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Mutant crystal structures of outer surface protein A

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Introduction

Outer surface protein A (OspA) from *Borrelia burgdorferi* has been an excellent model system for studying β-sheet folding and peptide self-assembly. OspA has an unusual dumbbell shape composed of sequential 21 antiparallel β-strands followed by a single α-helix at the C terminus. In the middle of the molecule is a single-layer β-sheet (SLB) segment. Both faces of this SLB segment are exposed to the solvent, and consequently the segment is not associated with a hydrophobic core. The amino acid sequences of the SLB segment are dominated by polar and charged amino acids, and they do not exhibit an alternating binary pattern of polar and nonpolar amino acid residues typical of an amphipathic β-sheet. We previously have reported the high-resolution crystal structure[1] by using surface entropy reduction method[2]. Using this crystallization scaffold, we have reported several OspA mutants thus far.

Here, we report the mutant X-ray crystal structures of (1) the tryptophan replaced mutant in the N-terminal domain for the folding study and (2) the aromatics packing mutant whose one face of SLB is mutated to the bulky aromatics.

Results

(1) Crystal structure of the tryptophan replaced mutant in the N-terminal domain

We have been studying the folding mechanism of OspA and we found that the folding undergoes via the formation of the on-pathway intermediate. We found that there is a lag phase during the formation of the N state detected by tryptophan fluorescence change. The presence of the lag phase is strong evidence for the formation of an on-pathway intermediate and we conclude that the kinetic folding of OspA also follows the three-state mechanism with the productive intermediate. To observe the N-terminal domain formation directly using the tryptophan fluorescence change during its refolding process, we introduced tryptophan residue in the N-terminal domain by replacing tyrosine 102. To confirm that this mutation has no undesired effect for the structure, we determined the Y102W mutant crystal structure on the crystallization scaffold with the resolution 1.86Å. Figure 1 shows the overall structure of the mutant superposing onto the wild-type structure. We found that even within the local mutation site there is almost no structure difference except for the mutated side chain (figure 1 right). Thus, the Y102W mutation can be a good probe for detecting the N-terminal domain formation.

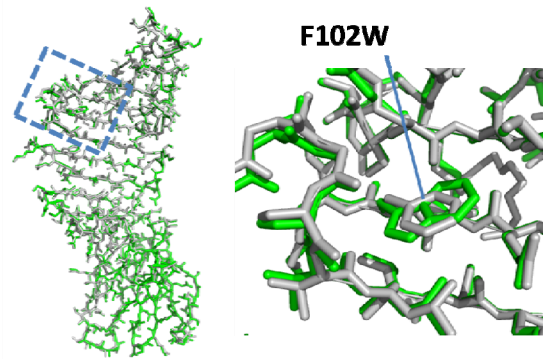

Figure 1. left: crystal structure of F102W mutant (green) and wild type (gray). right: expanded view of the mutated site.

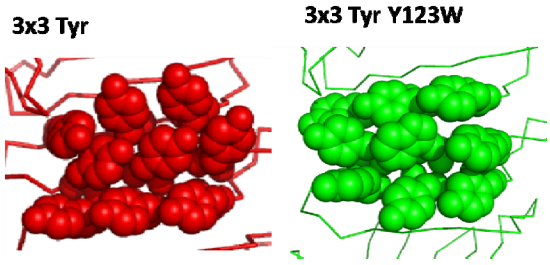

Figure 2. Side chain packing of the aromatics packing mutants (red: the nine tyrosine mutant, green: Y123W mutant)

(2) Crystal structure of the aromatics packing mutant on one face of SLB

We previously determined the crystal structure of the OspA mutant whose side chains on the one face of SLB were mutated to nine Tyr residues (Makabe, Yan, and Koide; unpublished data). We found that these bulky nine tyrosine side chains were nicely packed on the SLB surface. To see how much bulky side chains can be fit on the SLB surface, we additionally mutated the central tyrosine residue to tryptophan and determined its crystal structure (figure 2). Though the surface is crowded with these side chains, the side chains can be packed by adjusting their positions.

References

[1] K. Makabe et al., Protein. Sci. 15, 1907 (2006).
[2] ZS Derewenda et al., Structure. 12, 529 (2004).

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