residue is not fully exposed to the solvent. The observed red shift in the emission maximum upon Ca^{2+} addition implies that tryptophan now moves to a more polar environment, in agreement with our difference spectral observations, where the tryptophan residue was blue shifted in the presence of Ca^{2+}. Existence on S-100a of two sets of Ca^{2+} binding sites with K_d values of 5.5×10^{-5} M and 2.5×10^{-4} M at pH 8.3 and one binding site with a K_d of 1.2×10^{-4} M at pH 7.5 is very similar to the S-100b system (Mani et al., 1983). These results imply that the affinity of both proteins for Ca^{2+} is similar, as well as being pH dependent, and yet subtle differences exist in the microenvironment of specific chromophores. It is conceivable that these chromophores and the subtle differences in their response to Ca^{2+} may be exploited as specific probes in future structure–function studies on the S-100 proteins.

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Registry No. Calcium, 7440-70-2; potassium, 7440-09-7.

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Purification and Some Physicochemical Properties of Toxic-Shock Toxin†

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ABSTRACT: A procedure for the purification of a protein marker for the staphylococci isolated from toxic-shock syndrome patients has been developed. The purification procedure involves the removal of the toxic protein from culture supernatant fluids of toxic-shock syndrome associated *Staphylococcus aureus* strains FRI-1169 and FRI-1183 by batch absorption with CG-50 resin, ion-exchange chromatography on CM-Sepharose CL-6B, and gel permeation chromatography on Sephacryl S-200. The purified toxin is a simple protein with a molecular weight of 24 000 ± 500 as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The isoelectric point of the major band is 7.0 as determined by isoelectric focusing in polyacrylamide gels. The TS-toxin's reactivity with its specific antibody is not affected by tryptic digestion at pH 8.0 but is slowly reduced by treatment with pepsin at pH 4.5. The TS-toxin consists of 188 amino acid residues. Serine was shown to be the NH_2-terminal amino acid residue by end-group analysis. Initial studies indicated the protein was emetic; thus tentatively it was called staphylococcal enterotoxin F. In this paper it is called TS-toxin because the emetic action in monkeys has not been confirmed.

The staphylococci produce a number of toxic proteins that have been implicated in a variety of staphylococcal diseases, for example, gastric enteritis (Surgalla & Dack, 1955), scalded-skin syndrome (Melish & Glasgow, 1970), and toxic-shock syndrome (Todd et al., 1978). The signs and symptoms of the toxic-shock syndrome (Davis et al. 1980) are almost identical with those demonstrated in rhesus monkeys when staphylococcal enterotoxin B was injected intravenously (Beisel, 1972). Because of our interest in the role of the

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staphylococcal enterotoxins in staphylococcal diseases, an investigation was initiated on the possibility of enterotoxin involvement in the toxic-shock syndrome. Initial studies showed that only about 50% of the *Staphylococcus aureus* strains isolated from toxic-shock syndrome patients produced identifiable enterotoxins (Bergdoll et al., 1981). Further testing in monkeys (per os) (Surgalla et al., 1953) of culture supernatant fluids from several of the strains which did not produce known enterotoxins showed them to produce an emetic substance that we concluded was an enterotoxin (Bergdoll et al., 1981). An antigenic protein was isolated, and specific antibodies to it were prepared in rabbits. Over 90% of the strains isolated in connection with the toxic-shock syndrome produced this toxin, and it was shown that most of the toxic-shock syndrome patients had no or low antibody titers to it (Bergdoll et al., 1981).

In this paper we report the purification and some of the physicochemical properties of the TS-toxin[1] that we found associated with the *S. aureus* strains isolated from the sites of infection of toxic-shock syndrome patients.

Experimental Procedures

Materials. Reagents include acrylamide, *N,N,N′,N′*-tetramethylenediamine, Coomassie brilliant blue R-250, $NaDodSO_4$ (Bio-Rad Laboratories, Richmond, CA), CM-Sepharose CL-6B, Sephacryl S-200 superfine (Pharmacia Fine Chemicals, Piscataway, NJ), Amberlite CG-50 resin (100–200 mesh) (Mallinckrodt Chemical Works, St. Louis, MO), and poly(ethylene glycol) compound 20 M (Carbowax) (Union Carbide, Chicago, IL). Ampholytes for isoelectric focusing were purchased from Serva (Heidelberg, Germany) (Servalytes), LKB (Ampholines), and Pharmacia (Pharmalytes). Standard kits for isoelectric focusing and $NaDodSO_4$–polyacrylamide gel electrophoresis were purchased from Pharmacia Fine Chemicals. Bovine serum albumin, ovalbumin, α-chymotrypsinogen, soybean trypsin inhibitor, and cytochrome *c* were purchased from Sigma Chemical Co. (St. Louis, MO) for standards in the gel permeation molecular weight determinations. Phenol-extracted *Escherichia coli* lipopolysaccharide (endotoxin) was purchased from Sigma Chemical Co.

Estimation of Protein and Ultraviolet Absorption. During the early stages of purification, the protein concentration was estimated by using the extinction coefficient for enterotoxin C_1 ($E^{1\%}_{1cm}$ at 277 nm = 12.1) (Borja & Bergdoll, 1967). The highly purified TS-toxin has an $E^{1\%}_{1cm}$ of 9.5 at 280 nm and 9.7 at 277 nm (maximum absorption).

Antigen Production. The crude TS-toxin was prepared by inoculating 2-L Erlenmeyer flasks containing 400 mL of 3% N-Z Amine NAK (Humko-Sheffield Chemical) and 1% yeast extract (Difco) with 4 mL of a 24-h shake flask culture of a TS-toxin-associated *S. aureus* strain. The inoculated flasks were incubated on a Model V gyratory shaker (New Brunswick Scientific Co.) at 280 rpm in a 37 °C incubator for 20 h. The starting pH of the media was 6.5–6.6.

Antisera to the Toxic-Shock Antigen. Antisera to the highly purified TS-toxin were prepared in New Zealand white rabbits (Bergdoll, 1977).

Detection and Assay of the Toxic-Shock Antigen. Fractions from the different purification steps were tested for serological activity by the Ouchterlony plate method as modified by Bergdoll et al. (1965). The single gel diffusion tube method was used to quantitate the TS-toxin (Kato et al., 1966).

Protein Purity Determination. The purity of the TS-toxin was estimated by disc $NaDodSO_4$–polyacrylamide gel electrophoresis (Laemmli, 1979) and by the cathodic disc polyacrylamide gel electrophoresis procedure described by Reisfield et al. (1962). Also, the purity was determined by immunodiffusion techniques which utilized multivalent nonspecific antisera prepared against (1) preparations of TS-toxin and (2) the multiantigen-containing supernatant fluid of nonenterotoxigenic-non-TS-toxin *S. aureus* strain FRI-184. In addition, the TS-toxin was tested against the specific antisera for each of the identified enterotoxins by the optimum-sensitivity-plate method (Robbins et al., (1974).

Staphylococcal enterotoxin C_3,[2] which may be produced in low amounts, also was tested for by a modification of the radioimmunoassay procedure of Miller et al. (1978). Labeled [I^{125}]-enterotoxin C_3 competing with unlabeled enterotoxin C_3 vs. antibody to enterotoxin C_1 was used. Staphyloccoccal protein A which may interfere with the radioimmunoassay was removed as follows: (1) a chromatographic column, 1 × 3 cm (approximate bed volume 3 mL), was poured by using cyanogen bromide activated Sepharose 4B (Pharmacia Fine Chemical) to which 5 mg of human IgG/mL of gel had been coupled according to instructions in the Pharmacia bulletin; (2) 2–5 mL of culture supernatant fluid was percolated through the column; (3) the center of the plateau region of the through peak was collected for enterotoxin C_3 analysis.

Molecular Weight Determination. The molecular weight of the TS-toxin, after reduction with mercaptoethanol, was determined in disc $NaDodSO_4$–polyacrylamide gel electrophoresis by the method of Laemmli (1979) by using Bio-Rad Protean Cell vertical slab gel apparatus and the Pharmacia low molecular weight standards.

Gel permeation chromatography on Sephacryl S-200 (2.5 × 117 cm column) using proteins of known molecular weights was employed also for molecular weight determination. The purified protein standards and the TS-toxin were chromatographed in the denatured form (in 0.05 M sodium phosphate buffer containing 6 M urea, pH 6.8) and in the native state (in 0.05 M sodium phosphate buffer containing 0.5 M NaCl, pH 6.8).

Isoelectric Focusing. Slab gels (2 mm thick) were cast by using the gel casting kit provided with the LKB Multiphor unit. The following formulation was used for one slab: 39 mL of distilled water containing 7.5 g of sucrose, 18 mL of acrylamide–bis(acrylamide) (30:0.8), 3 mL of appropriate ampholyte, 0.2 mL of 10% ammonium persulfate, and 15 μL of *N,N,N′,N′*-tetramethylenediamine.

The precast polyacrylamide plates (p*I* = 3.5–9.5) (PAG-plate, LKB) were run according to instructions at 10 °C with 1 N NaOH as the cathode solution and 1 M H_3PO_4 as the anode solution. Good separation was obtained by using an initial setting at 210 V and 50 MA for 1.5 h and a final setting of 500 V and 10 MA for 3 additional h after the removal of the sample applicator. The broad range calibration kit for p*I* determination (Pharmacia) was used to estimate the p*I* of the TS-toxin.

Gels for isoelectric focusing in tubes were prepared as follows: two parts distilled water containing the sample, 1.5 parts 60% acrylamide, and 0.5 mL of appropriate ampholyte. The resulting solution was mixed with the catalyst (0.28% ammonium persulfate) at a ratio of 1:1, and the tube gels were cast [2 mL of gel/tube (5 mm × 110 mm)]. The anode buffer

[1] Abbreviations: TS-toxin, toxic-shock toxin; $NaDodSO_4$, sodium dodecyl sulfate; CM, carboxymethyl; disc, discontinuous; FRI, Food Research Institute.

[2] Unpublished data.

for the focusing was 4.25% H_3PO_4, and the cathode buffer was 5% ethylenediamine. The power supply voltage was set in the constant voltage mode, and the initial voltage was determined by using a starting amperage of 7 mA/tube. After 1 h under these conditions the amperage was increased by 2.4 mA/tube and the run continued for an additional 4 h at the voltage reached at the time of amperage increase. After completion of the run, one of the two tubes per sample was stained with Coomassie blue R-250 and the other sliced into 2-mm disks. The disks were placed in 0.5 mL of boiled distilled water to elute the TS-toxin for immunodiffusion studies and p*I* determination.

Amino Acid Composition. The amino acid composition of the TS–toxin was determined by Dr. Daniel Omilianowski, Biophysics Laboratory, University of Wisconsin, Madison, by the procedure of Moore (1972). Cystine as cysteic acid was determined by the technique described by Moore (1963). The tryptophan content of the TS-toxin was determined spectrophotometrically by the Edelhoch technique (Edelhoch, 1967).

NH_2-Terminal Analysis. The dinitrophenyl derivative of the toxic-shock antigen was prepared by the 1-fluoro-2,4-dinitrobenzene method of Sanger (1945) as modified by Fraenkel-Conrat et al. (1955). The dinitrophenyl amino acids were analyzed for by the thin-layer chromatography system described by Brenner et al. (1969).

Biological Activity Testing. The crude material and fractions obtained from the various purification steps were monitored for toxicity by intragastric injection of groups of four cynomolgus and rhesus monkeys (1.5–3.0 kg) (Surgalla et al., 1953). For injections intravenously into monkeys and rabbits and intraperitoneally into mice (ICR), the TS-toxin was dissolved in pyrogen-free bacteriostatic sodium chloride for injection (Elkins-Sinn, Inc., Cherry Hill, NC). The procedures of Sugiyama et al. (1964) were used to test lethality and endotoxin enhancement. The method of Burrows & Musteikis (1966) was used to test for ileal loop response, and the skin permeability assay of Glatz et al. (1974) was used for testing for vascular permeability changes. The mitogenic activity of the TS-toxin was determined by the method of Archer et al. (1979).

Results

All purification steps were performed at room temperature.

Step I: Removal of the Toxic-Shock Antigen from Culture Supernatant Fluids. The cultures were centrifuged at 13 000 rpm (27578*g*) at 0 °C in a Sorvall refrigerated centrifuge equipped with a GSA rotor (Du Pont Instruments, Newark, NJ). Ten liters of the supernatant fluid was adjusted to pH 5.6 with 6 N HCl (from approximately 7.8) and diluted 5-fold with distilled water. Amberlite CG-50 resin (150 mL, wet volume) was added to the diluted supernatant fluid. The resin had been precycled (pH 11.0 follod by ph 2.0), washed with distilled water, and equilibrated with 5 mM sodium phosphate buffer at pH 5.6. The mixture was stirred for a minimum of 1 h. After the mixture was stirred, the resin was allowed to settle, and the spent supernatant fluid was decanted and discarded. The resin was transferred to a chromatographic column (4 × 25 cm) and washed with 500 mL of water. The adsorbed materials including the TS-toxin were eluted with 2 L of 0.5 M sodium phosphate buffer containing 0.5 M NaCl at pH 6.2 at a flow rate of 2 mL/min. The entire eluate containing the TS-toxin was dialyzed against Carbowax in water (approximately 1:1 w/v) to reduce the volume 4-fold. The concentrate (approximately 500 mL) was further dialyzed against 5 mM phosphate buffer at pH 5.6 to prepare it for step II. On the basis of the amount of the TS-toxin in the culture supernatant fluid (determined by single gel diffusion), recovery of the toxin was about 40% with a purity of approximately 15%.

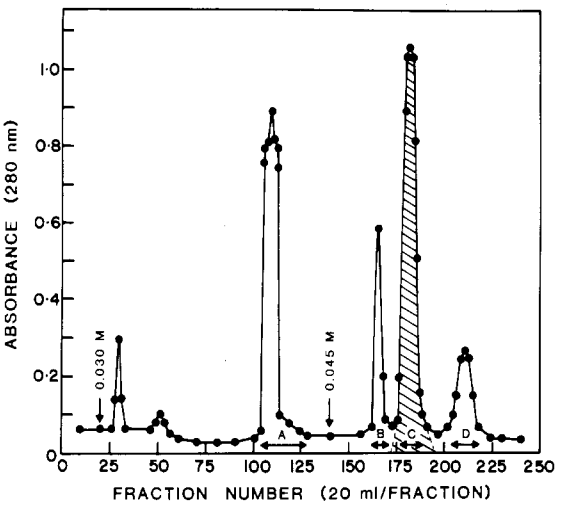

FIGURE 1: Ion-exchange chromatography of crude TS-toxin from step I on CM-Sepharose CL-6B column (2.5 × 45 cm) by stepwise elution with increasing concentration and pH of sodium phosphate buffer. Flow rate was 1 mL/min. Lined area, presence of TS-toxin.

Step II: Ion-Exchange Chromatography on CM-Sepharose CL-6B. The dialyzed concentrate from step I was clarified by centrifugation, applied to a column of CM-Sepharose CL-6B (2.5 × 45 cm) (precycled, washed, and equilibrated with 5 mM phosphate buffer at pH 5.6), and washed with 500 mL of 5 mM phosphate buffer at pH 6.0 at a rate of 1 mL/min. The TS-toxin was removed from the Sepharose with 2250 mL of 0.03 M phosphate buffer at pH 6.0 followed by 200 mL of 0.045 M phosphate buffer at pH 6.4. The effluent was continually monitored at 280 nm. A typical elution pattern is shown in Figure 1. The fractions eluted by the 0.03 M phosphate (including pool A) were discarded. Fraction pools B and D eluted by the 0.045 M phosphate buffer contained none of the TS-toxin and were discarded. Fraction pool C, which contained the major concentration of the TS-toxin, was collected and concentrated with Carbowax to approximately 15 mg of protein/mL for step III. The percentage of TS-toxin increased from about 15% to 71% with a recovery of 23%.

Step III: Gel Permeation Chromatography Using Sephacryl S-200. A column of Sephacryl S-200 (2.5 × 117 cm) was poured and washed overnight with 0.05 M phosphate buffer at pH 6.8 containing 1 M NaCl at a flow rate of approximately 1 mL/min. The concentrated TS-toxin from step II (2.5 mL maximum volume) was applied to the column followed by 0.05 M phosphate buffer at pH 6.8 containing 1 M NaCl and allowed to percolate at a flow rate of 1 mL/min. A typical elution profile is shown in Figure 2. Fractions 82–86 from the center portion of the major peak were combined, dialyzed against 5 mM phosphate buffer at pH 6.8, and lyophilized. The tailings of the peak were saved for reprocessing with those of other batches. The percentage of TS-toxin increased from 71% to 96% with an overall recovery of 17%.

Purity. The TS-toxin appears to be at least 96% pure by all techniques employed. No extraneous bands were visible in either the disc $NaDodSO_4$–polyacrylamide gel electrophoresis system or the disc polyacrylamide gel electrophoresis system for basic proteins when concentrations as high as 50 μg of protein per sample were applied. Immunodiffusion by the modified Ouchterlony technique using concentrations as high as 200 μg of TS-toxin showed only one minor line with

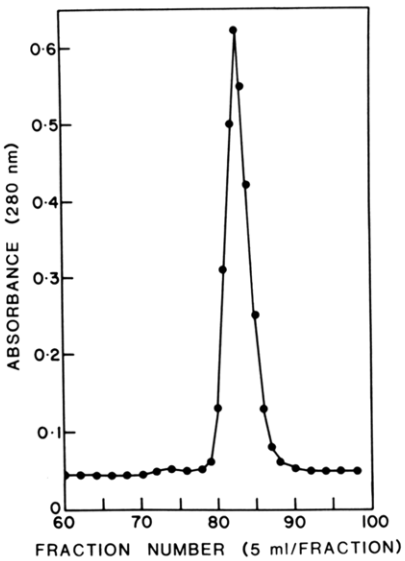

FIGURE 2: Elution profile from a column of Sephacryl S-200 (2.5 × 117 cm). The sample applied to the column was the fractions containing the TS-toxin from step II. Sodium phosphate buffer, 0.05 M and pH 6.8, containing 1 M NaCl was the eluting solution. Flow rate was 1 mL/min.

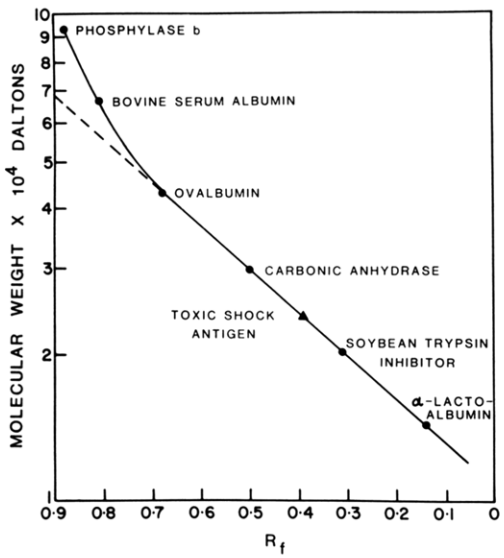

FIGURE 3: Semilog plot of the mobilities (average of duplicate runs) of six standard proteins obtained by disc $NaDodSO_4$–polyacrylamide gel electrophoresis (12% gel) against their molecular weights for determination of the molecular weight of the TS-toxin, indicates the mobility of the TS-toxin from the gel columns. See Experimental Procedures for details.

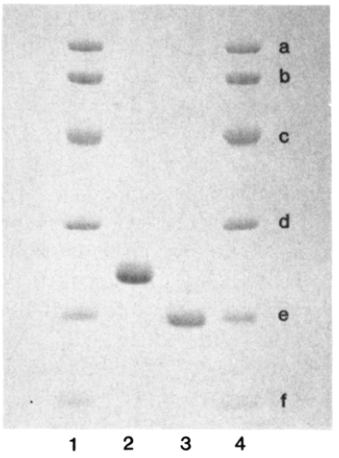

FIGURE 4: Disc $NaDodSO_4$–polyacrylamide gel electrophoresis (12% gel) for molecular weight determination of the TS-toxin. Conditions are described under Experimental Procedures. The cathode is at the top. Gel columns 1 and 4 are the Pharmacia standards: a, phosphorylase B, 94 000 daltons; b, bovine serum albumin, 67 000 daltons; c, ovalbumin, 43 000 daltons; d, carbonic anhydrase, 30 000 daltons; e, soybean trypsin inhibitor, 20 100 daltons; f, α-lactalbumin, 14 000 daltons. (Gel column 2) 10 μg of the TS-toxin. (Gel column 3) 10 μg of the soybean trypsin inhibitor.

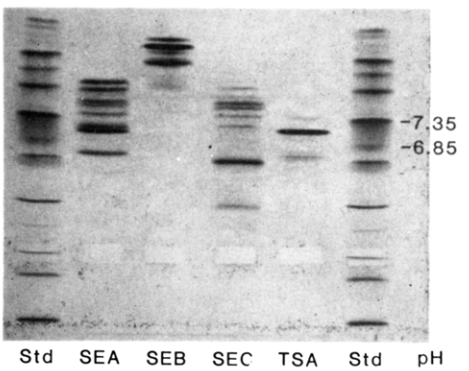

FIGURE 5: Isoelectric focusing of enterotoxin A, B, and C and the TS-toxin in precast polyacrylamide plates (p*I* = 3.5–9.5) (PAGplate, LKB). Conditions are described under Experimental Procedures. (Column 1 and 6) Standards: amyloglucosidase, p*I* = 3.5; soybean trypsin inhibitor, p*I* = 4.55; β-lactoglobulin A, p*I* = 5.2; bovine carbonic anhydrase B, p*I* = 5.85; human carbonic anhydrase B, p*I* = 6.55; horse myoglobin, p*I* = 6.85 and 7.35; lentil lectin, p*I* 8.5, 8.45, and 8.65; trypsinogen, p*I* = 9.3. (Column 2) Enterotoxin A (SEA). (Column 3) Enterotoxin B (SEB). (Column 4) Enterotoxin C_2 (SEC). (Column 5) TS-toxin.

antisera produced against the nonenterotoxigenic *S. aureus* strain FRI-184. This demonstrates that there are no contaminating staphylococcal antigens in excess of 1 μg in 200 μg of TS-toxin because this antisera contains the antibodies to at least 11 extraneous exoproteins produced by *S. aureus*.

Radioimmunoassay using the heterogeneous enterotoxin C system showed that purified TS-toxin from strain FRI-1183 had an enterotoxin C_3 concentration of 0.095% while the purified toxin from strain FRI-1169 showed no contamination with enterotoxin C_3. Subsequent testing of supernatant fluids from strains FRI-1169 and FRI-1183 (from which the protein A had been removed) by radioimmunoassay confirmed that enterotoxin C_3 was in fact produced by strain FRI-1183 at a very low level (<0.02 μg/mL) but not by strain FRI-1169.

Molecular Weight. The molecular weight of the TS-toxin was determined to be 24 000 ± 500 by disc $NaDodSO_4$–polyacrylamide gel electrophoresis (Figures 3 and 4). The molecular weight was calculated to be 20 000 ± 500 by gel permeation chromatography either in the presence or in the absence of 6 M urea. This indicates that the toxin may not have been completely unfolded in 6 M urea. When the 1.2% methionine content was used as a basis for calculating the minimum molecular weight, a value of 11 936 was obtained. If there are two methionine residues in the TS-toxin, the calculated molecular weight would be approximately 24 000.

Isoelectric Points. Isoelectric point determinations were performed by using each brand of ampholyte preparation. The range of values for the major TS-toxin band was 6.8–7.2. However, the TS-toxin band was consistently between the myoglobin standards (p*I* = 6.85 and 7.35) at the 7.0 position on slab gels; therefore, the TS-toxin p*I* is 7.0 (Figure 5). Staphylococcal enterotoxins A, B, C_1, and C_2 (Chang & Dickie, 1971; Chang et al., 1971; Schantz et al., 1972; Metzger et al., 1972) have been demonstrated to be heterogeneous by isoelectric focusing; therefore, it is not surprising that the

Table I: Amino Acid Composition of the TS-toxin

amino acid	amino acid residues (g/100 g of dry protein)	calcd residues[a]	nearest integral residues
lysine	12.0	22.5	23
histidine	2.8	4.9	5
arginine	2.7	4.2	4
aspartic acid	12.8	26.7	27
threonine	9.3[b]	22.1	22
serine	8.2[b]	22.6	23
glutamic acid	9.8	18.2	18
proline	5.3	13.1	13
glycine	3.6	15.2	15
alanine	1.2	4.1	4
half-cystine	0.7	1.7	2[c]
valine	2.4	5.8	6
methionine	1.2	2.2	2
isoleucine	7.7	16.4	16
leucine	8.1	17.2	17
tyrosine	7.0	10.3	10
phenylalanine	4.4	7.2	7
tryptophan	1.7	2.2	2[d]
amide nitrogen	1.3		

[a] Based on M_r 24 000. [b] Values extrapolated back to zero from 24- and 72-h hydrolysis. [c] Calculated from cystine as cysteic acid. [d] Estimated spectrophotometrically.

TS-toxin behaves in this manner.

Amino Acid Composition. The amino acid composition of the TS-toxin is presented in Table I. Each value is the average of three different preparations of the TS-toxin. The number of amino acid residues was calculated to be 188 based on a molecular weight of 24 000.

Amino-Terminal Amino Acid. Hydrolysis of the dinitrophenyl derivative of the TS-toxin yielded dinitrophenylserine.

Biological Activity Testing. Preliminary testing of the TS-toxin showed it to be emetic in cynomolgus monkeys. The group of animals used for this testing proved to be approximately 3 times more sensitive than monkeys previously used for staphylococcal enterotoxin testing on the basis of their reaction to staphylococcal enterotoxin A. However, later experiments with cynomolgus and rhesus monkeys in the Wisconsin Regional Primate Center, Madison, WI, gave negative results, even when the toxin was injected intravenously (30 μg/kg). The intravenous injection of 50 μg/kg of the TS-toxin or of enterotoxin B into rabbits followed by 0.05 μg/kg of endotoxin 4 h later gave negative results except for some weight loss which is normally experienced with the enterotoxins in rabbits. The intraperitoneal injection of 5, 10, and 15 μg/mouse of TS-toxin or enterotoxin A into mice followed by 150 μg/mouse of endotoxin 4 h later gave negative results. Also, the intraperitoneal injection of 50 and 100 μg/mouse of the TS-toxin or of enterotoxin A and 5, 10, 25, and 50 μg/mouse of entotoxin B into mice followed by 150, 300 and 450 μg/mouse of endotoxin 4 h later gave negative results. The TS-toxin did not elicite an ileal loop reaction nor did it show any vascular permeability changes when tested in rabbits; this is consistent with the results obtained with the staphylococcal enterotoxins.

The TS-toxin was equal in mitogenic activity to enterotoxin A, the most mitogenic of the enterotoxins (Archer et al., 1979). The TS-toxin was shown to produce the signs and symptoms of the toxic-shock syndrome in baboons and to be very mitogenic by Dr. Fred Quimby, Cornell Medical Center, New York, NY.[3]

[3] Unpublished data.

Stability and Other Characteristics. The lyophilized TS-toxin is a fluffy white powder which is readily soluble in distilled water. The ratio of the absorbance at 260/280 nm is approximately 0.5 which is typical of a simple protein lacking nucleotides or other substances which absorb at 260 nm. The TS-toxin is resistant to proteolytic digestion by trypsin but is susceptible to peptic digestion at pH 4.5. In sterile solution the TS-toxin is stable at neutral pH for several months and when lyophilized has shown no loss of serological activity after 1 year.

Discussion

The purification procedures described here make it possible to purify the TS-toxin in three steps: (1) adsorption of the antigen from culture supernatant fluids with CG-50 resin; (2) ion-exchange chromatography on CM-Sepharose CL-6B; (3) gel permeation chromatography on Sephacryl S-200. The use of the CM-Sepharose CL-6B in place of the CM-cellulose used in the purification of the staphylococcal enterotoxins appears to be the key to the purification of the TS-toxin; by use of the CM-cellulose it was impossible to separate the TS-toxin from some impurities present in the crude preparations. The TS-toxin appears to be homogeneous by the following criteria: (1) a single band in disc polyacrylamide gel electrophoresis (pH 4.5); (2) a single band in disc $NaDodSO_4$–polyacrylamide gel electrophoresis; (3) the detection of a single antigenic component over a wide range of concentrations with the use of multivalent antiserum prepared against the TS-toxin.

The TS-toxin is a simple protein consisting of a single polypeptide chain. This was confirmed when the reduced toxin gave a single band in $NaDodSO_4$–polyacrylamide gel electrophoresis. Additional evidence for this conclusion is the fact that only one NH_2-terminal amino acid (serine) was found.

The observation that staphylococcal enterotoxin B when injected intravenously into rhesus monkeys produced signs and symptoms almost identical with those observed in toxic-shock syndrome (Beisel, 1972) indicated that the causative factor in this disease may be an enterotoxin. Initial testing of strains from toxic-shock syndrome patients implicated an enterotoxin as the causative factor, as all strains tested produced an emetic substance (Bergdoll et al., 1981). Also, the initial testing of the purified TS-toxin indicated it to be emetic, the criterion used to identify proteins produced by the staphylococci as enterotoxins (Surgalla et al., 1953). Later testing in monkeys at the University of Wisconsin Primate Center, however, gave negative results, even when the TS-toxin was injected intravenously at levels of 30 μg/kg. The negative results from oral administration were understandable as it was shown that the TS-toxin was hydrolyzed by pepsin at pH 4.5, which is not the case with the staphylococcal enterotoxins. The lack of response of the monkeys to the toxin when administered intravenously cannot be explained at this time; however, the fact that the signs and symptoms observed in baboons included emesis may indicate a species difference. The staphylococcal enterotoxins provide an example of this in that enterotoxin C_1 is emetic in monkeys but 50-fold less so in cats when compared to the other enterotoxins (Casman et al., 1967).

The lack of evidence for emetic activity in monkeys makes the designation enterotoxin F questionable; however, because of the similarity of its physicochemical properties (amino acid composition, NH_2-terminal, basic protein, resistance to trypsin) to those of the staphylococcal enterotoxins, the term enterotoxin-like protein was applied (Bergdoll et al., 1982). Further, the TS-toxin is equal in mitogenicity and immunosuppression to staphylococcal enterotoxin A which may indicate relatedness to the staphylococcal enterotoxins. The results with the en-

dotoxin testing were inclusive because they did not show the effects of enterotoxin A on the lethality of the endotoxin in either mice or rabbits that had been observed previously (Sugiyama, 1964).

The TS-toxin undoubtedly serves as a marker for the toxic-shock syndrome (Bergdoll et al., 1981, 1982) as over 90% of the *S. aureus* strains isolated in connection with the toxic-shock syndrome produce the TS-toxin with approximately 40% of these producing no known staphylococcal enterotoxins (Bergdoll et al., 1982). In addition very few of the toxic-shock syndrome patients have an acute antibody titer to the TS-toxin. Many patients who do not develop antibody to the toxin have recurrence of the illness (Bergodoll et al., 1981). To date, no patient who has developed antibodies to the TS-toxin has had recurrence of the disease.

Schlievert et al. (1981) have reported the purification of a substance (pyrogenic exotoxin C) which also appears to be associated with the toxic-shock syndrome *S. aureus* strains. There exotoxin C preparation does react with the specific antibody to the TS-toxin but both immunological and NaDodSO$_4$–polyacrylamide gel electrophoresis analysis of this preparation in our laboratory indicated the presence of extraneous material.[4] There is little question that the active component of the enotoxin preparation is the same as the TS-toxin, but the presence of the impurities makes comparison of the two materials difficult. The fact that isoelectric focusing was used in the purification of the pyrogenic exotoxin C may account for the considerable difference in the amino acid composition reported for exotoxin C (Schlievert et al., 1981) and of the TS-toxin given here (Table I).

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