AD-A061 041 ARMY MEDICAL RESEARCH INST OF INFECTIOUS DISEASES FR--ETC F/G 6/1
N-TERMINAL AMINO ACID SEQUENCE OF EXFOLIATIVE TOXINS FROM TWO S--ETC(U)
OCT 78 J S CADES, A D JOHNSON, L SPERO

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JEFFREY S. CADES,* ANNA D. JOHNSON, AND LEONARD SPERO

Pathology Division, U. S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701

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ABSTRACT

N- terminal amino acid sequences of the exfoliative toxins from two strains of Staphylococcus aureus exhibited no obvious homology. However, a single unit alignment shift revealed significant similarities.

An exfoliative toxin from Staphylococcus aureus has been implicated as the cause of scalded-skin syndrome (5). In 1975 we reported the isolation and purification of one form of this toxin from S. aureus strain TA (3). In an accompanying article in this Journal (4) we have described the isolation from strain DI of a second form of exfoliatin. Although the two toxins have identical specific activities and differ only slightly in molecular weights and amino acid compositions, they show no serologic cross-reaction. As part of their characterization, we report here the primary structure of the amino-terminal portions of these toxins.

Intact protein from S. aureus strains TA and DI was subjected to automated degradation on a Beckman 890 C sequencer. The Beckman standard fast Quadrol protein program proved superior to the dimethylallylamine peptide program in determining the amino acid sequence for both proteins. Amino acid residues were identified directly from the phenythiohydantoin (PTH) derivatives. Gas chromatography (1), the ascending chromatographic method of Jeppsson and Sjöquist (2) and the gradient elution system of Tarr (6) were used. Acid hydrolysis of the PTH derivatives, followed by identification on a Beckman 121-MB amino acid analyzer of the products, was the final method of residue identification (6).

The amino acid sequence was obtained for the first 26 and 23 residues of the toxins from strains DI and TA, respectively. Both these sequences are given below.

| Strain | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DI | Lys | Glu | Tyr | Ala | Ala | Glu | Glu | Ile | Arg | Lys | Leu | Lys | Glu |
| TA | Glu | Val | Thr | Ala | Glu | Glu | Ile | Lys | Lys | His | Glu | Glu | Lys |

| | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DI | Lys | Phe | Glu | Val | Pro | Pro | Thr | Asp | Lys | Glu | Leu | Tyr | Thr |
| TA | Trp | Asp | Lys | Tyr | Tyr | Gly | Val | X | Asx | Phe | | | |

A direct comparison of these sequences shows little obvious homology. However, if the amino-terminal residue of the TA protein is compared with the second residue of the DI protein, and so on, substantial homology is observed. The results of this alignment are shown below.

| Strain | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DI | Lys | Glu | Tyr | Ala | Ala | Glu | Glu | Ile | Arg | Lys | Leu | Lys | Glu | Lys |
| TA | | Glu | Val | Thr | Ala | Glu | Glu | Ile | Lys | Lys | His | Glu | Glu | Lys |

Eight of the first 13 residues of the toxin from TA are now identical to those in the DI toxin. These are enclosed in boxes. Four additional residues at position 4, 9, 11 and 12 are underlined. Single base changes in the codons for these amino acids can account for the differences. The identical specific activity of the two molecular species of toxin suggests that additional segments possessing significant homology exist. Indeed one would anticipate that the molecules have

similar secondary and tertiary folding. The toxin from strain TA has been crystallized and preliminary X-ray diffraction patterns obtained (7). Establishment of its three-dimensional structure will be of great value in elucidation of the mechanism of exfoliation.

ACKNOWLEDGMENTS

The authors are grateful to Larry Smelkinson for his excellent technical assistance.

LITERATURE CITED

1. Hermodson, M. A., L. H. Ericsson, K. Titani, H. Neurath, and K. A. Walsh. 1972. Application of sequenator analyses to the study of proteins. Biochemistry 11:4493-4502.

2. Jeppsson, J., and J. Sjöquist. 1967. Thin-layer chromatography of PTH amino acids. Anal. Biochem. 18:264-269.

3. Johnson, A. D., J. F. Metzger, and L. Spero. 1975. Production, purification, and chemical characterization of Staphylococcus aureus exfoliative toxin. Infect. Immun. 12:1206-1210.

4. Johnson, A. D., L. Spero, and B. T. DeCicco. 1978. Purification and characterization of different types of exfoliative toxin from Staphylococcus aureus. Infect. Immun. Accompanying paper.

5. Melish, M. E., L. A. Glasgow, and M. D. Turner. 1972. The staphylococcal scalded-skin syndrome: isolation and partial characterization of the exfoliative toxin. J. Infect. Dis. 125:129-140.

6. Tarr, G. E. 1977. Improved manual sequencing methods. Methods Enzymol. 47:335-357.

7. Yoo, C. S., B.-C. Wang, M. Say, and A. D. Johnson. 1978. Preliminary crystallographic data for Staphylococcus aureus exfoliative toxin (exfoliatin). J. Mol. Biol. in press.

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

## REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER (6)

2. GOVT ACCESSION NO.

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle) (10)
N-Terminal Amino Acid Sequence of Exfoliative Toxins from Two Strains of Staphylococcus aureus

5. TYPE OF REPORT & PERIOD COVERED (9)
Interim ≠ repts.

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)
Jeffrey S. Cades, Anna D. Johnson and Leonard Spero

8. CONTRACT OR GRANT NUMBER(s)

9. PERFORMING ORGANIZATION NAME AND ADDRESS
U.S. Army Medical REsearch Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701
SGRD-UIP-A

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS (16) (17)
61102B
3M161102 BS03 00 022

11. CONTROLLING OFFICE NAME AND ADDRESS
U.S. Army Medical Research and Development Command, Office of The Surgeon General
Department of the Army, Washington, DC 20314

12. REPORT DATE (11)
10 Oct 78

13. NUMBER OF PAGES
7 pages

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) (12) 9p.

15. SECURITY CLASS. (of this report)
UNCLASSIFIED

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)
Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES
Reprints bearing assigned AD number will be forwarded upon receipt. To be submitted for publication in Infection and Immunity.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
Exfoliative toxins, amino acid sequence, Staphylococcus aureus, homology

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
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DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

405 039

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