APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1990, p. 1067–1072
0099-2240/90/041067-06$02.00/0

Vol. 56, No. 4

Large-Scale Purification of Staphylococcal Enterotoxins A, B, and C_2 by Dye Ligand Affinity Chromatography

ROSSALYN D. BREHM,* HOWARD S. TRANTER, PETER HAMBLETON, AND JACK MELLING

Biologics Division, Center for Applied Microbiology and Research, Public Health Laboratory Service, Porton Down, Salisbury, Wiltshire SP4 0JG, England

Received 1 November 1989/Accepted 23 January 1990

A simple method for the purification of staphylococcal enterotoxins A (SEA), B (SEB), and C_2 (SEC_2) from fermentor-grown cultures was developed. The toxins were purified by pseudo-affinity chromatography by using the triazine textile dye "Red A" and gave overall yields of 49% (SEA), 44% (SEB), and 53% (SEC_2). The purified toxins were homogeneous when analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, but isoelectric focusing of the preparations revealed the microheterogeneity associated with these toxins. The SEA and SEB preparations each consisted of two isoelectric forms with pI values of 7.3 and 6.8 (SEA) and 8.9 and 8.55 (SEB); in contrast, SEC_2 contained five different isoelectric forms, with pI values ranging between 7.6 and 6.85. The pattern of elution of the isoelectric forms from the column indicated a cationic-exchange process involved in the binding of toxin to Red A. Such a method forms the basis of a high-yielding, rapid means of purifying the staphylococcal enterotoxins that can easily be adapted to large-scale production.

The staphylococcal enterotoxins, which cause staphylococcal food poisoning (27), are a group of seven extracellular, water-soluble proteins, designated A (10), B (10), C_1 (2), C_2 (2), C_3 (28), D (8), and E (3). They are single, unbranched polypeptide chains (molecular masses, 27 to 30 kilodaltons) which are highly resistant to physical and chemical treatments (4, 18, 20). The toxins are differentiated according to their reaction with specific antibodies, and this has led to the development of immunologically based assays (9, 17, 19, 23, 26, 30, 37, 38). Such assays require large amounts of purified toxins not only for use as standards in the assays but also for the production of homologous antisera.

Several procedures (Table 1) have been used to purify the staphylococcal enterotoxins A (SEA), B (SEB), and C_2 (SEC_2). Multistep procedures such as these are time consuming, generally give low yields of toxin, and cannot easily be adapted for large-scale purification.

Recently, the purification of SEA by a method in which a single chromatography step on a dye ligand matrix was used was reported by this laboratory (29). Dye ligand chromatography, a variant of affinity chromatography, uses synthetic textile dyes in place of a naturally immobilized ligand. These dyes frequently have no biological relation to the proteins to which they bind and are often referred to as "pseudo-ligands." The use of such dyes for large-scale affinity chromatography has several advantages over conventional affinity chromatography, including their ability to bind large amounts of protein and their resistance to chemical and enzymatic degradation. These properties mean that they can be used repeatedly with very little loss in binding capacity. In addition, they are relatively inexpensive. In the present study, the use of dye ligand chromatography has been extended to the purification of SEB and SEC_2 and to the large-scale production of types A, B, and C_2 toxins.

MATERIALS AND METHODS

Microorganisms. *Staphylococcus aureus* 722 and 361 were obtained from M. S. Bergdoll, Food Research Institute, Department of Food Science and Industry, University of Wisconsin, Madison, and were used for the production of toxins A and C_2, respectively. *S. aureus* S-6 (NCTC 10657) was used for the production of enterotoxin B. The cultures were stored (in culture medium plus 50% [vol/vol] glycerol) frozen in liquid nitrogen and were maintained on nutrient agar (Oxoid Ltd.) at 4°C as described previously (29).

Culture medium. The culture medium used for the production of the enterotoxins contained 20.0 g of Difco Proteose Peptone no. 3 (Difco Laboratories) per liter and 20.0 g of N-Z amine A (Sheffield Products) per liter. The medium was adjusted to pH 6.8 and sterilized by autoclaving (121°C for 20 min). Glucose and growth factors were added to the sterile medium as described previously (29).

Fermentation procedure. Ten liters of culture medium was sterilized (121°C for 20 min) in a 15-liter Biostat E fermentor (B. Braun Medical Ltd.). After sterilization, the temperature of the medium was reduced to 37°C and the dissolved oxygen concentration set at 20%, to be controlled by a combination of agitation and aeration (10 liters per min) as required. Occasionally a few drops of sterile polypropylene glycol 2000 antifoam (KW-Revai Chemicals Ltd.) was manually pumped into the fermentor vessel to reduce foaming. The pH of the culture was automatically controlled at 6.8 by the addition of 2 N HCl or 2 N NaOH. For each run, the fermentor was inoculated with 1 liter of seed inoculum previously prepared in two 2-liter flasks (29) and the culture was grown for 24 h, after which it was harvested into a 10-liter glass aspirator. The bacterial cells were removed, and the culture filtrate was concentrated to 0.5 to 1 liter by cross-flow filtration with the Pellicon system (Millipore UK Ltd.) as previously described (29). Finally, concentrated culture supernatant fluid was dialyzed overnight at 4°C against the appropriate molarity and pH of potassium phosphate buffer (Table 2).

Dye ligand affinity chromatography. A settled volume (500 ml) of "Red A" gel (Amicon Ltd.) was washed with 8 M urea containing 0.5 M NaOH, followed by equilibration buffer as described previously (29). The dialyzed culture filtrate (Table 2) was applied to the washed gel, packed into a column (4.5 by 31 cm; Wright-Amicon Ltd.). The column was washed with 6 to 8 volumes of equilibration buffer, and the

* Corresponding author.

TABLE 1. Examples of procedures used in the purification of SEA, SEB, and SEC_2

Toxin and source	Procedure	Recovery (%)
Enterotoxin A		
Chu et al. (12)	2 × CM-cellulose Sephadex G-100 Sephadex G-75	35
Schantz et al. (34)	CG-50 resin CM-cellulose Hydroxylapatite Sephadex G-75	20
Robern et al. (32)	2 × QAE Sephadex 2 × Sephadex G-100	30
Reynolds et al. (29)	Red A	55
Enterotoxin B		
Schantz et al. (33)	2 × CG-50 resin CM-cellulose	50–60
Ende et al. (16)	CG-50 resin Chromatofocusing Sephadex G-50	60
Enterotoxin C_2		
Avena and Bergdoll (1)	2 × CM-cellulose Sephadex G-75 Sephadex G-50	40
Robern et al. (32)	2 × QAE Sephadex 2 × Sephadex G-100	30

enterotoxin was eluted by using a stepwise increase in the molarity of phosphate buffer, for SEA and SEB, or KCl in phosphate buffer, in the case of SEC_2. Fractions were collected, and their protein content was estimated by measuring the A_{280} with a PU 8600 UV/VIS spectrophotometer (Pye-Unicam). The toxin content of these fractions was determined by radial immunodiffusion (29), and fractions containing enterotoxin were pooled. All chromatographic steps were performed at 4°C.

SDS-PAGE. The purity of the enterotoxin was determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) on gradient gels (PAA 4/30; Pharmacia Fine Chemicals) according to the instructions of the manufacturer (29). The gels were scanned (Chromoscan 3; Joyce Loebl) to determine the purity of the toxin preparations.

Isoelectric focusing. The pI of the enterotoxins was determined by isoelectric focusing on Ampholine PAG plate gels (pH 3.5 to 9.5; LKB Instruments Ltd.) as previously described (29).

RESULTS

Purification of SEA, SEB, and SEC_2 by dye ligand affinity chromatography. SEA, SEB, and SEC_2 were purified from fermentor-grown cultures, each by a single chromatography step on Red A. The amount of toxin per milliliter in the initial culture supernatants was 30 μg for SEA, 65 μg for SEB, and 83 μg for SEC_2, and the overall yields of purified toxins after concentration and chromatography were 49% (SEA), 44% (SEB), and 53% (SEC_2). SEA was eluted from the column as a large and small peak during the 150 mM phosphate buffer (pH 6.8) and 300 mM phosphate buffer (pH 6.8) washes, respectively (Fig. 1). SDS-PAGE analysis and gel scanning of the pooled fractions taken from the toxin peaks showed that the toxin was homogeneous (see Fig. 4), while that from the 300 mM peak was approximately 93% pure.

SEB was eluted as two peaks in the 60 mM phosphate buffer (pH 6.8; Fig. 2). Only the second, main peak was homogeneous (99%) by SDS-PAGE (see Fig. 4). The first toxin peak contained some impurities (5%) and was pooled and stored separately from the main peak.

SEC_2 was eluted as a single peak with 35 mM potassium chloride in 10 mM phosphate buffer (pH 6.5; Fig. 3). The majority of the peak was shown to be homogeneous by SDS-PAGE (100%; Fig. 4), but early fractions (440 to 490) taken from the toxin peak showed slight contamination (<10% by SDS-PAGE) and were pooled and stored separately from the pure fractions.

Isoelectric forms of SEA. When SEA was examined by isoelectric focusing, the enterotoxin was shown to consist of two isoelectric forms (Fig. 5), a major band (pI 7.3) and a minor band (pI 6.8). Analysis of the material from both the 150 and 300 mM phosphate buffer peaks showed that they both contained similar proportions of the two isoelectric forms.

Isoelectric forms of SEB. Generally, when material from the second peak of SEB was examined by isoelectric focusing, the enterotoxin was shown to consist of two isoelectric forms with pI values of 8.9 and 8.55 (Fig. 6). Material taken from the beginning of the toxin peak contained a major isoelectric form which focused at pH 8.9 and a minor band which focused at pH 8.55. Although analysis of fractions taken later in the toxin peak showed the presence of both isoelectric forms, the relative proportion of component 8.55 had increased compared with earlier fractions. Two other very minor forms having pI values of 9.1 and 9.2 sometimes were present in material at the end of the peak (data not presented).

Isoelectric forms of SEC_2. Material taken from the SEC_2 peak which had previously been shown to be homogeneous by SDS-PAGE contained at least five different components, with pI values ranging between 7.6 and 6.85 (Fig. 7).

TABLE 2. Purification of SEA, SEB, and SEC_2 by dye ligand chromatography with Red A

Entero-toxin	Production strain	Total toxin in initial culture (mg)	Total toxin after concentration and dialysis (mg)	Column equilibration and toxin-binding buffer (mM phosphate) (pH)	Toxin eluting buffer (pH)	Total toxin after chromatography (mg)	Total toxin after dialysis and concentration (mg)	Overall process yield (%)
SEA	FRI-722	240	200	20 (6.8)	150 mM phosphate (6.8)	157	118	49
SEB	NCTC 10657	552	463	5 (6.8)	60 mM phosphate (6.8)	265	240	44
SEC_2	FRI-361	747	536	10 (6.5)	35 mM KCl in 10 mM phosphate (6.5)	490	395	53

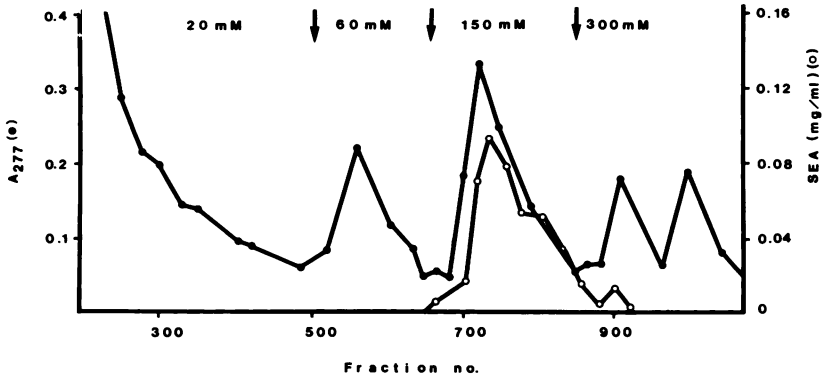

FIG. 1. Dye ligand affinity chromatography of SEA from 8.0 liters of *S. aureus* culture supernatant on Red A. The column (31 by 4.5 cm) of Red A was equilibrated in 20 mM potassium phosphate buffer (pH 6.8). The column was eluted with a stepwise increase in molarity of phosphate buffer (pH 6.8). Fractions (10 ml) were collected at a flow rate of 40 ml/h (during binding) and 200 ml/h (during elution).

Fractions taken from the early part of this peak contained a major band with a pI value of 6.85 and a minor band with a pI value of 7.1. In addition to these two forms, fractions from the middle of the peak contained two other major forms with pI values of 7.45 and 7.55, and a minor form which focused at pI 6.9. Analyses of fractions taken from the end of the toxin peak demonstrated the presence of all of these bands, with component 7.45 as the major form.

DISCUSSION

The use of single-step dye ligand chromatography, previously described for the purification of SEA (29), has now been used to obtain SEB and SEC_2 of a high degree of purity (>99%), with a final overall yield in excess of 43%.

Before beginning the purification process, it is necessary to reduce the volume of the crude toxin-containing supernatant. Dialysis against Carbowax (12) is not practical for large volumes, and acid precipitation is also impractical because the acid may denature the toxin (5). Batch adsorption of the toxin onto an ion-exchange resin, followed by transfer to a chromatography column for elution (34, 35), is widely used, although it is necessary to dilute the culture supernatant to lower its ionic strength (6), thus increasing the already large volumes. In this laboratory, enterotoxins are routinely purified from up to 30 liters of culture supernatant fluid by using tangential flow filtration to concentrate the fluid, which allows large volumes of culture to be processed rapidly and efficiently with a minimum of inconvenience. The major advantage of this purification procedure is that it can be used for the purification of all three toxins with only a slight variation in the buffers used. Initial small-scale studies using phosphate buffer containing different concentrations of KCl to elute SEC_2 from the dye ligand were successfully transcribed to larger-scale operations. We have no reason to suppose that SEC_2 would not be eluted at higher phosphate ion concentrations, but this has not been investigated. The dye ligands are readily available and relatively inexpensive; they generally have high protein-binding capacities and are resistant to chemical and enzymatic degradation, thus enabling them to be reused many times.

A number of theories for the binding of dye ligands to proteins in general have been suggested. One such possibility is that the chromophore moiety of the dye molecule is structurally analagous to the natural biological binding site of

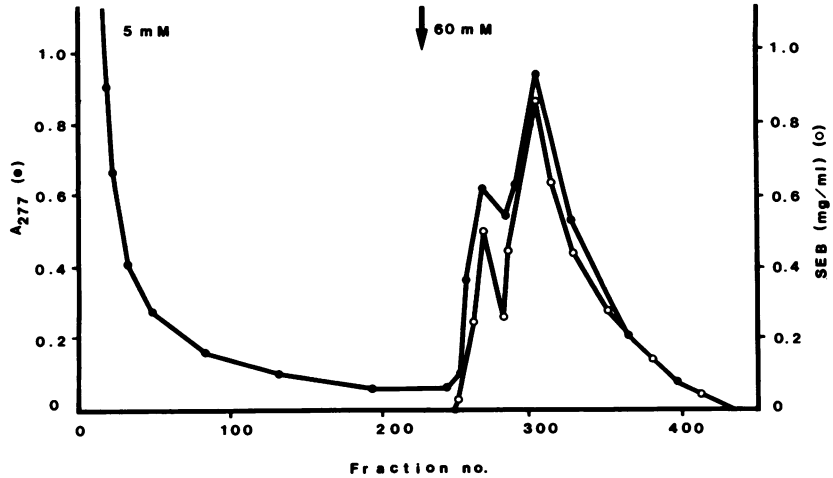

FIG. 2. Dye ligand affinity chromatography of SEB from 8.5 liters of *S. aureus* culture supernatant on Red A. The column (31 by 4.5 cm) of Red A was equilibrated in 5 mM potassium phosphate buffer (pH 6.8). The column was eluted with a stepwise increase in molarity of phosphate buffer (pH 6.8). Fractions (10 ml) were collected at a flow rate of 40 ml/h (during binding) and 200 ml/h (during elution).

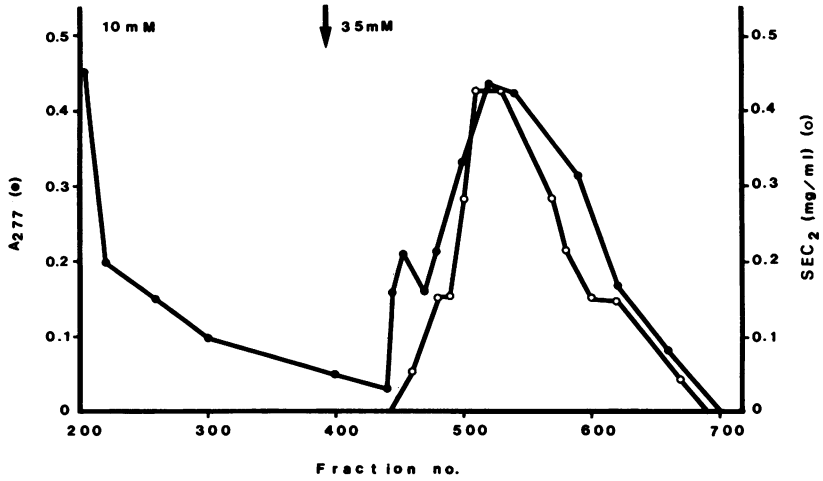

FIG. 3. Dye ligand affinity chromatography of SEC_2 from 9.0 liters of *S. aureus* culture supernatant on Red A. The column (31 by 4.5 cm) of Red A was equilibrated in 10 mM potassium phosphate buffer (pH 6.5). The column was eluted with a stepwise increase in molarity of KCl in 10 mM phosphate buffer (pH 6.5). Fractions (10 ml) were collected at a flow rate of 40 ml/h (during binding) and 200 ml/h (during elution).

the protein, and it is this that is responsible for the specific interactions (7, 13, 22). Edwards and Woody (15) proposed that since the dyes were aromatic compounds, as such they could be capable of binding proteins which possess a hydrophobic pocket in their surface structure. Another theory suggests that the specificity of the dye-protein interaction depends primarily on hydrophobic interactions, whereas electrostatic forces contribute to the stability of the complex (21). The present data suggest that the binding of the staphylococcal enterotoxins to Red A is likely to be due to cation exchange, because the pH of the buffer in which the toxin binds is lower than the isoelectric points of the toxins eluted from the column. If the pH of the binding buffer is increased above the pI of the toxins, they do not bind. If more specific interactions were involved, one would expect the ligand-toxin complex to remain stable over a range of pH values. The sulfonic acid residues found in the Procion Red HE-3B molecule (Fig. 8) may enable the Red A to act as a weak cation exchanger. This mechanism can also be seen in the binding of muscle pyruvate kinase to Cibacron Blue F3G-A and Procion Red HE-3B (21).

Isoelectric focusing of the enterotoxins purified by Red A revealed the microheterogeneity in the toxin preparations that has already been reported by several other groups (11, 14, 16, 24, 29, 31, 34, 36). By this method, SEA had pI values of 7.3 and 6.8, which have also been observed by Robern et al. (31) and Schantz et al. (34). Other isoelectic forms of SEA with pI values between 8.6 and 7.6, which were reported by the same authors as well as Reynolds et al. (29), were not found.

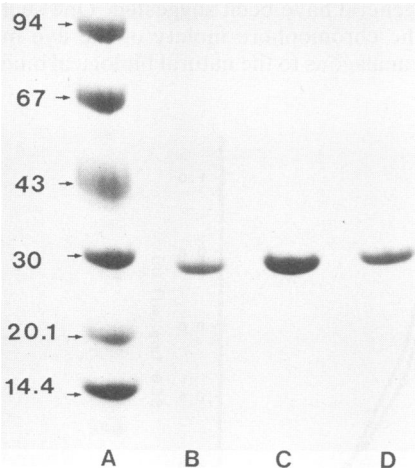

FIG. 4. SDS gradient-PAGE of SEA, SEB, and SEC_2. Lane A, Molecular mass markers (in kilodaltons): phosphorylase *b*, 94; albumin, 67; ovalbumin, 43; carbonic anhydrase, 30; trypsin inhibitor, 20.1; and α-lactalbumin, 14.4. Lane B, SEA eluted from a Red A column with 150 mM phosphate buffer (pH 6.8). Lane C, SEB eluted from a Red A column with 60 mM phosphate buffer (pH 6.8). Lane D, SEC_2 eluted from a Red A column with 35 mM KCl in 10 mM phosphate buffer (pH 6.5). Lanes B through D were loaded with 20 μl of sample containing 0.5 to 1.0 mg of total protein per ml.

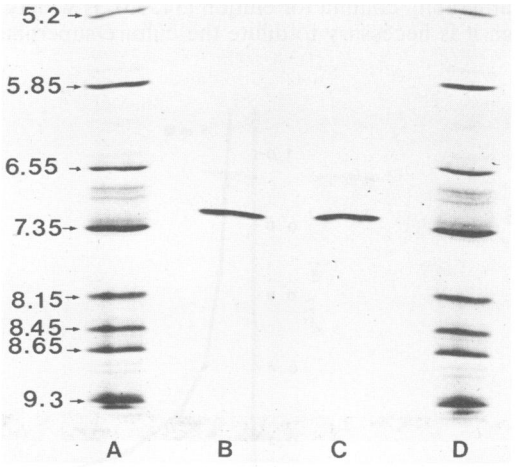

FIG. 5. Isoelectric focusing of SEA. Lanes A and D, pI markers: trypsinogen, 9.3; lentil lectin (basic band), 8.15; horse myoglobin (basic band), 7.35; human carbonic anhydrase B, 6.55; bovine carbonic anhydrase B, 5.85; and β-lactoglobulin A, 5.2. Lane B, Material from the first peak of SEA eluted from a Red A column with 150 mM phosphate buffer (pH 6.8). Lane C, Material from the second peak of SEA eluted from a Red A column with 300 mM phosphate buffer (pH 6.8).

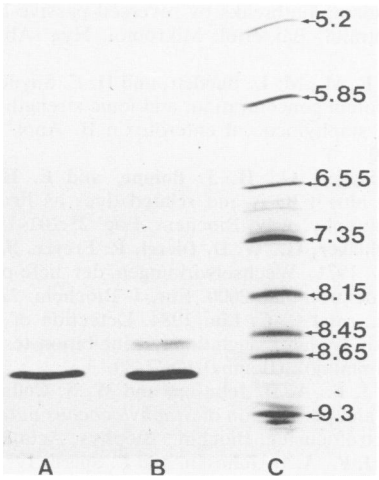

FIG. 6. Isoelectric focusing of SEB. Lane A, Material (fraction 350) from the early part of the main peak of SEB eluted from a Red A column with 60 mM phosphate buffer (pH 6.8). Lane B, Material (fraction 390) from the latter part of this peak. Lane C, pI markers as detailed for Fig. 5.

The absence of the more basic forms of SEB was also evident after isoelectric focusing of the toxin preparations purified by Red A. Toxin purified in this way gave pI values of 8.55, 8.9, 9.1, and 9.2. While the 8.55 and 9.1 forms probably correspond to the 8.55 (11, 16) and 9.05 (24, 36) observed by other workers, the more basic forms of 9.4, 9.56, and 9.6 observed by the same workers were not present in the preparations described here.

The microheterogeneity of SEC_2 observed by Dickie et al. (14) was confirmed by isoelectric focusing of the SEC_2 purified by Red A. The previous workers separated SEC_2 into as many as eight components with pI values of 7.35, 6.95, 6.75, 6.55, 6.2, 5.95, 5.7, and 5.5. A similar range of isoelectric forms were demonstrated by SEC_2 purified by Red A (7.55, 7.45, 7.1, 6.9, and 6.85). However, the more

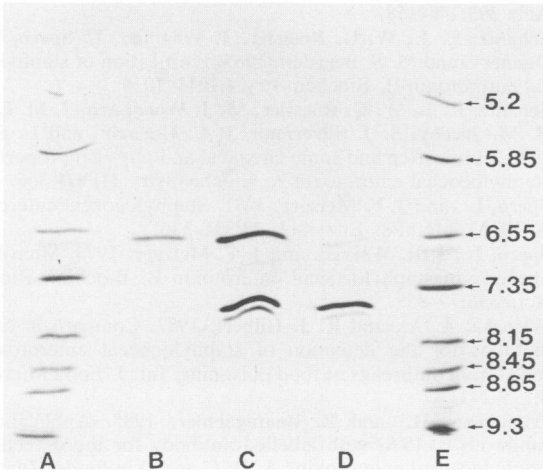

FIG. 7. Isoelectric focusing of SEC_2. Lanes A and E, pI markers as detailed for Fig. 5. Lane B, Material (fractions 440 to 490) from the early part of the peak of SEC_2 eluted from a Red A column with 150 mM phosphate buffer (pH 6.5). Lane C, Material from the middle of this peak (fractions 490 to 547). Lane D, Material from the end of this peak (fractions 547 to 690).

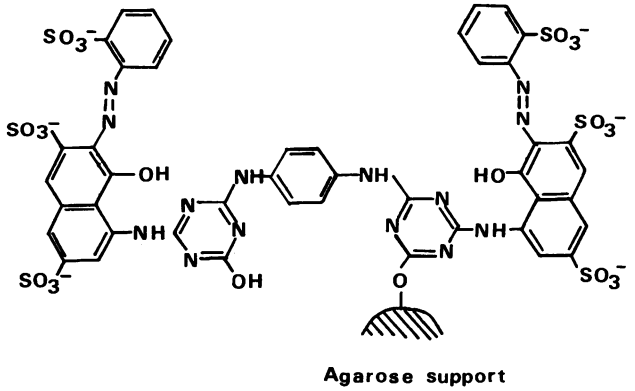

FIG. 8. The structure of Matrex Gel Red A, including the chemical structure of the dye ligand Procion Red HE-3B.

acidic forms below pI 6.5 would not be expected to bind to the dye ligand column under the running conditions used in this work and may have been washed through the column in the initial washing stage, although toxin was not detected in the washings and only 8% of the toxin loaded onto the column could not be accounted for. Again, the more basic forms of this toxin that have been reported by Metzger et al. (8.4 and 8.1 [25]) were not detected here.

In general, the profiles of the different isoelectric forms of the toxins eluted from the dye ligand column are consistent with the separation being a cationic-exchange process. However, the vastly increased purity of the enterotoxin preparations after one chromatographic step on Red A compared with an identical step on carboxymethyl (CM)-cellulose indicates that a mixture of interactions may be involved. Slight differences in the isoelectric points of the different forms observed between laboratories can be explained by differences in the isoelectric focusing methods used together with differences in the handling of the toxins and the running conditions used. For example, temperature and pH have both been shown to be important in the conversion of isoelectric forms of these toxins (36). However, the absence of any of the highly basic forms of the enterotoxins is difficult to explain. It is perhaps noteworthy that toxin production in this work was by fermentation and not shake-flask culture, as in most other reports, and that the profile of isoelectric forms may differ between the two systems. Indeed Metzger et al. (25) demonstrated that the basic forms of SEC_2 disappeared after continued fermentation of the toxin-producing strain for 72 h. These authors postulated that the conversion of basic to acidic forms was related to the production of a bacterial deamidase during fermentation, since the same conversion could not be repeated after alkaline treatment of the purified toxin.

The purification of SEA, SEB, and SEC_2 by the method reported here is a simple alternative to currently used procedures. The use of one chromatographic step on Red A for the purification of all three toxins (under slightly different conditions), together with its use in large-scale and small-scale purification, is a choice rarely found elsewhere. Recent work indicates that this purification procedure can be scaled up even further; indeed, as much as 0.8 g of SEA has been purified from a single 30-liter fermentor culture. This laboratory has used the dye ligand method under different conditions for the successful purification of small amounts of staphylococcal enterotoxins C_1 and E. Work is currently being undertaken to extend this method to the purification of

larger amounts of these toxins and to adapt this method for the purification of staphylococcal enterotoxin D.

ACKNOWLEDGMENTS

We thank R. Rhind-Tutt and H. Sheeley for skilled technical assistance with the fermentor work.

LITERATURE CITED

1. **Avena, R. M., and M. S. Bergdoll.** 1967. Purification and some physicochemical properties of enterotoxin C from *Staphylococcus aureus* strain 361. Biochemistry **6**:1474–1480.
2. **Bergdoll, M. S., C. R. Borja, and R. M. Avena.** 1965. Identification of a new enterotoxin as enterotoxin C. J. Bacteriol. **90**:1481–1485.
3. **Bergdoll, M. S., C. R. Borja, R. N. Robbins, and K. F. Weiss.** 1971. Identification of enterotoxin E. Infect. Immun. **4**:593–595.
4. **Bergdoll, M. S., I.-Y. Huang, and E. J. Schantz.** 1974. Chemistry of the staphylococcal enterotoxins. J. Agric. Food Chem. **22**:9–13.
5. **Bergdoll, M. S., H. Sugiyama, and G. M. Dack.** 1959. Staphylococcal enterotoxin I. Purification. Arch. Biochem. Biophys. **85**:62–69.
6. **Bergdoll, M. S., H. Sugiyama, and G. M. Dack.** 1961. The recovery of staphylococcal enterotoxin from bacterial culture supernatants by ion exchange. J. Biochem. Microbiol. Technol. Eng. **3**:41–50.
7. **Bohme, H. J., G. Kopperschlager, J. Schulz, and E. Hofmann.** 1972. Affinity chromatography of phosphofructokinase using cibacron blue F3G-A. J. Chromatogr. **69**:209–214.
8. **Casman, E. P., R. W. Bennett, A. E. Dorsey, and J. A. Issa.** 1967. Identification of a fourth staphylococcal enterotoxin, enterotoxin D. J. Bacteriol. **94**:1875–1882.
9. **Casman, E. P., R. W. Bennett, A. E. Dorsey, and J. E. Stone.** 1969. The microslide gel double diffusion test for the detection and assay of staphylococcal enterotoxin. Health Lab. Sci. **6**:185–198.
10. **Casman, E. P., M. S. Bergdoll, and J. Robinson.** 1963. Designation of staphylococcal enterotoxins. J. Bacteriol. **85**:715–716.
11. **Chang, P. C., and N. Dickie.** 1971. Fractionation of staphylococcal enterotoxin B by isoelectric focusing. Biochim. Biophys. Acta **236**:367–375.
12. **Chu, S. F., K. Thadhani, E. J. Schantz, and M. S. Bergdoll.** 1966. Purification and characterization of staphylococcal enterotoxin A. Biochemistry **5**:3281–3289.
13. **Cottreau, D., M. J. Levin, and A. Kahn.** 1979. Purification and partial characterisation of different forms of phosphofructokinase in man. Biochim. Biophys. Acta **568**:183–194.
14. **Dickie, N., Y. Yano, H. Robern, and S. Stavric.** 1972. On the heterogeneity of staphylococcal enterotoxin C_2. Can. J. Microbiol. **18**:801–804.
15. **Edwards, R. A., and R. W. Woody.** 1979. Spectroscopic studies of cibacron blue and Congo red bound to dehydrogenases and kinases. Evaluation of dyes as probes of the dinucleotide fold. Biochemistry **18**:5197–5204.
16. **Ende, I. A., G. Terplan, B. Kickhofen, and D. K. Hammer.** 1983. Chromatofocusing: a new method for purification of staphylococcal enterotoxins B and C_1. Appl. Environ. Microbiol. **46**: 1323–1330.
17. **Fey, H., H. Pfister, and O. Rüegg.** 1984. Comparative evaluation of different enzyme-linked immunosorbent assay systems for the detection of staphylococcal enterotoxins A, B, C, and D. J. Clin. Microbiol. **19**:34–38.
18. **Fung, D. Y. C., D. H. Steinberg, R. D. Millet, M. J. Kurantnick, and T. F. Murphy.** 1973. Thermal inactivation of staphylococcal enterotoxins B and C_1. Appl. Microbiol. **26**:938–942.
19. **Igarashi, H., N. Shingaki, H. Fujikawa, H. Ushioda, and T. Terayama.** 1985. Detection of staphylococcal enterotoxins in food poisoning outbreaks by reversed passive latex agglutination. Zentralbl. Bakteriol. Mikrobiol. Hyg. Abt. I Suppl. **14**: 255–257.
20. **Jamlang, E. M., M. L. Bartlett, and H. E. Snyder.** 1971. Effect of pH, protein concentration, and ionic strength on heat inactivation of staphylococcal enterotoxin B. Appl. Microbiol. **22**: 1034–1040.
21. **Kopperschlager, G., H. J. Bohme, and E. Hofmann.** 1982. Cibacron blue F3G-A and related dyes as ligands in affinity chromatography. Adv. Biochem. Eng. **25**:101–138.
22. **Kopperschlager, G., W. D. Diezel, R. Freyer, S. Liebe, and E. Hofmann.** 1971. Wechselwirkungen der hefe-phosphofrutokinase mit dextran blau 2000. Eur. J. Biochem. **22**:40–45.
23. **Lee, C.-L., and C.-C. Lin.** 1984. Detection of staphylococcal enterotoxin by latex agglutination inhibition test. Chin. J. Microbiol. Immunol. (Beijing) **17**:77–80.
24. **Metzger, J. F., A. D. Johnson, and W. S. Collins.** 1972. Fractionation and purification of *Staphylococcus aureus* enterotoxin B by electrofocusing. Biochim. Biophys. Acta **257**:183–186.
25. **Metzger, J. F., A. D. Johnson, and L. Spero.** 1975. Intrinsic and chemically produced microheterogeneity of *Staphylococcus aureus* enterotoxin type C. Infect. Immun. **12**:93–97.
26. **Meyer, R. F., and M. J. Palmieri.** 1980. Single radial immunodiffusion method for screening staphylococcal isolates for enterotoxin. Appl. Environ. Microbiol. **40**:1080–1085.
27. **Public Health Laboratory Service Communicable Disease Surveillance Centre and Office of Population Censuses and Surveys.** 1985. Annual review of communicable diseases. Public Health Laboratory Service Communicable Disease Surveillance Centre and Office of Population Censuses and Surveys, Wiltshire, England.
28. **Reiser, R. F., R. N. Robbins, A. L. Noleto, G. P. Khoe, and M. S. Bergdoll.** 1984. Identification, purification, and some physicochemical properties of staphylococcal enterotoxin C_3. Infect. Immun. **45**:625–630.
29. **Reynolds, D., H. S. Tranter, R. Sage, and P. Hambleton.** 1988. Novel method for the purification of staphylococcal enterotoxin A. Appl. Environ. Microbiol. **54**:1761–1765.
30. **Robbins, R., S. Gould, and M. S. Bergdoll.** 1974. Detecting the enterotoxigenicity of *Staphylococcus aureus* strains. Appl. Microbiol. **28**:946–950.
31. **Robern, H., S. Stavric, and N. Dickie.** 1974. Fractionation of staphylococcal enterotoxin A by isoelectric focusing. Experientia **30**:1087–1089.
32. **Robern, H., S. Stavric, and N. Dickie.** 1975. The application of QAE-Sephadex for the purification of two staphylococcal enterotoxins. I. Purification of enterotoxin C_2. Biochim. Biophys. Acta **393**:148–158.
33. **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.
34. **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.
35. **Spero, L., and J. F. Metzger.** 1981. Staphylococcal enterotoxin A (SEA). Methods Enzymol. **78**:331–336.
36. **Spero, L., J. R. Warren, and J. F. Metzger.** 1974. Microheterogeneity of staphylococcal enterotoxin B. Biochim. Biophys. Acta **336**:79–85.
37. **Wieneke, A. A., and R. J. Gilbert.** 1987. Comparison of four methods for the detection of staphylococcal enterotoxin in foods from outbreaks of food poisoning. Int. J. Food Microbiol. **4**:135–143.
38. **Windeman, H., and E. Baumgartner.** 1985. Application of sandwich ELISA, with labelled antibody for the detection of staphylococcal enterotoxins A, B, C and D in foods. Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. B **181**:345–363.