THE EFFECTS OF ALANINE INSERTIONS IN β-SHEET OF STAPHYLOCOCCAL NUCLEASE

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INTRODUCTION

Staphylococcal nuclease(SNase) is a small soluble protein. It has been used for folding studies as a model protein. We have examined the effect of one alanine insertion into the third β-sheet of SNase to understand the protein design principle. For the purpose, we prepared alanine insertion mutants systematically. Most insertion mutants take non-native structure under a physiological condition, but some can fold into a native structure upon addition of an inhibitor[1][2][3]. Solution structure of alanine insertion mutants were examined by solution X-ray scattering.

EXPERIMENTAL

The created insertion mutants are 31A32(the mutant SNase with alanine insertion between the 31-st and 32-nd residues), 32A33, 33A34…36A37. SNase and its mutants were expressed in E.coli and isolated. The proteins were purified by urea extraction, ethanol precitiation and ion-exchange chromatography [1]. The samples were dialyzed against 10mM MOPS buffer. Small-angle X-ray solution scattering(SAXS) measurements were performed with SAXES installed at BL10C.

RESULT

We examined the solution structures of WT SNase and the insertion mutants under a physiological condition in the presence and the absence of an inhibitor prAp. Further we estimated secondary structures using CD spectrum. The mutant with an insertion near the loop(36A37) can take native-like conformation, while the mutants with an insertion near the center of the β-sheet take a non-native conformation. Among the mutants lost its native conformation, some can fold into a native structure upon addition of an inhibitor, prAp. However, two mutants, 34A35 and 35A36, do not show the inhibitor-induced folding. The effect of the insertion differed from position to position. In the previous study, it was suggested that functionability is closely related to the foldability, and non-native state determines the stability of native structure, thus the folding destination. From the present result, we hypothesized that a protein is functionable and foldable if the topology of the active site in native conformation is completed at non-native state. Otherwise, a protein would be neither functionable nor foldable.

Table:The structure and foldability of WT and mutants

	structure	foldability
WT	N	—
31A32	D	**O**
32A33	I	**O**
33A34	D	**O**
34A35	D	×
35A36	I	×
36A37	N	—

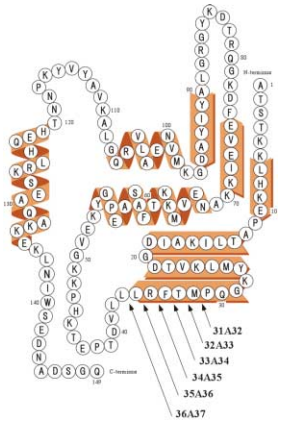

Figure:The secondary structure of WT and insertion psition

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