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Structure and Function of the ETD mutant by the X-ray Crystallography

Katsuo KATAYANAGI*, Yasunori KAMIYA, JingJie MAO, Yoshihiko HORI, Kenji YAMAMOTO, Fuki HAGIWARA, Hiromi KITTAKA

Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, Higashi-Hiroshima 739-8526, Japan

Introduction

Exfoliative toxin (ET) is one of the exotoxin, which was secreted by staphylococcal species, and it causes blisters in animal and human skin. Exfoliative toxins (ET's), serotypes A (ETA) and B (ETB), from *Staphylococcal aureus* cause staphylococcal scaled skin syndrome (SSSS), or Ritter disease, and bullous impetigo in neonates. Both types cause intraepidermal cleavage in the granular layer, without epidarmal necrolysis or inflammatory response of skin. Recently, the mechanism of Exfoliative Toxin was found as act as serine protease. The crystal structures of ETA and ETB have been determined in 1999, and both types were shown to structurally belong to the chymotrypsin family of serine proteases. Moreover, it has been shown that ETA digests desmoglein I, which is one of the major desmosome proteins present in the epidermal layer of human skin. On the other hand, Motoyuki Sugai *et al*. found new serotype ET in the process of analyzing *S. aureus* genes, and designated as Exfoliative Toxin D (ETD).

Results and Discussion

We have determined the three-dimensional structure of ETD, and clarified that its structure is closer to ETB rather than ETA. In this study, we have crystallized the active site mutant S189A, and we will elucidate the structural background of these unknown functions of the new ETD.

The crystal of ETD S189A was obtained as wild type. But high resolution diffraction was obtained only from large (more than 0.3mm diameter) crystal. Unfortunately, such large crystal was obtained only 1 batch among more than 40 batches. Other ones were quite thin needle crystals of only 0.01 mm diameter. Such needle crystals diffracted less than 5 Å resolutions. Finally, 2.4 Å resolution data were obtained. This crystal belong to the space group $P6_1$, and the lattice parameter was a=b=122.10 Å, c=122.67 Å, and the R_{sym} was 0.102.

The molecular replacement method using AMoRe and MolRep were applied to determine the initial phase. By comparing ETA and ETB, a quite unique loop (155-163), which locates near the cataritic triad, was newly found in the determined 3D structure of ETD. Thus, S189A (S189 is one of the catalytic triad) structure will show the relationship of serine protease activity and exfoliative activity.

Moreover, in order to clarify the structure-function relationship, we made a crystal of loop modified ETD mutant. The loop was selected as discussed above (155-163), and amino acid sequence of ETD loop was modified as that of ETB. The obtained crystal of this loop mutant was quite thin less than 1 μm (area was 200x400 μm), it diffracted x-ray over 2.7 Å resolution. This crystal belong to the space group $P2_12_12_1$, and the lattice parameter was a=69.12 Å, b=104.01 Å, c=108.25 Å, and the R_{sym} was 0.102. Structural analysis was performed using molecular replacement method. According to the result of the structural analysis, the loop structure of ETD loop mutant became almost identical to the structure of ETB.

We have determined the new exfoliative toxin ETD , its active site mutant, and ETB like loop modified mutant. These structures are the start point to identify the structural exfoliative activity from serin protease activity. Based on these results, we are now expanding to make ET-dsg1 co-crystal.

* kkata@sci.hiroshima-u.ac.jp