants and 2 substitution mutants. We investigated their solution structures by small angle X-ray scattering (SAXS).

## Experimental

The deletion and substitution mutants we constructed are Δ142-149/S141A, Δ142-149/S141N and Δ141-149/W140A. We also constructed the substitution mutants, which are S141A and W140A. SNase and its mutants were expressed in *E. coli* and purified by urea extraction. [1] The samples were dialyzed against 10mM MOPS buffer at pH 7 overnight and concentrated to 12mg/ml. SAXS measurements were preformed with SAXS installed at BL-10C.

## Result

*Substitution effect on Trp140*

We carried out SAXS measurements on W140A and Δ141-149/W140A. Figure 1 shows Kratky plot of SAXS data of these two mutants, compared with WT, Δ140-149 and Δ141-149. The scattering profile of Δ141-149 has an intermediate property between WT and Δ140-149. However, the curve for Δ141-149/W140A is essentially identical to the curve of Δ140-149, indicating that the deletion effect of W140 and the substitution effect of W140A are the same on Δ141-149.

The curve of W140A is largely different form that of WT and is similar to that of Δ140-149. The Rg value of W140A is 22Å, which is also similar to the Rg value of Δ140-149. These results suggest that W140A takes compact denatured structure. The side-chain of 140th residue, tryptophan, is important to keep the native structure.

*Substitution effect on Ser141*

Figure 2 shows the SAXS profiles of WT, S141A, Δ142-149/S141N and Δ142-149/S141A. Profiles of S141A, Δ142-149/S141N and Δ142-149/S141A are identical to that of WT, suggesting that these mutants take native structure. This result suggests that the 141st reside should not be serine. However Δ141-149 takes non-native structure. Therefore the existence of the 141st reside is important to keep the native structure.

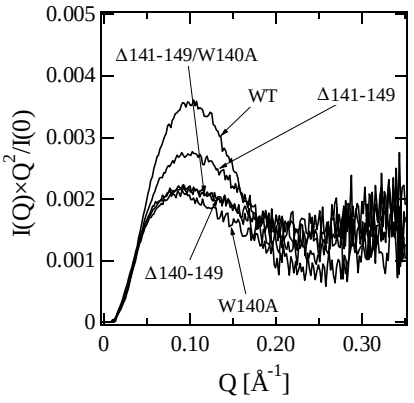


Figure 1
Kratky plot of SAXS profiles for W140A and Δ141-149/W140A, in 10mM MOPS at pH7, 25ºC.

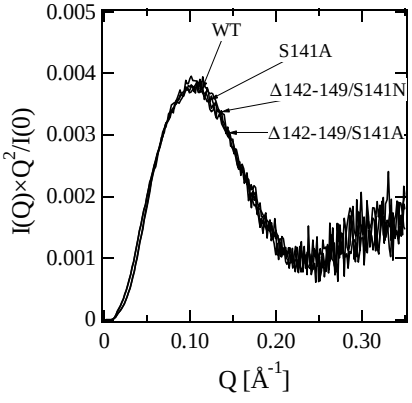


Figure 2
Kratky plot of SAXS profiles for S141A, Δ142-149/S140N and Δ142-149/S141A in 10mM MOPS at pH7, 25ºC.

## Reference

[1] Shortle, D., & Meeker, A., *Biochemistry.* , 28, 936-944 (1989)
[2] Flanagan, J. M. *et al., Proc. Natl. Acad. Sic. USA*, 89, 748-752 (1992)
[3] Hirano, S. *et al., PF Activity Report 1999*,17, 26
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