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# Preliminary X-ray studies on two new crystal forms of staphylococcal enterotoxin C2.

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## Abstract

Two new crystral forms of staphylococcal enterotoxin C2 have been obtained by vapor-diffusion methods. Form 1 crystals are monoclinic in space group $P2_1$ with cell dimensions $a = 43.43$, $b = 69.92$, $c = 42.22$ Å, $\beta = 90.1°$ and diffract to at least 2.7 Å resolution. Form 2 crystals are tetragonal in space group $P4_12_12$ or $P4_32_12$ with cell dimensions $a = b = 42.98$, $c = 289.92$ Å and diffract to 1.9 Å resolution.

## Introduction

Staphylococcal enterotoxins produced by *Staphylococcus aureus* are among the common causes of food poisoning (Bergdoll, 1985). These toxins cause vomiting and diarrhoea in humans. There are five serologically distinct types of staphylococcal enterotoxins and they are classified as SEA to SEE all ranging in molecular weights from 26 000 to 29 000 Da. SEC itself cold be subdivided into SEC1, SEC2 and SEC3 because of minor epitope differences. These toxins have come to the forefront of microbiological research because of their ability to stimulate massive T-cell responses when presented by major histocompatibility complex class II molecules (MHCII) for which reason they are called superantigens (White *et al.*, 1989). This massive stimulation occurs since they activate all T-cells bearing particular types of $V_\beta$ elements. Moreover, unlike ordinary antigens, superantigens bind as intact molecules to MHCII molecules. Three-dimensional structures of SEB (Swaminathan, Furey, Pletcher & Sax, 1992), toxic shock syndrome toxin, TSST-1 (Prasad *et al.*, 1993; Acharya *et al.*, 1994), SEC3 (Hoffmann *et al.*, 1994) and MHCII-SEB complex (Jardetsky *et al.*, 1994) have been reported. As has been suggested (Swaminathan *et al.*, 1992) these enterotoxin molecules which have limited sequence homology possess a common folding pattern, *viz* the 'SE fold'. Staphylococcal enterotoxins could be divided into two groups with SEA, SED and SEE forming one group and SEB and SEC1-3 forming the other. Staphylococcal enterotoxins within one group share higher sequence homology than with those in the other group (Marrack & Kappler, 1990). Moreover, $V_\beta$ specificity for T-cell activation is different for toxins within the same group. The three-dimensional structure determination of these proteins should show the common features among these toxins possessing similar biological properties and highlight the differences responsible for different specificities. Crystallization of SEC2 has recently been reported (Passalacqua, Brehm, Acharya & Tranter, 1993). Here we report the crystallization of two new crystal forms of SEC2. The molecular weight of SEC2 is 26 000 Da and it consists of a single chain with 239 amino-acid residues. The amino-acid sequence has been reported (Bohach & Schlievert, 1989). While circular dichroism studies have been performed on SEC1 (Singh, Evenson & Bergdoll, 1988) no such work has been reported for SEC2.

## Methods and results

Samples of SEC2 were obtained from USAMRIID, Fort Detrick, USA. Initial trials were carried out by vapor-diffusion methods using crystal screens from Hampton Research and 24-well Linbro plastic culture plates. 2 µl of 5 mg ml$^{-1}$ protein in distilled water were combined with 2 µl of reservoir solution on a siliconized coverslip which was then inverted and placed over the reservoir well and sealed with vacuum grease. Initial attempts at crystallizing this toxin were not successful. Since the protein as obtained from USAMRIID contained 0.01 *M* phosphate, 4 mg of lyophilized protein was dissolved in 1 ml of distilled water and dialyzed against 2 l of de-ionized water for about 5 h. This was done to remove or reduce the phosphate content in the solution. Crysallization experiments were repeated with this new sample of toxin. Form 1 crystals were obtained with 20% PEG 6000 and 0.1 *M* Hepes buffer at pH 7.0 and 20% PEG 6000 and 0.1 *M* Tris at pH 8.0. These crystals appeared within a week and grew to their full size within two weeks as reactangular plates of 0.6 × 0.15 × 0.05 mm. Crystals of better quality could be obtained if the concentration of PEG 6000 in the reservoir solution was increased slowly from 10 to 20% by adding concentrated reservoir solution in small aliquots. Form 2 crystals were obtained using 20% PEG 8000, 0.2 *M* magnesium acetate and 0.1 *M* sodium cacodylate buffer at pH 6.5. These crystals also appeared within a week and grew to their full size in about three weeks. These crystals were of dimensions 0.25 × 0.25 × 0.1 mm.

Crystals were mounted in thin-walled glass capillaries for X-ray diffraction data collection. Data were collected with a Siemens area detector mounted on a Rigaku rot ting-anode X-ray generator with focal size 0.2 × 2.0 mm operating at 42 kV and 65 mA. Monochromatic Cu $K\alpha$ radiation was obtained using an Ni filter and Franks double focusing mirrors. Form 1 crystals belong to the monoclinic system in space group $P2_1$ with cell dimensions $a = 43.43$, $b = 69.92$, $c = 42.22$ Å and $\beta = 90.1°$. Assuming two molecules in the unit cell the Matthews coefficient is 2.47 Å$^3$ Da$^{-1}$. These crystals contain 50% solvent and diffract at least to 2.7 Å with some reflections in the 2.7–2.5 Å shell. An electronic image equivalent to 2° oscillation is shown in Fig. 1. The *XENGEN* package (Howard, Gilliland, Finzel & Poulos, 1987) was used for data processing and locally modified programs of Weissman (1982) were used for merging and scaling the data. A total of 16 415 reflections was merged to give 7124 unique reflections. The merging $R$ factor ($R_m = \sum|I-\langle I\rangle|/\sum\langle I\rangle$) is equal to 5.27%. This data set is 83% complete to 2.6 Å resolution for reflections with $I \geq 1\sigma(I)$. A derivative data set with a crystal soaked in

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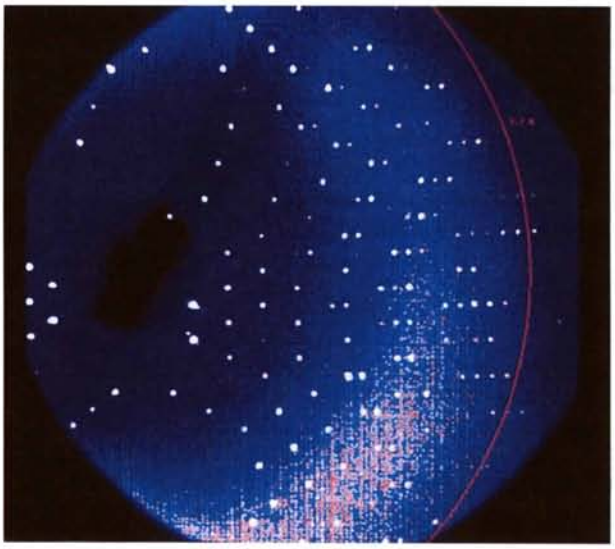

Fig. 1. An electronic image of the diffraction pattern of a form 1 crystal covering 2° oscillation. Crystal-to-detector distance was 12 cm with the detector offset by 14° on $2\theta$ axis. The red arc indicates 2.7 Å resolution limit.

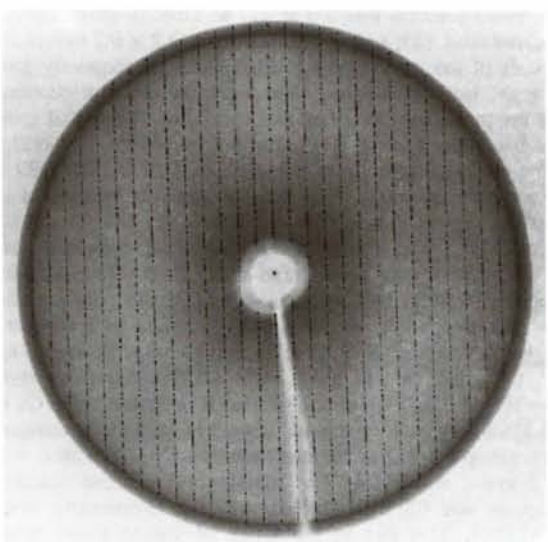

Fig. 2. A precession photograph of $0kl$ zone of form 2 crystals. Crystal-to-film distance was 10 cm and the precession angle $\mu = 15°$. The resolution limit along the edge of the pattern is 3.0 Å.

platinum diamino dichloride has also been collected to 3 Å resolution. The structure determination is in progress.

Form 2 crystals are tetragonal in space group $P4_12_12$ or $P4_32_12$ with cell dimensions $a = b = 42.98$ and $c = 289.92$ Å. Crystals reported earlier (Passalacqua *et al.*, 1993) have the same cell lengths but are in space group $P4_122$; however, those crystals were obtained using 0.15 *M* ammonium sulfate and 25 to 28% PEG 8000 as coprecipitants at pH 6.5. A precession photograph of the $0kl$ zone is shown in Fig. 2. Matthews coefficient is 2.57 $Å^3\ Da^{-1}$ assuming eight molecules per unit cell. These crystals diffract to better than 1.9 Å resolution. A preliminary native data set has been collected to 3.2 Å resolution with crystal-to-detector distance equal to 22 cm. However, the very long *c* axis is a limiting factor in collecting higher resolution data for this form with the in-house facility. A full data set will be collected using synchrotron radiation in the near future. These crystals are amazingly stable in the beam for over 72 h.

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