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Amino Acid Sequence of a Deltalike Toxin from *Staphylococcus epidermidis*

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A deltalike toxin produced by a clinical isolate of *Staphylococcus epidermidis* was purified, and the amino acid sequence was determined. The toxin molecule consisted of 25 amino acid residues and shared a high degree of molecular homology with delta toxin purified from a *Staphylococcus aureus* human isolate.

The pathogenic role of coagulase-negative staphylococci (CNS) in certain clinical infections is now well established (1, 7, 15). Scheifele et al. (15) reported evidence implicating delta-toxin-producing CNS in neonatal necrotizing enterocolitis. As most CNS and *Staphylococcus aureus* isolates produce deltalike hemolysins, the relatedness of these toxins is of interest. *S. aureus* delta toxin is a heat-stable, surface-active peptide with lytic activity on most kinds of membranes (2, 13). The toxin monomer has an M_r of 2,977 and has been shown to consist of 26 amino acid residues (5). It contains no arginine, proline, histidine, tyrosine, or cysteine but contains a high proportion of hydrophobic amino acids (5).

Purification of a deltalike toxin from CNS has been reported by various groups (8, 15, 16). Its biological properties resemble those of *S. aureus* delta toxin, but its physical properties differ among the preparations (8, 16). The studies described here were performed to establish whether *S. aureus* delta toxin and *Staphylococcus epidermidis* deltalike toxin are homologous.

S. epidermidis B52b isolated from the stool of an infant with necrotizing enterocolitis was used for the purification of deltalike toxin. This isolate does not produce either alpha or beta hemolysin. Toxin was purified from broth culture supernatants by the solvent transfer method described by Heatley (10). Culture medium and growth conditions were as described previously (15). To examine the purity of material obtained by this method, sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out with a 4% stacking gel and a 20% separating gel as described by Laemmli (11), at a constant current of 30 mA. After electrophoresis, gels were fixed and stained by a silver-staining procedure (bulletin no. 1089, Bio-Rad Laboratories, Richmond, Calif.) originated by Merril et al. (12). Protein concentrations were measured by the method of Bradford (4).

For hydrophobic column chromatography (14), 4 ml (850 μg/ml) of toxin purified as described above was applied to a column (1 by 15 cm) of phenyl-Sepharose (Pharmacia, Uppsala, Sweden). The column was washed with 0.05 M NH_4OH, and bound protein was eluted with water followed by a gradient of 0 to 100% ethanol. Fractions were lyophilized, suspended in 0.75 ml of water, and assayed for A_{280} and for hemolytic activity. Assessment of the purity of hemolytic fractions from the phenyl-Sepharose column was undertaken by reversed-phase high-performance liquid chromatography (RP-HPLC). A 100-μl volume (500 μg/ml) of a single-column fraction was applied in 0.5% (vol/vol) trifluoroacetic acid (Sigma Chemical Co., St. Louis, Mo.) to an Altex Ultrapore RPSC C-3 column (Beckman Instruments, Palo Alto, Calif.) equilibrated in 0.05% trifluoroacetic acid on 2150 HPLC pump (LKB, Bromma, Sweden). Elution was with a linear acetonitrile (Millipore Corp., Bedford, Mass.) gradient (4%/min at a flow rate of 1 ml/min). Fractions (0.5 ml) were collected and monitored for A_{206}. The procedure was repeated six times. Three peak fractions were pooled and concentrated; acetonitrile was removed by evaporation.

Toxin preparations were tested in microdilution trays for hemolytic activity against human erythrocytes (3). For calculation of specific hemolytic activity, 1 hemolytic unit was defined as the amount of toxin which caused the release of 50% of the hemoglobin from the cells in 1 ml of a 0.5% suspension of erythrocytes.

To unblock the N-terminal methionine, purified toxin preparations were incubated in 1 ml of 70% (vol/vol) formic acid. The solution was treated with an excess (25-fold; wt/wt) of cyanogen bromide (Sigma) overnight at ambient temperature (9). The reaction mixture was diluted with a

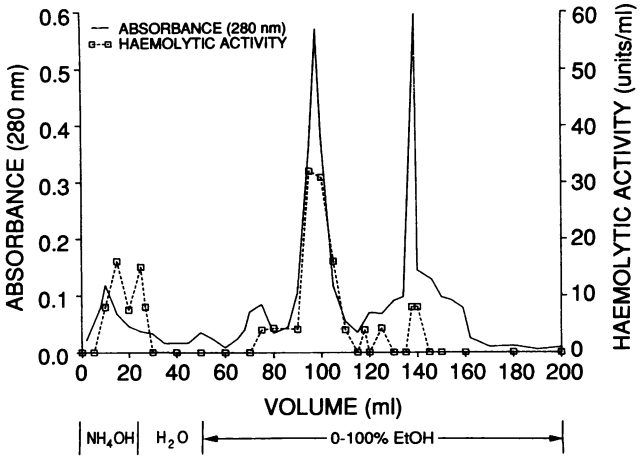

FIG. 1. Phenyl-Sepharose chromatography of deltalike toxin. Material (4 ml; 850 μg/ml) obtained by the solvent transfer method (10) was applied to a column of phenyl-Sepharose CL-4B. The column was washed with 0.05 N NH_4OH. Bound protein was eluted with water followed by a water-ethanol gradient (0 to 100%). EtOH, Ethanol.

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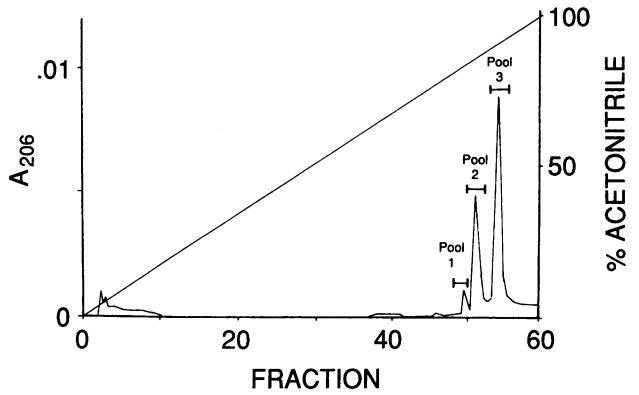

FIG. 2. RP-HPLC of deltalike toxin. Material from a single peak fraction (100 μl; 500 μg/ml) from a phenyl-Sepharose column which eluted at 33% ethanol was applied to a C-3 column equilibrated in 0.05% trifluoroacetic acid. Elution was with an acetonitrile gradient.

10-fold excess of distilled water to stop the reaction and then lyophilized.

Purified samples of toxin (10 μg) were hydrolyzed with gas-phase 6 N HCl under argon at 165°C for 1 h. Amino acid compositional analysis was conducted by using an amino acid derivatizer-analyzer (model 420; Applied Biosystems, Foster City, Calif.).

Protein microsequence analysis (500 pmol of toxin) was performed on a gas-phase protein sequencer (Applied Biosystems model 470A) with an on-line HPLC phenylthiohydantoin analyzer (Applied Biosystems model 120).

The solvent transfer method of toxin purification yielded a product with a specific hemolytic activity of 150 hemolytic units per mg. A silver-stained sodium dodecyl sulfate-polyacrylamide gel of this material contained a single diffuse band identical to the one obtained with purified *S. aureus* delta toxin kindly donated by N. Heatley (data not shown). Attempts to obtain the amino acid sequence of this product indicated that more than one component was present. Further separation was achieved by using hydrophobic interaction chromatography on a phenyl-Sepharose column (Fig. 1). The major hemolytic peak eluted at approximately 33% ethanol. RP-HPLC of material from this fraction revealed three peaks (Fig. 2), but only fractions from pool 2 had hemolytic activity after removal of acetonitrile.

TABLE 1. Amino acid compositions of *S. epidermidis* deltalike toxin[a] and *S. aureus* delta toxin

Amino acid	No. of residues/mol of protein		
	S. epidermidis toxin		*S. aureus* toxin[b]
	Actual[c]	Found in sequence	
Aspartic acid	3.74 (4)	4	4
Threonine	1.26 (2)	2	3
Serine	0.44 (1)	1	1
Glutamic acid	0.11 (0)	0	1
Proline	0.0 (0)	0	0
Glycine	1.96 (2)	1	1
Alanine	2.00 (2)	2	1
Cysteine	0.0 (0)	0	0
Valine	1.93 (2)	2	2
Methionine	0.35 (1)	1	1
Isoleucine	3.39 (4)	5	5
Leucine	0.80 (1)	1	1
Tyrosine	0.04 (0)	0	0
Phenylalanine	0.92 (1)	1	1
Histidine	0.0 (0)	0	0
Lysine	4.35 (4)	4	4
Arginine	0.0 (0)	0	0
Tryptophan	1.0 (1)	1	1

[a] From a phenyl-Sepharose column.
[b] Analysis of *S. aureus* delta toxin published by Fritton et al. (5).
[c] Based on an apparent M_r of 3,000. Residue whole numbers are shown in parentheses.

TABLE 2. Amino acid sequences of *S. epidermidis* B52b deltalike toxin and *S. aureus* 186X delta toxin (5)[a]

Strain	Amino acid at residue no.:			
	1	10	20	25
S. epidermidis B52b	MAADISTIGDLVKWIIDTVNKFKK			
S. aureus 186X	MAQDISTIGDLVKWIIDTVNKFTKK			

[a] Note dissimilarities at residues 3, 24, and 26.

The amino acid compositions of toxins obtained from the major peak fractions both from the phenyl-Sepharose column and from pool 2 from the RP-HPLC column proved to be identical; in Table 1, this composition is compared with that of *S. aureus* delta toxin purified from strain 186X (5). Neither toxin contained proline, cysteine, tyrosine, histidine, or arginine. Compositional analysis indicated four isoleucine residues in the CNS toxin, but subsequent sequence analysis showed that five isoleucine residues were present in the molecule.

Attempts to determine the N-terminal sequence of our purified toxin preparations were unsuccessful, suggesting that the terminal amino acid was blocked, as in delta toxin from *S. aureus* 186X (5). After treatment of the deltalike toxin with cyanogen bromide (9) to remove N-terminal methionine, subsequent N-terminal sequence analysis of toxins from both phenyl-Sepharose and RP-HPLC columns revealed an identical amino acid sequence. This sequence (GenBank accession no. A33058) differed from that obtained by Fitton et al. (5) for *S. aureus* delta toxin purified from strain 186X (Table 2) in that alanine (*S. epidermidis* deltalike toxin) was substituted for glutamine (*S. aureus* delta toxin) at position 3 and threonine at position 24 was absent in the *S. epidermidis* protein.

The results reported here show that a deltalike toxin produced by a human isolate of *S. epidermidis* shares a high degree of molecular homology with delta toxin purified from an *S. aureus* human isolate (5). Homology was greater than that between delta toxins produced by *S. aureus* isolates of human and canine origin (6), which differed in 9 of 26 residues. We suggest that the toxin purified in this report warrants designation as *S. epidermidis* delta, rather than deltalike, toxin.

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