AD_____

MIPR NO: 93MM3556

TITLE: THE 3D STRUCTURE OF STAPHYLOCOCCAL ENTEROTOXINS

PRINCIPAL INVESTIGATOR: Martin Sax, Ph.D.

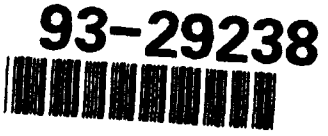
CONTRACTING ORGANIZATION: Veterans Administration Medical Center
Acquisition & Materiel Management (90)
University Drive C
Pittsburgh, Pennsylvania 15240

REPORT DATE: October 15, 1993

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and
Development Command, Fort Detrick
Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited



REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	15 Oct 93	Annual Report (5/1/93-9/30/93)

4. TITLE AND SUBTITLE
The 3D Structure of Staphylococcal Enterotoxins

5. FUNDING NUMBERS
MIPR No.
93MM3556

6. AUTHOR(S)
Martin Sax, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Veterans Administration Medical Center
Acquisition & Materiel Management (90)
University Drive C
Pittsburgh, Pennsylvania 15240

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research & Development Command
Fort Detrick
Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The overall objective is to determine the crystal structures of the staphylococcal enterotoxins. The 3D structure of SEB has been determined and functional sites on the molecule have been identified. Efforts are underway currently to purify and to crystallize other members of the family, particularly SEA and SEB. The mode of binding of the latter two to MHC class II molecules differs from that of SEB, and this implies a difference in 3D structure could be a likely possibility, although the overall main chain fold of the entire family is expected to be the same. Refinements are in progress on a mitant of SEB and on another polymorph of SEB.

14. SUBJECT TERMS
Staphylococcal enterotoxins; 3D structures; x-ray diffraction; protein crystallography; superantigen; Toxins; Enterotoxins; BD; BL2; RAD IV

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____Where copyrighted material is quoted, permission has been obtained to use such material.

____Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

____Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

____In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

____For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

[signature] Oct 15, 1993

(PI Signature) (Date)

TABLE OF CONTENTS

THE 3D STRUCTURE OF STAPHYLOCOCCAL ENTEROTOXINS

Introduction:

Staphylococcal enterotoxins are diarrheal producing bacterial toxins. The five serologically distinct S. enterotoxins (labeled A to E) all induce the same biological effects namely, emesis, diarrhea and mitogenesis. Considerable biochemical knowledge has been accrued over the years about the biological activities of these toxins. Segments responsible for immunogenic, serologic and emetic activities have been assigned in these toxins. An important recent discovery is that these molecules act as superantigens. Superantigens which induce massive immune responses are similar to antigens in some respects but differ in others. Intact superantigen forms a binary complex with the major histocompatibility complex class II molecule before being presented to T-cells for T-cell activation. The superantigen-MHCII binary complex forms a ternary complex with the appropriate T-cell receptor to activate the T-cell. Since the antigenic fragment interacts only with the variable (Vβ) elements of TCR, all TCRs bearing a particular type of Vβ elements are activated causing a massive proliferation of TCRs. Mutational studies are being carried out in order to understand the mechanism of action of superantigens.

Inspite of all these data, the main link namely the 3D structure of staphylococcal enterotoxins was missing to clearly identify different binding pockets. The present work was undertaken to provide this important data for understanding the

mechanism of intoxication resulting from food poisoning and also to identify the stereochemical details of superantigenicity.

Work Accomplished:

The 3D structure of staphylococcal enterotoxin B (SEB) has been determined (Objective (a) of Technical Objectives). The SEB molecule consists of two domains. Domain 1 comprises residues 1 to 120. It contains two β-sheets curving around to form a β cylinder capped by a small α helix. Domain 2 is made up of residues 127 to 239 and contains a five stranded twisted β sheet and two α helices. Using results from mutational studies together with the 3D structure, the TCR binding was identified in the molecule. A binding site where an MHCII molecule can bind has also been identified. These two sites are adjacent to each other on SEB molecule. Three stacked loops comprising residues from both domain 1 and domain 2 form a characteristic structural element of SEB which is involved in TCR binding. Some of the residues in these loops were predicted to define the specificity of TCR binding. This prediction was shown to be true by later mutational studies.

Work In Progress:

1. It has been pointed out that (Murzin,1993) that the unusual folding motif of domain 1 has been observed in five other structures and that all of them bind to either oligonucleotides or oligosaccharides. Accordingly, this motif is called the oligomer binding fold (OB fold). In two cases where structural

evidence is available, the binding site falls on the same side of the β cylinder in domain 1. We are now testing this hypothesis crystallographically by cocrystallizing SEB with lactose, nucleotide etc. Lactose was chosen since it forms the head of glycosphingolipid which has been observed to bind to SEB. X-ray diffraction data were collected from an SEB-lactose crystal and a difference Fourier was computed. The difference map shows electron density near domain 1 and we are currently trying to fit lactose molecule into the density.

2. X-ray diffraction data have been collected on a mutant of SEB in which phenylalanine 44 is replaced with serine. Mutation at this position is known to affect MHCII binding. Refinement of this mutant structure is in progress.

3. Earlier we had reported 3D structure of Form II crystals of SEB. We have now collected X-ray diffraction data on Form III crystals of SEB which diffract to 1.95A. The structure was refined and solvent molecules located. The structure analysis is in progress.

4. A new FPLC system for purifying proteins was purchased. We are currently using it to purify toxins that are bought commercially.

Work Plan for Next Year:

The purified samples of toxins will be used in the crystallization experiments. Once suitable crystals are obtained X-ray data will be collected and the structures solved by the molecular replacement method using SEB as model. If this method

fails we will use the multiple isomorphous method by collecting heavy atom derivative data. The crystal structures now in progress will be completed and published.

Reference:

1. A.G. Murzin, The EMBO Journal,12, 861-867, 1993.

Personnel

M. Sax, J. Pletcher, Wm. Furey, S. Swaminathan, L. Abrams, T. Umland and L. Wingert