Structure of streptococcal pyrogenic exotoxin A

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Introduction

Gram-positive bacteria are responsible for a number of diseases ranging from food poisoning to toxic shock syndrome (TSS); these diseases are caused by a group of toxins referred to as pyrogenic toxin superantigens (PTSAg's). This group includes enterotoxins types A through J (SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, and SEJ) excluding F and toxic shock syndrome toxin 1 (TSST-1) in *Staphylococcus aureus*. It also includes exotoxins type A through J (SPEA, SPEB, SPEC, SPEF, SPEG, SPEH, and SPEJ) excluding D, E, and I, and streptococcal superantigen (SSA) in *Streptococcus pyogenes*. These toxins are so named because of their ability to stimulate the proliferation of T-cells displaying particular Vβ elements (superantigenicity) and their ability to induce fever in their hosts (pyrogenicity). Other properties commonly associated with these molecules are the ability to cause capillary leakage and the enhancement of endotoxin toxicity (lethality). Structural analyses thus far have indicated that all the PTSAg's have similar folds.

SPEA is a secreted protein with a molecular weight of approximately 25,000 after removal of its signal peptide. SPEA shares 50–66% primary sequence similarity with SEB and SEC. Here we report structural analysis SPEA in the P212121 crystal form to 1.9 Å resolution in the presence of 10 mM CdCl$_2$.

Methods and Materials

Crystals of SPEA were obtained using the hanging drop method. Two microliter aliquots of 10 mg/mL toxin in 20 mM HEPES (pH = 7.5) were mixed with 2 μL of well solution containing 0.25–1.0 M LiCl, 5–20 mM CdCl$_2$, 4–20% PEG 8000, and 50 mM Na acetate (pH = 3–7). After incubation at 18°C crystals were obtained. Diffraction data were collected in house at room temperature using a Bruker-Siemens High Star area detector mounted on a Rigaku RU200 rotating anode generator producing monochromatized Cu-Kα radiation. These data were scaled and merged using the program XENGEN. Diffraction data were also collected at 77 K using α = 1.034 Å radiation produced at the 19-ID beamline at the Advanced Photon Source at Argonne National Laboratory. These data were scaled and merged using the program DENZO. The structure of the P212121 crystal form was solved using molecular replacement with the program XPLOR [1] using the structure of SEC3 as a probe [2]. Refinement was carried out using XPLOR and CNS [3] with 5% of the data set aside from the start of refinement to assess the progress of refinement using the free R value [4].

Results and Discussion

Structure of SPEA

The final structural model of SPEA contains 7287 protein atoms, 626 solvent molecules, and 15 cadmium cations. The side chain of Lys 221 has its occupancy set to zero. The main chain torsion angles for all residues are in the most favored or additionally allowed regions of the Ramachandran plot with the exception of Asn 178A, Leu 86C, and Leu 129D, which are in the generously allowed region. The overall fold is typical of the PTSAg's .There are two structural domains. The smaller, more amino terminal domain (Domain 1) contains five β-strands in a barrel. The larger carboxyl terminal domain (Domain 2) has a four-turn α-helix resting against a three-strand β-sheet. Structural superpositions of the homologous portions of the entire molecules indicate that SPEA is most similar to SEC, SEC3, and SEB; 0.92 Å, 0.97 Å, and 0.98 Å RMS differences over 218, 215, and 216 Cα's, respectively.

Metal sites

Electron density maps from initial refinement cycles of the P212121 crystal form (using a model of only protein atoms) had several clusters of 10–33σ difference electron density features. Since SPEA was crystallized from a mother liquor containing cadmium, the largest difference features were modeled as cadmium cations. The four clusters are found near the center of the tetramer (two on one side and two on the other) near local two-fold axes. The clusters can be best described a forming a tetrahedron where the average Cd-Cd distance is 4.06 ± 0.22 Å. Above five of the six edges of the tetrahedron are features that have been interpreted as water molecules. The average distance of these molecules from the cadmiums is 2.65 ± 0.20 Å. Above the sixth edge is the Sγ of Cys 90 2.55 ± 0.03 Å from both cadmiums. Off three of the peaks of the tetrahedrons is Glu 91' making a bidentate interaction (d = 2.42 ± 0.11 Å), Asp 39 (d = 2.08 ± 0.23 Å), and a solvent molecule (d = 2.41 ± 0.08 Å). The prime on Glu 91' indicates that the residue is from the symmetry-related monomer. Several of the bridging water molecules have very low thermal parameters and might be chloride anions. The presence of such anions would help neutralize the +8 charge from the cadmiums.

References

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