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PRINCIPAL INVESTIGATOR(S): M. Sax, Ph.D.
J. Pletcher, Ph.D.
S. Swaminathan, Ph.D.

CONTRACTING ORGANIZATION: Veterans Administration Medical Center
Pittsburgh, Pennsylvania 15240

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The aim of this research was to determine the crystal structures of staphylococcal enterotoxins. In the past year we solved the crystal structure of SEC2, and we modeled the 3-D structures of SEA and SEE based on our previous results for SEB and SEC2. As was predicted from the first structure of this family of proteins to have been determined & from their sequence homology, they share a common folding pattern, the SE fold. An important outcome of these studies was the identification of residues determining the V beta specifications of SEB, SEC, SEA and SEE. The crystal structure of a complex of SEB and 3-N-acetylneuramin-lactose was solved. The trisaccharide is the head group of glycosphin-golipid, GM3.

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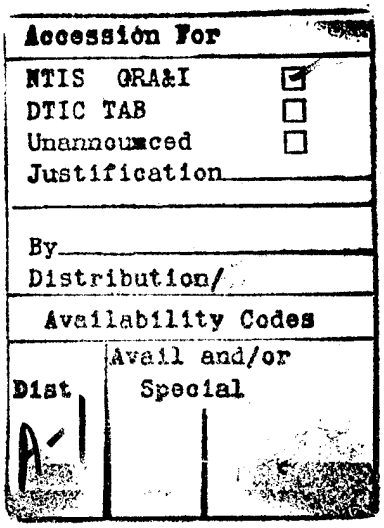

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STAPHYLOCOCCAL ENTEROTOXINS - ANNUAL REPORT

INTRODUCTION:

Staphylococcal enterotoxins secreted by *staphylococcus aureus* are both toxins and superantigens. The five distinct serotypes of s. enterotoxins, labeled SEA through SEE are divided into two groups based on their sequence homology. SEA, SED, and SEE form one group while SEB and SEC form the other. SEC itself could be further subdivided into SEC1-3 depending on the epitope variation. These toxins act as superantigens when presented by major histocompatibility complex class II (MHCII) molecules and induce massive proliferation of T cells bearing particular types of variable (Vβ) chains by forming a ternary complex of MHCII, superantigen and T-cell receptor (TCR). Unlike ordinary processed antigens, intact superantigens bind to a wide variety of MHCII molecules but with different degree of affinity. Binding studies of s. enterotoxins to MHCII molecules have shown that the binding mode of different s. enterotoxins to MHCII are different. While some of them require zinc to bind to MHCII, others do not. Similarly s. enterotoxins within the same group have different Vβ specificities though there may be some overlap. Though a model has been proposed for the formation of the ternary complex, recent results indicate that the model could be different depending on the type of s. enterotoxin. The crystal structure of MHCII has revealed that its structure is very similar to the MHC class I molecule. Crystal structure of soluble Vβ chain has been solved recently. The crystal structures of SEB, toxic shock syndrome toxin (TSST) , SEC2 and SEC3 have been determined. It is now possible to model a ternary complex for different serotypes of s. enterotoxin.

BODY:

The crystal structure of SEB was determined in this laboratory. The molecule consists of two domains (see figure 1); domain 1 is made up of a five stranded β cylinder with one end of the cylinder capped by a small α helix (α3). Domain 2 mainly consists of two α helices with a five stranded twisted β sheet covering one side of both helices. Based on the topology of the molecule and on the results of mutational studies, regions and sites relevant for MHCII and TCR binding were proposed. The TCR binding site is located at the top of the molecule and is at the interface of the two domains . MHC binding site was proposed to be the entire front side of the molecule (on the side of α5 helix). It was also proposed that in spite of their limited sequence homology all s. enterotoxins will possess a common folding pattern similar to SEB. This hypothesis has been proved to be right by the crystal structure determinations of TSST-1, SEC3 and SEC2 and this fold is now called the SE-fold. The three dimensional structure of SEB has helped scientists in designing

experiments for elucidating the mechanism of action of the enterotoxin and for identifying epitopes for developing vaccines (Jett et al., 1994).

The major aim of this project was to determine the crystal structure of all s.enterotoxins. In the past year we have crystallized SEC2 and determined its crystal structure. SEC2 was crystallized in two forms. Form 1 crystals are in space group $P2_1$with cell dimensions a = 43.43, b = 69.92, c = 42.22 Å and $\beta = 90.1^\circ$. Form 2 crystals are in tetragonal space group $P4_32_12$ with cell dimensions a = b = 42.98 and c = 289.92 Å. Form 1 crystals were crystallized at pH 7.0 while form 2 at pH 6.5. We have now crystallized a third form at pH 8.0 using conditions similar to form 1. Form 3 crystals are also in space group $P2_1$with cell dimensions a = 43.3, b=70.4, c= 42.2 Å and $\beta = 90.3^\circ$. The crystal structure of form 1 crystals was solved at 2.7 Å resolution using a combination of the molecular replacement and the isomorphous replacement methods. As predicted earlier the folding is similar to SEB folding. On the basis of the homology, the three dimensional structures of SEA and SEE were modeled and a paper has been published (reprints enclosed) describing these results. An important outcome of this study was the identification of residues determining the $V\beta$ specificity of these s. enterotoxins. Since form 1 crystals did not diffract to very high resolution, the crystal structure analysis of form 2 crystals which diffract to 2.0 Å was carried out. The diffraction data for these crystals with one very long dimension was collected with our newly acquired area detector system. The crystal structure was solved by the molecular replacement method using SEC2 structure from the monoclinic form as the starting model. An important discovery in this high resolution structure determination is the identification of a zinc binding site in SEC2 (Figure 2) which is different from that proposed for SEA or SEE (Figure 3). The structure has now been refined to 2.0 Å resolution using simulated annealing method. The final model now includes 235 residues, one zinc ion and 90 water molecules. The final R factor is 0.23 for 10969 reflections with $I > 1.0\sigma(I)$ in the resolution range 10 - 2.2 Å . The RMSD in bond lengths and bond angles are 0.016 Å and 2.3° respectively. It is now suggested that this zinc ion might play a role in the binding of SEC2 to MHCII. As seen in figures 2 and 3 the two zinc sites are different suggesting the mode of binding to MHCII may be different. A detailed paper on the three dimensional structure of SEC2 is in preparation.

As reported in the last annual report, domain 1 of SEB has been identified as oligomer or oligonucleotide binding site (OB fold). SEB has been shown to bind to glycosphingolipids in kidney cells. We have determined the crystal structure of SEB cocrystallized with lactose which is the head group of lactosylceramide. Lactose is bound to

SEB but not exactly at the same site as reported for other toxins possessing the OB-fold; it appears to act as a cross link between SEB molecules.

Crystal structure of SEB and 3'-N-acetylneuramin-lactose complex has also been solved. This trisaccharide is the head group of another glycosphingolipid, GM3. This structure reveals a different binding site for this trisaccharide than lactose which shows that while the OB-fold is general the binding site is specific for different sugars. The structure is being analyzed.

CONCLUSION:

1. Efforts are still being made to improve the quality of SED crystals. Crystallization of other s.enterotoxins will be continued.

2. The structure - function relationships in SEB which were described first by us (Swaminathan et al., 1992) were deduced from the 3 dimensional structure analysis of SEB and from the available mutational data. We now are extending the technique to other members of the SE family of proteins. The goal is to correlate structural differences with variations in the biological activities of the member proteins, in order to gain further precision in defining the stereochemical factors influencing their activities.

3. We were invited to write a review article on the structure of staphylococcal enterotoxins which will form a chapter in a book titled "Toxin Structures".

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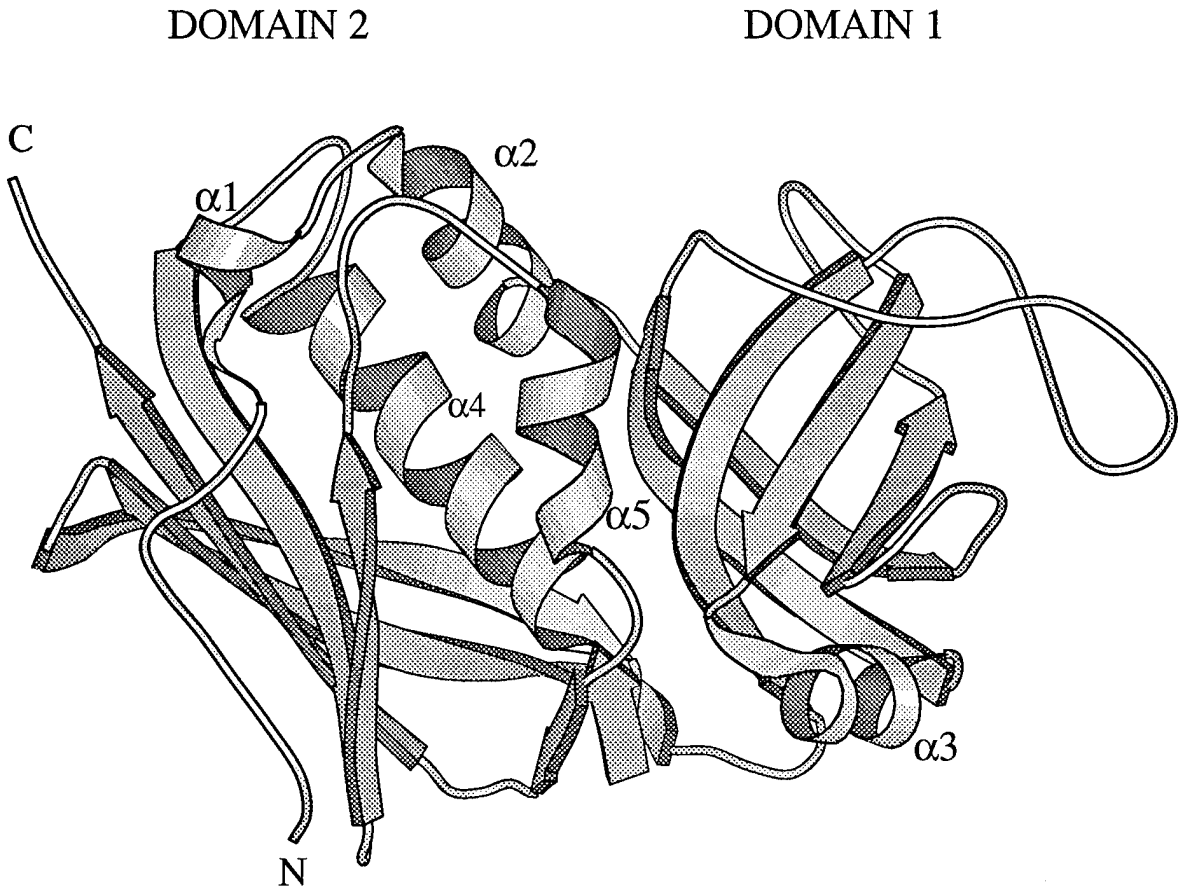

FIGURE 1: SEB

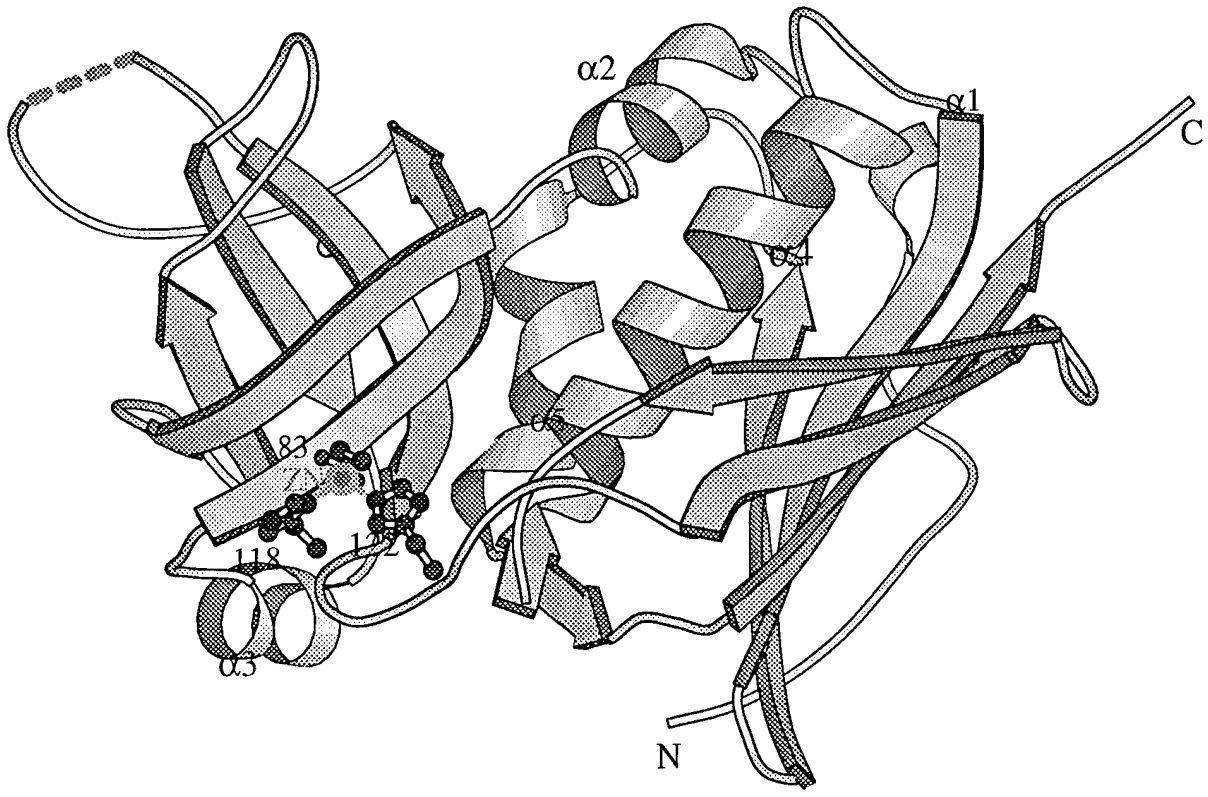

FIGURE 2: SEC2

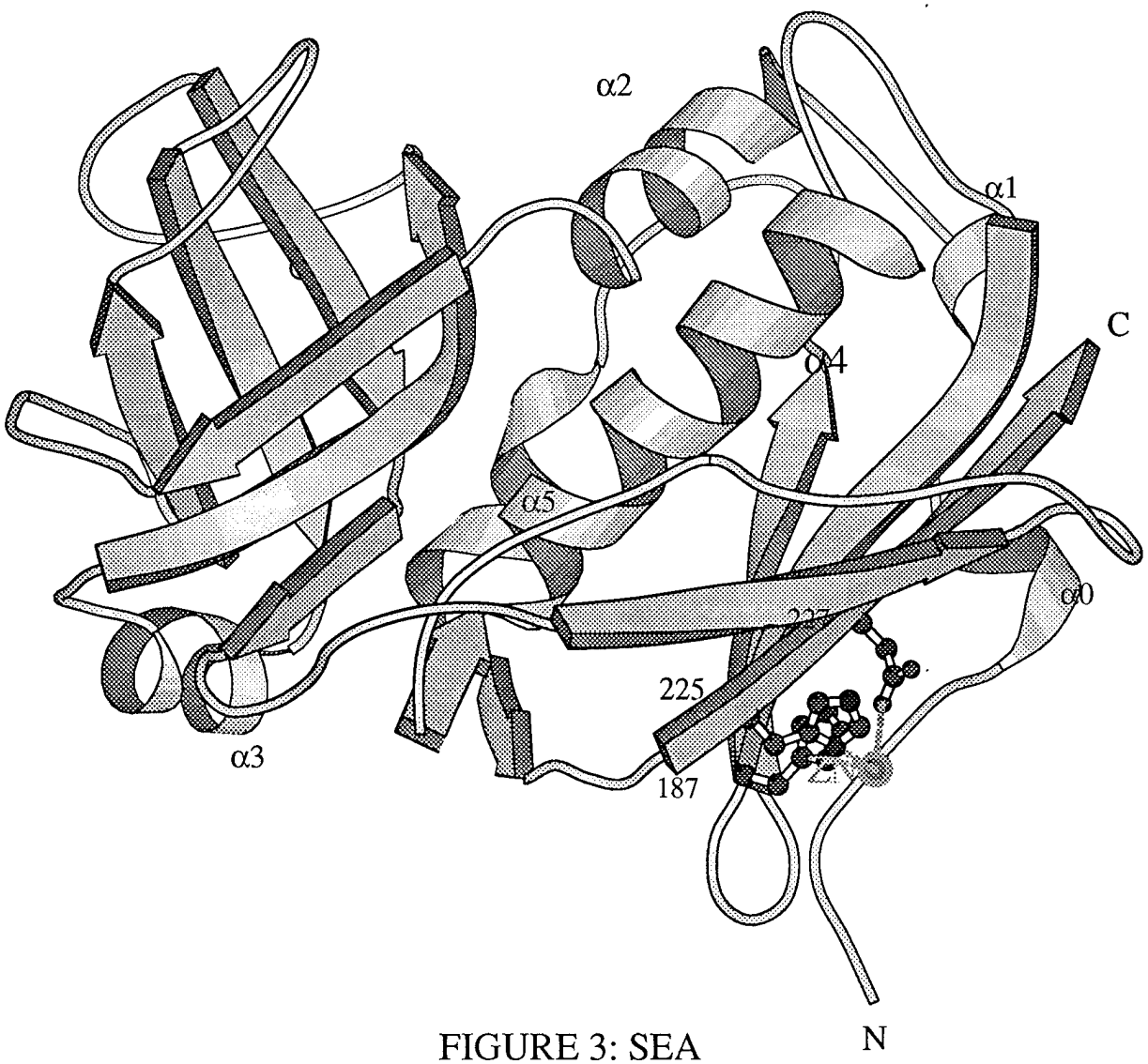

FIGURE 3: SEA