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Rapid Purification of Staphylococcal Enterotoxin B by High-Pressure Liquid Chromatography

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The *Staphylococcus aureus* enterotoxins represent a group of proteins that cause emesis and diarrhea in humans and other primates. We have developed a rapid two-step high-pressure liquid chromatography (HPLC) procedure for purification of staphylococcal enterotoxin B (SEB). Sterile filtrates (2.5 liters) of strain 10-275 were adsorbed directly onto a reversed-phase column (50 mm by 30 cm Delta Pak; 300 Å [30 nm], 15 μm, C18). SEB was obtained by using a unique sequential gradient system. First, an aqueous ammonium acetate to acetonitrile gradient followed by an aqueous trifluoroacetic acid (TFA) wash was used to remove contaminants. A subsequent TFA to acetonitrile-TFA gradient eluted the bound SEB. Further purification was obtained by rechromatography on a cation-exchange column. From 35 to 45% of the SEB in starting filtrates was recovered. Analysis by immunoblotting of samples separated on sodium dodecyl sulfate-polyacrylamide gels indicated that HPLC-purified SEB exhibited immunological and biochemical properties similar to those of the SEB standard. Induction of an emetic response in rhesus monkeys showed that the HPLC-purified toxin also retained biological activity.

Staphylococcal enterotoxins, a closely related group of proteins produced by some strains of *Staphylococcus aureus*, cause food poisoning in humans and other primates that is characterized by emesis and diarrhea (1). On the basis of serological reactivities with specific antibodies (1), they have been divided into several classes, designated staphylococcal enterotoxin A (SEA), SEB, SEC, SED, and SEE. In addition to provoking food poisoning, these proteins are mitogenic and pyrogenic, can modulate immune responses, and can enhance sensitivity to endotoxic shock (1, 3, 19, 24). The molecular and cellular basis for these toxin activities remains obscure. Enterotoxins A, B, and C1 have been extensively characterized for their chemical and physical properties, including homogeneity, molecular size, nucleotide sequence, and primary structure. Staphylococcal enterotoxins have molecular masses in the range of 26 to 30 kilodaltons (kDa) and possess similar structures, consisting of a single polypeptide chain which contains a single disulfide loop (1, 2, 6, 8–11, 14, 20–22).

Multistep, conventional ion-exchange and gel filtration chromatographic procedures for purification of staphylococcal enterotoxins have been developed (1, 21, 22). These procedures yield essentially pure toxin which behaves as a single protein component when analyzed via chromatographic and electrophoretic procedures. Some microheterogeneity, shown to reflect interconversion of enterotoxin into several forms, has been demonstrated by isoelectric focusing techniques (5, 25). More recent studies have defined additional approaches for purification of these proteins by chromatofocusing fractions recovered from initial chromatography (7, 12, 17, 18). In addition, early efforts with high-pressure liquid chromatographic (HPLC) procedures for purification of SEB revealed that this toxin was closely associated with several low-molecular-weight proteins that could be separated following sodium dodecyl sulfate (SDS) denaturation of the toxin preparation (29).

The development of information on the molecular mode of action of staphylococcal enterotoxins remains of paramount importance to our understanding of their role in pathogenesis. Structure-function studies of these bacterial proteins would be greatly facilitated by rapid and simple procedures for their purification and analysis. We have recently extended the application of HPLC techniques for isolation of SEB (M. P. Strickler, M. J. Stone, T. Grebas, G. Tkalcevic, R. Neill, and P. Gemski, Abstr. 7th Int. Symp. HPLC of Proteins, Peptides, and Polynucleotides, 1987, abstr. 829, p. 41; P. Strickler, M. J. Stone, T. Grebas, G. Tkalcevic, R. Neill, and P. Gemski, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, B63, p. 40). In the present paper, we describe in detail an efficient, simple, and rapid two-step HPLC method for purification of SEB from crude bacterium-free filtrates by use of reversed-phase and cation-exchange techniques. Immunochemical, biochemical, and biological characterization of the toxin obtained by this method showed that its properties were similar to those of an SEB standard purified previously by conventional means.

MATERIALS AND METHODS

Preparation of SEB. Culture filtrates of *S. aureus* 10-275 served as a source of crude SEB. Strain 10-275 is a high-SEB-producing mutant derivative of strain S6 (4, 27, 28). Bacterial cultures were grown in 1.0-liter batches of L broth (1% tryptone [Difco Laboratories], 0.5% yeast extract [Difco], and 0.5% NaCl) contained in 2-liter Erlenmeyer flasks at 37°C on a rotary shaker (New Brunswick Scientific Co., Edison, N.J.; model 25; 200 rpm) for 20 h. Cells were removed by centrifugation (6,000 × *g*, 4°C, 30 min), and bacterium-free filtrates were then prepared by filtration of the supernatant through membranes (0.45-μm pore size; Minitan Ultrafiltration System, Millipore Corp., Bedford, Mass.). These crude bacterial filtrates served as starting material for purification of SEB.

SEB reference standard. A solution (1 mg/ml) of purified SEB, previously isolated at the U.S. Army Research Institute of Infectious Diseases, Frederick, Md., by the method

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of Schantz et al. (21), was used as a reference standard in our study.

Chromatographic procedures. All chromatographic columns and equipment were from Waters Chromatography Division, Millipore Corp., Milford, Mass. All buffers and solvents were HPLC grade (Fisher Scientific Co., Pittsburgh, Pa.) and were filtered through an HA membrane (0.45-μm pore size) prior to use. Parameters for reversed-phase chromatography with a sequential gradient were first developed on a Delta Pak column (3.9 mm by 30 cm; 300 Å [30 nm], 15 μm, C18). Buffer A was 0.01 M ammonium acetate, pH 5.5; buffer B was acetonitrile; buffer C was 0.1% aqueous trifluoroacetic acid (TFA); and buffer D was acetonitrile with 0.1% TFA. The solvent flow rate was 0.5 ml/min. One milliliter of bacterium-free filtrate was injected onto the column, followed by a 15-min linear gradient from 100% buffer A to 80% B–20% A. After a 15-min wash with 100% buffer C, a 40-min linear gradient from 100% C to 70% D–30% C eluted the SEB.

Preparative-scale reversed-phase chromatography was performed on a Delta Prep HPLC equipped with a model 481 variable-wavelength detector with a semi-preparative flow cell and a Delta Pak column (50 mm by 30 cm). Eluting buffers were those described above. The column was equilibrated in 100% buffer A, and the sample was loaded directly through a port on the solvent delivery system at a flow rate of 80 ml/min. After sample loading, the column was washed with buffer A until the A_{280} was zero. A 15-min linear gradient at a flow rate of 80 ml/min from 100% buffer A to 80% B–20% A was followed by a 15-min wash in 100% buffer C. A 40-min linear gradient from 100% buffer C to 70% D–30% C at a flow rate of 80 ml/min eluted the SEB. Fractions (40 ml) were collected.

Preparative-scale cation-exchange chromatography was performed with a Protein Pak SP-5PW column (21.5 mm by 15 cm). An 80-min linear gradient from 20 mM sodium phosphate (pH 6.0) to 20 mM sodium phosphate (pH 6.0)–0.5 M sodium chloride was run at a flow rate of 4 ml/min.

Analysis of fractions from the preparative reversed-phase separation were performed on (i) a μBondapak C18 reversed-phase column (3.9 mm by 15 cm) with a 15-min linear gradient from 0.1% aqueous TFA to 70% acetonitrile–30% aqueous TFA at a flow rate of 1 ml/min and (ii) a Protein Pak SP-5PW cation-exchange column (7.5 mm by 7.5 cm) with a 30-min linear gradient from 20 mM sodium phosphate (pH 6.0) to 20 mM sodium phosphate (pH 6.0)–0.3 M sodium chloride at a flow rate of 1 ml/min. Samples (100 ml) from the preparative cation-exchange separation were also analyzed on a Protein Pak SP-5PW column (7.5 mm by 7.5 cm) with a 40-min linear gradient from 20 mM sodium phosphate (pH 6.0) to 20 mM sodium phosphate (pH 6.0)–0.3 M sodium chloride at a flow rate of 1 ml/min. Gel filtration HPLC analysis was carried out on a Protein Pak 300 SW column (7.5 mm by 30 cm) with 0.1% aqueous TFA as the eluent at a flow rate of 1 ml/min.

PAGE and immunoblot analysis. Protein samples were subjected to polyacrylamide gel electrophoresis (PAGE) under reducing and denaturing conditions by the method of Laemmli (16) with 12% polyacrylamide–SDS gels. Following electrophoresis, gels were either stained (16) or analyzed by immunoblotting techniques as described previously (23). For immunoblot analyses, a 1:100 dilution of rabbit anti-SEB serum (Sigma Chemical Co., St. Louis, Mo.) was used as the primary antibody, and a 1:200 dilution of alkaline phosphatase-labeled goat anti-rabbit serum (Kirkegaard and Perry, Gaithersburg, Md.) was used as the secondary antibody.

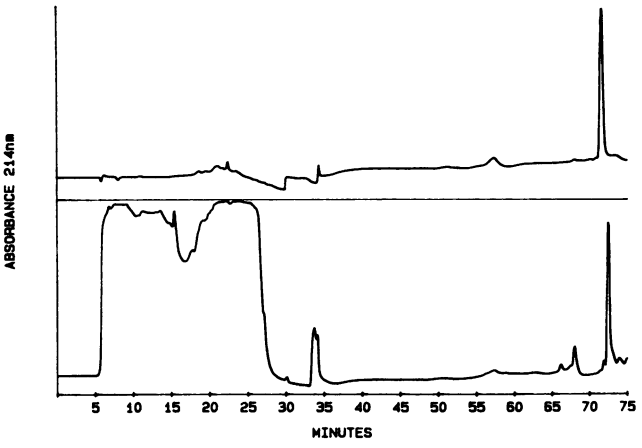

FIG. 1. Analytical reversed-phase HPLC separation of SEB. Samples were eluted from a Delta Pak column (3.9 mm by 30 cm) as described in Materials and Methods. The top panel shows the elution of 50 μg of the SEB standard. The bottom panel shows the separation of SEB from 1 ml of filtrate. Detection was at 214 nm at 2.1 absorbance units full scale.

Test for biological activity. Rhesus monkeys (*Macaca mulatta*) weighing 3.5 to 5.0 kg were anesthetized with 50 mg of ketamine hydrochloride per animal. Animals were then held in a sitting position and a no. 8 infant feeding tube was passed for the nasogastric instillation of 150 μg of SEB. After passage of the nasogastric tube, 2 ml of sterile water was administered, followed by SEB. The SEB was followed by an 8-ml sterile water nasogastric tube flush. Animals were returned to their cages for recovery and observation. As promulgated by the Walter Reed Army Institute of Research Laboratory Animal Care and Use Committee, this research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health publication 86-23, 1985 edition).

RESULTS

HPLC analysis of bacterial filtrates. We devised a unique sequential buffer gradient system for isolation of SEB from crude bacterium-free culture filtrates by reversed-phase HPLC. Under these conditions, from 60 to 90% of the SEB present in these filtrates was eluted (Fig. 1) from a Delta Pak C18 column as a distinct peak with a retention time similar to that of the SEB standard. The results of immunoblot analysis on material eluting in this peak revealed high reactivity with antibody specific for SEB, confirming that this peak represented SEB (data not shown). When the peak fraction was collected from such a separation of 1.0 ml of culture filtrate and then rechromatographed, the material behaved as a single homogeneous component (data not shown).

Preparative HPLC isolation of SEB. The results of the adaptation of this sequential buffer gradient system to a preparative scale (2.5 liters of culture filtrate applied to the column) are shown in Fig. 2. Except for reversed-phase column capacity and size, as well as a proportional increase in flow rate (based on column dimension), the chromatographic conditions for the preparative-scale isolation were

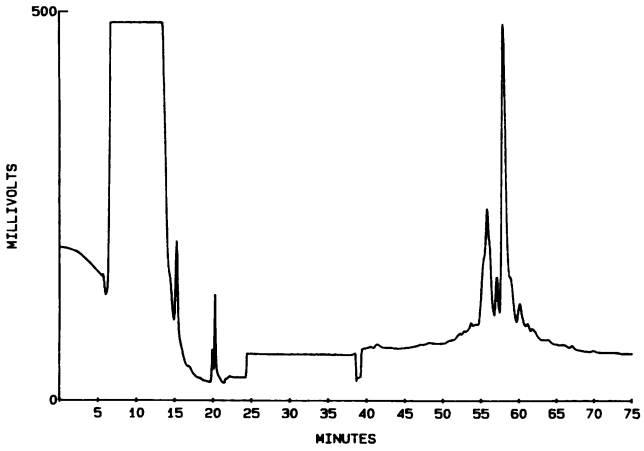

FIG. 2. Preparative reversed-phase HPLC of culture filtrate. Culture filtrate (2.5 liters) was loaded onto a Delta Pak column (50 mm by 30 cm), and SEB was eluted as described in Materials and Methods. Detection was at 280 nm at 0.5 absorbance units full scale.

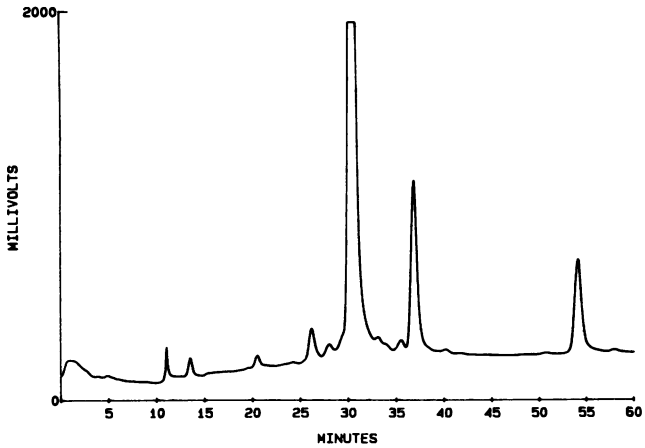

FIG. 4. Rechromatography of SEB-containing fraction RP-57.5 from preparative reversed-phase chromatography. An 8-ml amount of a 1:5 dilution of fraction RP-57.5 in water was injected onto a Protein Pak SP-5PW column (21.5 mm by 15 cm) and chromatographed as described in Materials and Methods. Detection was at 214 nm at 2.5 absorbance units full scale.

essentially unchanged from those used at the analytical level. A major peak as well as several minor peaks (detected at 280 nm) with retention times of 55 to 59 min were resolved by the column. Fractions (40 ml) were collected at 30-s intervals for further study. Analysis of fractions RP-55.5 through RP-59 by rechromatography on a reversed-phase column or on a cation-exchange column indicated that fractions RP-57.5 and RP-58 contained in substantially pure form the bulk of SEB present in the 2.5 liters of starting material.

The purity of fractions RP-55.5 through RP-59 was also characterized by PAGE in the presence of SDS (SDS-PAGE). Both staining (Fig. 3A) and immunoblotting (Fig. 3B) techniques were used. The purified SEB standard (lane 1) and crude bacterial culture filtrate (lane 2) were included for comparison in this analysis. The molecular mass of SEB was calculated to be 27.5 kDa under the conditions of our procedures, a value close to the molecular mass (28.4 kDa) derived for SEB from amino acid sequencing analysis. As has been observed by previous investigators, the purified SEB standard was found to contain some native nicked toxin material similar in size (13 to 16 kDa) but not identical to peptides generated by experimental trypsin hydrolysis. A fragment of about 26.5 kDa was also present. These particular fragments represented nicked forms of SEB and not some extraneous contaminants because they reacted in an immunoblot (data not shown) with a monoclonal antibody (2B [26]) specific for SEB. Similar forms of SEB were found to be components of fractions RP-57.5 and RP-58 (lanes 6 and 7, Fig. 3A) recovered by reversed-phase preparative chromatography. However, another minor protein component of about 32 kDa which did appear to be a contaminant was also detected in these fractions. It was serologically unrelated to SEB because it was unreactive in the rabbit anti-SEB immunoblot analysis (lanes 6 and 8, Fig. 3B).

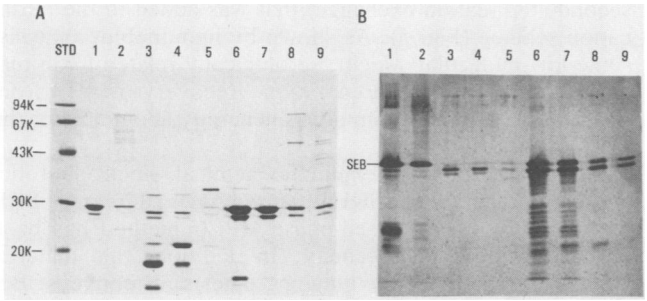

FIG. 3. Immunoblot analysis of fractions from preparative reversed-phase HPLC. Samples of fractions were electrophoresed on 12% polyacrylamide–SDS gels and either stained (A) or transferred by electroelution onto nitrocellulose and reacted with specific antisera prepared against purified SEB (B). Lane STD contains 4 μg of protein molecular mass standards (sizes shown in kilodaltons). Lanes 1 contain 8 μg (A) or 1 μg (B) of purified SEB. Lanes 2 contain 40 μl (A) or 25 μl (B) of culture filtrate. Remaining lanes contain 15 μl (A) or 0.5 μl (B) of fractions 55.5 (lanes 3), 56 (lanes 4), 57 (lanes 5), 57.5 (lanes 6), 58 (lanes 7), 59.5 (lanes 8), and 59 (lanes 9).

Cation-exchange rechromatography of SEB recovered from reversed-phase HPLC. Fraction RP-57.5 was next subjected to cation-exchange HPLC to purify SEB more thoroughly. As shown in Fig. 4, several major peaks (retention times of 31, 37, and 55 min) could be resolved by the preparative cation-exchange separation step. Subsequent analysis of the fractions that were recovered revealed that fraction CE-31 contained most of the SEB contained in fraction RP-57.5. Approximately 50% of the SEB in fraction RP-57.5 was recovered in fraction CE-31.

Fractions isolated during the cation-exchange step were also characterized for purity by SDS-PAGE and subsequent immunoblotting with rabbit anti-SEB (Fig. 5). The purified SEB standard (lane 1) and fraction RP-57.5 (lane 2) have been included for comparison. It is apparent from these analyses that the cation-exchange step eliminated the serologically unrelated 34-kDa contaminant present in fraction RP-57.5 from SEB. Fractions CE-30 (lane 4), CE-31 (lane 5), and CE-32 (lane 6) consisted of SEB at a high level of purity equal to that of the SEB standard isolated by conventional, multistep techniques (lane 1). The nicked forms of SEB were also present in these fractions as well as fraction CE-37. Fraction CE-31 contained a preponderance of native SEB (27.5 kDa) with a lesser amount of the 26.5-kDa form, whereas the reverse was true for fraction CE-37. When subjected to gel filtration analysis, the SEB in fraction CE-31

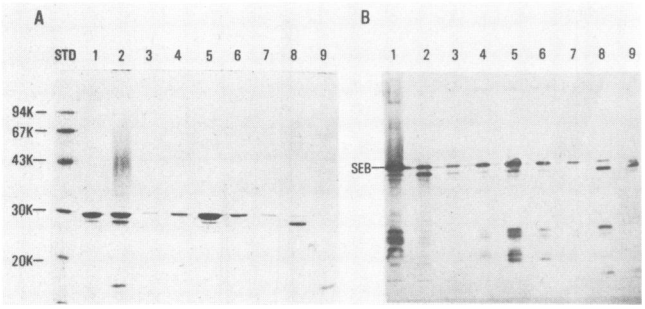

FIG. 5. Immunoblot analysis of fractions from preparative cation-exchange chromatography. Samples of fractions were electrophoresed on 12% acrylamide–SDS gels and either stained (A) or transferred by electroelution onto nitrocellulose and reacted with specific antisera prepared against purified SEB (B). Lane STD contains 4 μg of protein molecular mass standards (sizes shown in kilodaltons). Lanes 1 contain 8 μg (A) or 1 μg (B) of purified SEB. Lanes 2 contain 10 μl (A) or 0.5 μl (B) of fraction 57.5 (Fig. 2). Remaining lanes contain 35 μl (A) or 1.5 μl (B) of fractions 27 (lanes 3), 30 (lanes 4), 31 (lanes 5), 32 (lanes 6), 33 (lanes 7), 37 (lanes 8), and 55 (lanes 9).

behaved as a homogeneous protein with a molecular weight of about 30,000 (Fig. 6).

Biological activity. A sample of SEB that we purified by this two-step HPLC procedure was tested for its ability to induce an emetic response in rhesus monkeys. This assay represents the standard for determining staphylococcal enterotoxin activity (15) and thus served as a means of assessing whether the SEB purified by our HPLC method retained toxic activity. Between 2 and 7 h following oral administration of 150 μg of HPLC-purified SEB, five of six monkeys exhibited vomiting and one of these five also developed mild diarrhea. In comparison, six of eight monkeys exhibited an emetic response following administration of 150 μg of SEB purified by the method of Schantz et al. (21).

DISCUSSION

A rapid, two-step procedure for isolation of SEB has been devised and found to yield a product of high purity. The simplicity and speed of our purification method facilitate isolation of biologically active toxin from crude bacterium-free filtrates of *S. aureus*. For example, starting from 2.5 liters of culture filtrate of *S. aureus* 10-275 grown in L broth, we generally recover 60 to 70 mg of SEB with a quality consistent with that of SEB produced by conventional, multistep ion-exchange and gel filtration chromatographic procedures (Fig. 5). Very likely, this yield could be improved further by controlled propagation of the organism in specialized medium containing the proteolytic digests of casein that have been described for optimum production of SEB (21).

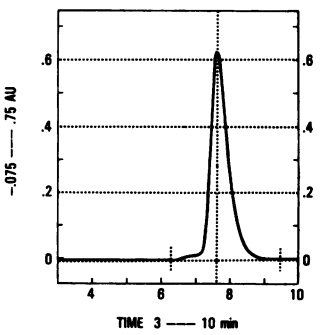

FIG. 6. Gel filtration HPLC analysis of SEB from preparative cation-exchange separation. A 100-μl sample of fraction 31 was chromatographed on a Protein Pak 300 SW column (7.5 mm by 30 cm) with 0.1% aqueous TFA as the eluent at a flow rate of 1 ml/min. Detection was at 214 nm at 0.75 absorbance units full scale. Retention time of the SEB standard was 7.6 min. AU, Absorbance units.

Previously described methods for purification of staphylococcal enterotoxins have been faced with the need to reduce large volumes of bacterial culture filtrates to manageable levels. Generally, dialysis of this material against polyethylene glycol or its bulk slurry adsorption to ion-exchange resins has been used for the initial concentration step (7, 17). The use of a preparative reversed-phase C18 column capable of adsorbing up to 1 g of protein in the first step of our isolation procedure eliminated this problem in SEB purification. Under the conditions that we have specified for adsorption and elution of SEB with sequential buffer gradients, the initial step of loading the column with a large volume (2.5 liters) of unconcentrated filtrate accomplishes both enrichment and substantial purification of the toxin. Estimates of mass recovery of SEB, based on spectrophotometric analysis, indicate that from 60 to 90% of the SEB originally present in the starting filtrate can be recovered in this first step. For example, in one experiment, 133 mg of SEB was recovered from 2.5 liters of filtrate which originally contained 150 mg of SEB. Past efforts at isolating SEB via application of HPLC procedures revealed the presence of several low-molecular-mass contaminating proteins (17- to 26-kDa range) that could be separated following denaturation by preincubation in SDS-glycerol-mercaptoethanol buffer (29). The denaturing effects of this incubation step generally yielded an essentially homogeneous 28-kDa SEB protein which, as expected, lacked biological activity (29). Under the conditions that we have employed for purification, such contaminating proteins in the 17- to 26-kDa range were also present in SEB-containing fractions recovered from reversed-phase chromatography (Fig. 3). However, these contaminants plus a serologically unrelated contaminating peptide of about 32 kDa were readily eliminated by a second step (cation exchange) that was added to the purification scheme (Fig. 5). As shown by immunoblot analysis, some low-molecular-weight, serologically related toxin material remained in the preparation. Recovery of SEB from the cation-exchange column was generally about 50% of the toxin applied.

Within the limits of the immunochemical, electrophoretic, and chromatographic criteria that we have addressed, SEB recovered by the reversed-phase HPLC method has been purified to near homogeneity. In addition, this material retains toxic activity for inducing emesis in monkeys. Because SEA and SEC1 share a degree of relatedness to the protein structure and amino acid sequence of SEB, the method described here may be applicable to purification of these other staphylococcal enterotoxins. Moreover, efforts are under way to develop HPLC procedures for rapid isolation of SEB peptide fragments generated by digestion with enzymes and other agents. Such systems have an important role in development of information on the structure and functional relationships of staphylococcal enterotoxins.

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LITERATURE CITED

1. **Bergdoll, M. S.** 1972. The enterotoxins, p. 187–331. *In* J. O. Cohen (ed.), The staphylococci. Wiley Interscience Publishers, New York.
2. **Betley, M., and J. J. Mekalanos.** 1988. Nucleotide sequence of type A staphylococcal enterotoxin gene. J. Bacteriol. **170:** 34–41.
3. **Brunson, K. W., and D. W. Watson.** 1974. Pyrogenic specificity of staphylococcal enterotoxin and gram-negative endotoxin. Infect. Immun. **10:**347–351.
4. **Casman, E. P., M. S. Bergdoll, and J. Robinson.** 1963. Designation of staphylococcal enterotoxins. J. Bacteriol. **85:**715–716.
5. **Chang, P. C., and N. Dickie.** 1971. Fractionation of staphylococcal enterotoxin B by isoelectric focusing. Biochim. Biophys. Acta **236:**367–375.
6. **Couch, J. L., M. T. Soltis, and M. J. Betley.** 1988. Cloning and nucleotide sequence of the type E staphylococcal enterotoxin gene. J. Bacteriol. **170:**2954–2960.
7. **Ende, I. A., G. Terplan, B. Kickhofen, and D. Hammer.** 1983. Chromatofocusing: a new method for purification of staphylococcal enterotoxins B and C1. Appl. Environ. Microbiol. **46:** 1323–1330.
8. **Huang, I.-Y., and M. S. Bergdoll.** 1970. The primary structure of staphylococcal enterotoxin B. I. Isolation, composition and sequence of tryptic peptides from oxidized enterotoxin B. J. Biol. Chem. **245:**3493–3510.
9. **Huang, I.-Y., and M. S. Bergdoll.** 1970. The primary structure of staphylococcal enterotoxin B. II. Isolation, composition and sequence of chymotryptic peptides. J. Biol. Chem. **245:**3511–3517.
10. **Huang, I.-Y., and M. S. Bergdoll.** 1970. The primary structure of staphylococcal enterotoxin B. III. The cyanogen bromide peptides of reduced and amino-acylated enterotoxin B and the complete amino acid sequence. J. Biol. Chem. **245:**3518–3525.
11. **Huang, I.-Y., J. L. Hughes, M. S. Bergdoll, and E. J. Schantz.** 1987. Complete amino acid sequence of staphylococcal enterotoxin A. J. Biol. Chem. **262:**7006–7013.
12. **Janin, F., C. Lapeyre, M. L. de Buyser, F. Dilasser, and M. Borel.** 1984. Purification of *S. aureus* enterotoxins A, C1 and D by fast protein liquid chromatography, p. 251–252. *In* J. Alouf, F. Fehrenbach, J. Freer, and J. Jeljaszewica (ed.), Bacterial protein toxins. Academic Press, Inc. (London), Ltd., London.
13. **Johnson, D. A., J. W. Gautsch, J. R. Sportsman, and J. H. Elder.** 1984. Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. Gene Anal. Tech. **1:**3–8.
14. **Jones, C. L., and S. A. Khan.** 1986. Nucleotide sequence of the enterotoxin B gene from *Staphylococcus aureus*. J. Bacteriol. **166:**29–33.
15. **Kent, T. H.** 1966. Staphylococcal enterotoxin gastroenteritis in rhesus monkeys. Am. J. Pathol. **48:**387–407.
16. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
17. **Lei, Z., R. F. Reiser, and M. S. Bergdoll.** 1988. Chromatofocusing in the purification of staphylococcal enterotoxin D. J. Clin. Microbiol. **26:**1236–1237.
18. **Metzger, J. F., A. D. Johnson, and W. S. Collins II.** 1972. Fractionation and purification of *Staphylococcus aureus* enterotoxin B by electrofocusing. Biochim. Biophys. Acta **257:**183–186.
19. **Peavy, D. L., W. H. Adler, and R. T. Smith.** 1970. The mitogenic effects of endotoxin and staphylococcal enterotoxin B on mouse spleen cells and human peripheral lymphocytes. J. Immunol. **105:**1453–1458.
20. **Ranelli, D. M., C. L. Jones, M. B. Johns, G. L. Mussey, and S. A. Kahn.** 1985. Molecular cloning of staphylococcal enterotoxin B gene in *Escherichia coli* and *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA **82:**5850–5854.
21. **Schantz, E. J., W. G. Roessler, J. Wagman, L. Spero, D. A. Dunnery, and M. S. Bergdoll.** 1965. Purification of staphylococcal enterotoxin B. Biochemistry **4:**1011–1016.
22. **Schantz, E. J., W. G. Roessler, M. J. Woodburn, J. M. Lynch, H. M. Jacoby, S. J. Silverman, J. C. Gorman, and L. Spero.** 1972. Purification and some chemical and physical properties of staphylococcal enterotoxin A. Biochemistry **11:**360–366.
23. **Sidberry, H., B. Kaufman, D. C. Wright, and J. Sadoff.** 1985. Immunoenzymatic analysis by monoclonal antibodies of bacterial lysopolysaccharides after transfer to nitrocellulose. J. Immunol. Methods **76:**299–305.
24. **Smith, B. G., and H. M. Johnson.** 1975. The effect of staphylococcal enterotoxins on the primary in vitro immune response. J. Immunol. **115:**575–578.
25. **Spero, L., J. R. Warren, and J. F. Metzger.** 1974. Microheterogeneity of staphylococcal enterotoxin B. Biochim. Biophys. Acta **336:**79–85.
26. **Thompson, N. E., M. J. Ketterhagen, and M. S. Bergdoll.** 1984. Monoclonal antibodies to staphylococcal enterotoxins B and C: cross-reactivity and localization of epitopes on tryptic fragments. Infect. Immun. **45:**281–285.
27. **Tweten, R. K., and J. Iandolo.** 1981. Purification and partial characterization of a putative precursor of staphylococcal enterotoxin B. Infect. Immun. **34:**900–907.
28. **Warren, J. R., L. Spero, and J. F. Metzger.** 1974. Isothermal denaturation of aqueous staphylococcal enterotoxin B by guanidine hydrochloride, urea and acid pH. Biochemistry **13:**1678–1683.
29. **Williams, R. T., C. T. Wehr, T. J. Rogers, and R. Bennett.** 1983. High-performance liquid chromatography of staphylococcal enterotoxin B. J. Chromatogr. **266:**179–186.