Identification, Purification, and Some Physicochemical Properties of Staphylococcal Enterotoxin C₃

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A third staphylococcal enterotoxin C₃ has been identified, purified, and characterized. Staphylococcal enterotoxin C₃ was identified from a Staphylococcus aureus isolate received from England. The purified toxin was determined by gel permeation chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis to be a simple protein with a molecular weight of 26,900. The isoelectric point of the major band was determined by isoelectric focusing in polyacrylamide gels to be 8.15. The reaction of enterotoxin C₃ with its specific antibody was not affected by trypsin digestion at pH 8.0 or peptic digestion at pH 4.5. The enterotoxin C₃ consisted of 236 amino acid residues. Serine was shown to be the NH₂-terminal amino acid residue by end group analysis. The protein was highly emetic in cynomolgus monkeys both per os and intravenously.

There are six known serologically distinct staphylococcal enterotoxins: A, B, C₁, C₂, D, and E (SEA, SEB, SEC₁, SEC₂, SED, and SEE, respectively). Enterotoxins C₁ (4) and C₂ (1) were purified as individual enterotoxins before it was discovered that they react with the same antibody; thus, they were both labeled SEC (2). Subsequently, they were labeled SEC₁ and SEC₂ when it was demonstrated that each had antibodies that reacted with minor determinants (3, 12). This posed little problem in the detection of the SECs, as antibodies raised to either of the toxins could be used for detection of Staphylococcus aureus strains that produce SEC; however, it was noted that under certain conditions SEC production by some strains might be missed when the antibodies to SEC₁, but not those to SEC₂, were used in gel diffusion analyses. The SEC produced by one S. aureus strain that reacted poorly in gel diffusion tests with SEC₁ antibodies but adequately with SEC₂ antibodies reacted heterogeneously with both SEC₁ and SEC₂ antibodies in quantitative studies with radioimmunoassay (RIA), indicating that this was a third SEC.

This communication reports the identification, purification, and some of the physicochemical and serological properties of enterotoxin C₃.

MATERIALS AND METHODS

Materials. Reagents included acrylamide, N,N,N',N'-tetramethyleyldiamine, Coomassie brilliant blue R-250, sodium dodecyl sulfate (SDS) (Bio-Rad Laboratories, Richmond, Calif.), carboxymethyl cellulose (CM-cellulose) type 20 (Schleicher & Schuell, Inc., Keene, N.H.), Amberlite CG-50 resin (100 to 200 mesh) (Mallinkrodt Chemical Works, St. Louis, Mo.), Sephadex G-75 and Sephadex S-200 superfine (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.), and polyethylene glycol compound 20 M (Carbowax; Union Carbide Corp., Chicago, Ill.). Ampholytes for isoelectric focusing were Ampholines (LKB Instruments, Inc., Gaithersburg, Md.) and Pharmalytes (Pharmacia). Standard kits for isoelectric focusing and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from Pharmacia and used according to the instructions of the manufacturer. Bovine serum albumin, ovalbumin, α-chymotrypsin, soybean trypsin inhibitor, and cytochrome c were purchased from Sigma Chemical Co., St. Louis, Mo., and used as standards in the gel permeation molecular weight determination. Staphylococcal protein A was purchased from Sigma, and carrier-free 125I was purchased from New England Nuclear Corp., Boston, Mass., for preparing 125I-protein A and 125I-SECs. Purified SEC₁ and SEC₂ antisera against the various enterotoxins and the toxic shock toxin (TST) were prepared at the Food Research Institute, University of Wisconsin, Madison.

Estimation of protein and UV absorption. During the early stages of purification, the protein concentration was estimated by using the extinction coefficient for SEC₁ (ε₃₄ₐ₅₅₄ at 277 nm = 12.1) (4). The highly purified SEC₁ has an extinction coefficient of 12.1 at 277 nm, its maximum absorption.

Antigen production. The crude SEC₁ was prepared by inoculating 2-liter Erlenmeyer flasks containing 400 ml of 3% N-Z Amine NAK (Humko-Sheffield Chemical, Memphis, Tenn.) and 17% yeast extract (Difco Laboratories, Detroit, Mich.) with 4 ml of an 18-h shake-flask culture of S. aureus FRI-913. The inoculated flasks were incubated on a Gyrotory shaker (New Brunswick Scientific Co., Inc., Edison, N.J.) at 280 rpm in a 37°C incubator for 20 h. The starting pH of the medium was 6.5 to 6.6.

Monoclonal antibodies. Monoclonal antibody 1C₃ was prepared by the methods of Thompson et al. (25). This antibody was prepared against SEC₁, but it cross-reacts with SEC₁ and SEC₂.

Detection and assay of the SECs. Fractions from the different purification steps were initially tested for serological activity by the optimal-sensitivity-plate method (19) with SEC₂ reagents. The RIA procedure of Miller et al. (14) as modified by Reiser et al. (17) was used to demonstrate identity. The single-gel diffusion tube method (10) was used to quantify the SEC₁ after specific antiserum was available.

Protein purity determination. The purity of the SEC₁ was estimated by disc PAGE (11) and by the cathodic disc-PAGE procedure described by Reisfeld et al. (18). The SEC₁ immunological integrity was determined by the optimal-sensitivity-plate method (19) employing reagents for the detection of SEA, SEB, SED, SEE, TST, and the multiantigenic system for S. aureus FRI-184. Relationships between SEC₁, SEC₂, and SEC₃ were studied on double-gel diffusion plates and single-gel diffusion tubes and by RIA.

Molecular weight determination. The molecular weight of

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SEC3 after the reduction with mercaptoethanol was estimated in the disc-SDS-PAGE system described by Laemmlli (11) with the Bio-Rad Protein Cell vertical slab gel apparatus and the Pharmacia low-molecular-weight standards.

Gel permeation chromatography on a Sephacryl S-200 column (2.5 by 117 cm) with proteins of known molecular weight also was employed for molecular weight determination. The purified-protein standards and the SEC3 were chromatographed in the denatured form (in 0.05 M sodium phosphate containing 6 M urea, pH 6.8).

Isoelectric focusing. Slab gels (2 mm thick) were cast using the gel-casting kit provided with the LKB Multiphor unit. The formation per slab gel was as follows: 39 ml of distilled water containing 7.5 g of sucrose, 18 ml of acrylamide-bisacrylamide (30:0.8), 3 ml of the appropriate ampholyte, 0.2 ml of a 10% ammonium sulfate solution, and 15 μl of N,N,N',N'-tetramethylenediamine. The solution of sucrose, acrylamide, and Ampholine was mixed and degassed before the addition of the persulfate and N,N,N',N'-tetramethylenediamine.

Gel staining. When necessary, both isoelectric focusing and disc-polyacrylamide gels were stained with Coomassie brilliant blue R-250.

Electrophoretic transfer and autoradiography. Electrophoretic transfers of the SEC3s from gels to nitrocellulose sheets were performed under basic conditions by the procedures of Towbin et al. (26). The immunonautographic technique of Symington et al. (24) was used for the detection of the proteins on the nitrocellulose sheet with the substitution of 3% bovine hemoglobin for gelatin as the blocking protein in the blocking buffer. Undiluted polyclonal rabbit antisera (20 μl) or 50 μl of undiluted 1C1 monoclonal antibody per 50 ml of blocking buffer was found adequate for the nitrocellulose blot. The 125I-labeled protein A concentration (14) used to probe the blot was 20,000 cpm/ml of hemoglobin blocking buffer with shaking at room temperature overnight.

Amino acid composition. The determination of the amino acid composition of the SEC3 and of cystine as cysteic acid was performed by Daniel Omilianowski, Biophysics Laboratory, University of Wisconsin-Madison, employing the techniques of Moore (15, 16). The tryptophan content was determined spectrophotometrically by the Edelhoch technique (8).

NH2-terminal analysis. The dinitrophenyl derivative of the SEC3 was prepared by the Fraenkel-Conrat et al. modification (9) of the Sanger method (20). We analyzed for the presence of the dinitrophenyl derivative by the technique of Brenner et al. (5).

Biological activity testing. The toxicity of the purified SEC3 was demonstrated both intravenously and per os with cynomolgus monkeys (1.5 to 3.0 kg) (23). The intravenous testing was done with animals at the Wisconsin Regional Primate Center, Madison, Wis.

The hemolytic activity of the various preparations during the purification steps was monitored by mixing equal volumes (50 μl) of sample serially diluted in phosphate-buffered saline with a 2% suspension of 0.9% saline-washed rabbit erythrocytes. (The phosphate-buffered saline was prepared by adjusting the pH of 0.02 M NaH2PO4 containing 0.9% NaCl to pH 6.8 with 0.02 M KH2PO4 containing 0.9% NaCl and adding 1 ml of a buffer solution containing 5% MgSO4, 0.5% MnCl2, and 0.5% FeCl3·6H2O in 0.001 M H2SO4 per liter to provide trace salts.) The assay was incubated at 37°C for 60 min, and the activity was expressed as the reciprocal of the last dilution giving complete hemolysis of the erythrocytes.

**RESULTS**

Identification of SEC3. Supernatant fluid from a 24-h shake-flask culture of *S. aureus* FRI-913 was processed to remove staphylococcal protein A by the method of Reiser et al. (17). The processed supernatant fluid was tested for the presence of staphylococcal enterotoxins by the optimal-sensitivity plate method, by the single-gel diffusion tube method, and by RIA. The results from the optimal-sensitivity-plate method indicated that the strain produced four staphylococcal toxins: SEA, SEC, SEE, and TST. The results from the single-gel diffusion tube method indicated that the SEC was not SEC1 but might have been SEC2. The results from the RIA analysis showed a lack of parallelism for either SEC1 or SEC2, indicating nonidentity with either of the two toxins. In RIA, identity is indicated when the slopes of the lines generated by the data from the unknowns are parallel to the slope of the line generated by the data from the standards.

**Purification of SEC.** All purification steps were performed at room temperature.

(i) Step 1. Removal of the SEC3 from culture supernatant fluids. The Amberlite CG-50 resin adsorption technique of Reiser et al. (17) was utilized for step 1 of the purification procedure. Briefly, 200 ml (wet volume) of packed resin equilibrated with 5 mM sodium phosphate buffer, pH 5.6, was stirred with a 1:5 dilution of 10 liters of culture supernatant fluid for a minimum of 1 h. The resin-bound toxin was allowed to settle, the spent supernatant fluid was decanted, and the resin was packed in a chromatographic column (4.0 by 25 cm). The attached proteins including the SEC3 were eluted with 0.5 M sodium phosphate buffer containing 0.5 M NaCl, pH 6.2. The entire eluate was concentrated approximately fourfold by dialysis against Carbowax and further dialysis against 5 mM sodium phosphate buffer, pH 5.6, to prepare it for step 2. The recovery at this stage was ca. 45% with a purity of 20%.

(ii) Step 2. Ion-exchange chromatography on CM-cellulose. The dialyzed, concentrated toxin pool from the previous step was clarified by centrifugation, applied to a column of CM-cellulose (4.0 by 30 cm; prewashed with 0.1 N NaOH and 0.1 N HCl, washed, and equilibrated with 5 mM sodium phosphate buffer [pH 5.6]), and washed with 5 mM sodium phosphate buffer, pH 5.6, until the absorbance monitor at 280 nm (Isco model UA-5, Isco Laboratories, Lincoln, Nebr.) returned to the base line (ca. 500 ml). The column was eluted stepwise, first with 0.03 M sodium phosphate buffer, pH 6.0 (2,000 ml), and then with 0.045 M sodium phosphate buffer, pH 6.2 (1,500 ml). The compound first peak, which contained some SEC, some SEA, hemolysin, and other extraneous proteins, was discarded. The compound peak that eluted with the 0.045 M buffer contained most of the SEC3, SEA, SEE, and TST but no hemolysin. Fractions 42 to 48 of this peak were pooled (Fig. 1); most of the SEA, SEE, and TST were in fractions 49 to 58 of this peak and thus were eliminated. The percentage of SEC3 increased from ca. 20 to 80% with a recovery of 27%. The pooled fractions were concentrated with Carbowax to ca. 15 to 20 mg of protein per ml for step 3.

(iii) Step 3. Gel permeation chromatography with Sephadex G-75. A column (4.0 by 96 cm) of Sephadex G-75 was poured and washed overnight with 0.05 M sodium phosphate buffer, pH 6.8, containing 1 M NaCl at a flow rate of ca. 1 ml/min. The concentrated pool from step 2 (maximum volume, 6.0 ml) was applied to the column and eluted by the addition of 0.05 M sodium phosphate buffer containing 1 M NaCl, at the rate of 1 ml/min. The center portion of the major peak
VOL. rate was an overall 6.8; the native protein appeared high as to be sodium phosphate (fractions 43 to 49 Carbowax; of blot cated that both the native the molecular weight bands evident were enterotoxins monoclonal antibody mately 1.C3). Reprobing with polyclonal anti-SEC3 rabbit serum indicated that both fragments reacted to the antibody. The sum of the molecular weight of the two proteins was approximately equal to the molecular weight of SEC3. When the enterotoxins were run in the native state, the lower-molecular-weight bands evident on the SDS gel were absent.

Immunodiffusion of SEC3 (50 μg/ml) by the optimal-sensitivity-plate method failed to show any contamination with SEA or SEE; however, when the SEC3 preparation was tested by RIA, the SEC3 was found to contain 0.7% SEA and 0.2% SEE. The amount of TST in the purified SEC3 was observed to be 1 to 2% by the optimal-sensitivity-method and autoradiography.

Analysis of as much as 50 μg of the purified SEC3 for hemolysin was negative. Analysis for extraneous staphylococcal antigens by using antisera produced against the multiantigen nonenterotoxigenic supernatant fluid of S. aureus FRI-184 failed to indicate any miscellaneous antigens present when as much as 200 μg of the SEC3 per ml was used in the test.

Subsequent purification with S. aureus FRI-1230, which produces only SEC3, yielded purified SEC3 which was free of any SEA, SEE, TST, and hemolysin.

Comparison of the purified SEC3 to SEC1 and SEC2 in RIA revealed dissimilarities of the toxins (Fig. 4).

Comparison of the SECs on double-gel diffusion plates by using undiluted antisera (or a 1:4 dilution) with appropriate SEC concentrations revealed differences in the antigenic structures of these toxins. In the SEC1-homologous system (Fig. 5a), the precipitin line with SEC2 extended over the joining line (spur) with SEC2 and SEC3 showing the presence

![Figure 1](https://example.com/figure1.png)

**FIG. 1.** Ion-exchange chromatography of crude SEC1 from step 1 on a CM-cellulose column (4.0 by 30 cm) by stepwise elution with sodium phosphate buffer of increasing concentration and pH. The flow rate was 1 ml/min.

![Figure 2](https://example.com/figure2.png)

**FIG. 2.** Elution profile from a column of Sephadex G-75 (4.0 by 96 cm). The sample applied to the column was the fractions containing the SEC1 from step 2. Sodium phosphate buffer (0.05 M, pH 6.8) containing 1 M NaCl was the eluting solution. The flow rate was ca. 1 ml/min.

![Figure 3a](https://example.com/figure3a.png)

**FIG. 3a.** Amido black-stained electroblot transfer of disc SDS-PAGE (12% gel) for comparison of reduced (R) and native (N) SEC3 (25 μg). The cathode is at the top. The standards (Std) are the Pharmacia standards. From top to bottom: phosphorylase b, 94,000 daltons; bovine serum albumin, 67,000 daltons; ovalbumin, 43,000 daltons; carbonic anhydrase, 30,000 daltons; soybean trypsin inhibitor, 20,000 daltons; and α-lactalbumin, 14,000 daltons. (b) Autoradiogram of a nitrocellulose duplicate blot probed with monoclonal antibody IC3 and 125I-SEC3.
of antibodies specific for SEC1. In the SEC2-homologous system (Fig. 5b), a spur was formed over the precipitin line with SEC1 but not over the line with SEC3, showing the presence of antibodies that distinguished SEC2 from SEC1 but not from SEC3. In the SEC1-homologous system (Fig. 5c), no differences were noted in the reaction of the three toxins with the SEC1 antibodies, indicating that no determinants not found on SEC1 and SEC2 were detectable. It is possible, of course, that SEC2 and SEC3 produce specific antibodies that either are at a level too low to detect or are nonprecipitating. The latter is indicated by the results of the reactions of these two toxins in homologous and heterologous systems by RIA.

The quantitative assaying of the SECs by the single-gel diffusion tube method (10) with the three homologous systems (e.g., SEC1 versus anti-SEC1) showed differences in the reactions of the toxins in the homologous and heterologous systems. By this method, the amount of serological difference is determined by the length of the precipitin zone; the longer the zone, the greater is the difference. The standard used is that of the toxin against the antibodies prepared against it (e.g., SEC1 versus anti-SEC1). The following results indicate serological differences: (i) SEC1 gave a longer zone against anti-SEC2 than against anti-SEC1; (ii) SEC2 gave a longer zone against anti-SEC1 and anti-SEC3 than against anti-SEC2; and (iii) SEC3 gave a longer zone against anti-SEC1 and anti-SEC2 than against anti-SEC3. This showed that certain antibodies to the homologous toxin present in the homologous system did not react with the heterologous toxin, thus allowing the toxin in the heterologous systems to diffuse more rapidly than that in the homologous systems. This indicates that the SECs are different antigenically.

Molecular weight. The molecular weight of SEC1 was determined by gel permeation chromatography in the presence of 6 M urea to be 26,900 ± 1,900. By disc SDS-PAGE, the molecular weight appeared to be the same (Fig. 3a). The molecular weight calculated from the amino acid composition data yielded a value of 27,111.

Amino acid composition. The amino acid composition of SEC1 is presented in Table 1. The amount of cystine present was confirmed by subsequent analysis of cystine as cysteic acid. Each value is the average of two different preparations of SEC1. The number of amino acid residues was calculated to be 236 based on a molecular weight of 26,900.

Amino-terminal amino acid. Hydrolysis of the dinitrophenyl derivative of the SEC1 yielded dinitrophenylserine.

Isoelectric point. The isoelectric points of the various microheterogeneous components ranged from 6.55 to ca. 8.25 with the apparent major component at 8.15 (Fig. 6a). The various microheterogeneous components of the SECs all reacted with monoclonal antibody 1C3 as is indicated on the nitrocellulose-blot autoradiogram (Fig. 6b).

![Figure 4](image1.png)

**FIG. 4.** Log logit plot of RIA data with 125I-SEC1 and SEC3 antiserum versus unlabeled SEC1, SEC2, and SEC3. Nonidentity is indicated by nonparallel lines. %B/Bo, ratio of uptake of 125I-SEC1 in the presence of unlabeled toxin to uptake of 125I-SEC1 in the absence of unlabeled toxin.

![Figure 5](image2.png)

**FIG. 5.** (a) Comparative reaction of SEC1, SEC2, and SEC3 with anti-SEC1. Formation of spurs with SEC1 shows the presence of antibodies specific for SEC1 in the anti-SEC1. (b) Comparative reaction of SEC2, SEC1, and SEC3 with anti-SEC2. Formation of spurs with SEC2 over the SEC3 precipitin line shows the presence of antibodies in the anti-SEC1 that do not react with SEC1. All precipitating antibodies present in the anti-SEC2 react with both SEC1 and SEC2. (c) Comparative reaction of SEC1, SEC2, and SEC3 with anti-SEC3. The results indicate that all precipitating antibodies present in the anti-SEC3 react with all of the SECs.

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**TABLE 1.** The amino acid composition of SEC1, SEC2, and SEC3

<table>
<thead>
<tr>
<th>Amino acid residues (g/100 g of dry protein) for enterotoxin:</th>
<th>No. of residuesa in SEC1, SEC2, and SEC3 (nearest integer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>14.4</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.9</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.7</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>17.9</td>
</tr>
<tr>
<td>Threonineb</td>
<td>5.3</td>
</tr>
<tr>
<td>Serinec</td>
<td>4.6</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>9.0</td>
</tr>
<tr>
<td>Proline</td>
<td>2.2</td>
</tr>
<tr>
<td>Glycine</td>
<td>3.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>1.9</td>
</tr>
<tr>
<td>Half-cystinec</td>
<td>0.79</td>
</tr>
<tr>
<td>Valine</td>
<td>6.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>3.2</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.1</td>
</tr>
<tr>
<td>Leucine</td>
<td>6.5</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>9.8</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>5.4</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.0</td>
</tr>
<tr>
<td>Amide NH2</td>
<td>1.7</td>
</tr>
</tbody>
</table>

a Based on a 26,900 molecular weight.
b Extrapolated to zero from 24- and 72-h hydrolysis.
c Calculated from cystine as cysteic acid.
d Estimated spectrophotometrically.
Biological activity. The emetic 50% effective dose in cynomolgus monkeys was less than 10 μg per os and 0.05 μg/kg by the intravenous route (Table 2).

Stability and other characteristics. The freeze-dried SEC3 is a fluffy white powder which is readily soluble in water. The ratio of absorbance at 260/277 was ca. 0.5 (typical of simple proteins lacking nucleotides or other substances that absorb at 260 nm). Treatment with pepsin (pH 4.5) and trypsin (pH 8.0) had no effect on the toxicity of SEC3, although it is known that the SECs can be nicked in the cystine loop and at another site near the NH2 terminus by trypsin; only after reduction of the cystine residue can the parts be separated (22). At pH 6.8 in sterile solution, the SEC3 was stable for several months and showed no loss of serological activity when stored lyophilized for over 1 year.

DISCUSSION

The original identification and isolation of SEC3 were made from a strain of S. aureus (FRI-913) which was isolated from prawns by Richard Gilbert and co-workers at the Public Health Laboratories in England. We received the strain as an SEA, SEC, and SEE producer. No reference was made at the time as to which SEC was produced. We subsequently demonstrated that the SEC produced was a previously unidentified SEC, i.e., SEC3. Subsequent to the identification and purification of SEC3, strain FRI-913 was shown to produce TST as well.

The purification procedure outlined here makes it possible to purify SEC3 from strain FRI-913 to at least 96% purity in three steps: (i) adsorption from culture supernatant fluids with CG-50 resin, (ii) ion-exchange chromatography on CM-cellulose, and (iii) gel permeation chromatography through Sephadex G-75. The SEC3 appears to be homogeneous in that it gives (i) a single band of disc PAGE at pH 4.5, (ii) a major band and two nicked component bands in disc SDS-PAGE under reduced conditions, and (iii) a single N-terminal amino acid residue (serine).

It was demonstrated by Chang et al. (6) that SEC2 is microheterogeneous under conditions of isoelectric focusing. These workers suggested that the heterogeneity was due to a difference in amide groups as recombining the toxin yielded bands at the same pl or lower but never at a higher pl. Metzger et al. (13) were able to increase the microheterogeneity of SEC by treatment with alkali as demonstrated by isoelectric focusing. Purified SEC2 was more heterogeneous than SEC1 and was affected very little by treatment with alkali. Prolonged incubation of SEC2 produced changes that could be related to bacterial deamidases. They showed that SEC1 and SEC2 are distinct toxins and not interconvertible by alkali treatment. We have demonstrated here that SEC3 has similar properties. It is evident that SEC3 is more like SEC2 than SEC1 in isoelectric focusing, single-gel diffusion, and activity.

### TABLE 2. Effect of SEC3 on cynomolgus monkeys by route of administration

<table>
<thead>
<tr>
<th>Route</th>
<th>Amt administered</th>
<th>Result (no. positive/no. tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intragastric</td>
<td>10.0</td>
<td>4/4</td>
</tr>
<tr>
<td>Intravenous</td>
<td>0.5</td>
<td>4/4</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>4/4</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>1/4</td>
</tr>
</tbody>
</table>

*Micrograms per animal (intragastric route) or per kilogram (intravenous route).*
and double-gel diffusion characteristics but is not identical to either.

As can be seen in Table 1, the amino acid compositions of the SECs (grams per 100 g of protein) are within the range of experimental error of each other considering the fact that they were performed by three independent groups at different times. The number of residues calculated for SEC1 (236) is only three less than the number revealed for SEC1 (239) from the sequencing data of SEC1 (21). The calculated molecular weight for SEC1 (27,111) is almost identical to that calculated for SEC1 from the sequencing data (27,293). Perhaps the most distinguishing difference is that the SEC1 has serine as the N-terminal residue, whereas the SEC1 and SEC2 have glutamic acid as the N-terminal residue with serine as the next residue in the sequence (21).

The toxicity of the SEC1 is equal to that of the staphylococcal SEA, SEB, SEC1, and SEC2, both per os and intravenously (2, 7). This fact coupled with its resistance to proteolytic enzymes makes SEC1 a potential hazard that could cause food intoxications and infections. It was the only enterotoxin produced by an S. aureus strain implicated by Chow et al. (Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami Beach, Fla., abstr. no. 369, 1982) in an illness of a mother and her infant daughter that was similar to toxic shock syndrome.

There is no question that SEC1 is an enterotoxin. It is serologically and chemically similar to SEC1 and SEC2; however, as shown in isoelectric focusing, RIA, and N-terminal analysis, it is not identical to them. Thus, the name enterotoxin C is applied.

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LITERATURE CITED