Chromatofocusing in the Purification of Staphylococcal Enterotoxin D

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A chromatofocusing procedure for the purification of staphylococcal enterotoxin D was developed. The purification included the removal of the toxic protein from culture supernatant fluids of Staphylococcus aureus 1151m by batch adsorption with CG-50 resin, chromatofocusing on Polybuffer Exchanger 94, and gel permeation chromatography on Sephacryl S-200. The purity of the staphylococcal enterotoxin D obtained was approximately 98%.

Staphylococcus aureus produces a group of enterotoxins (A, B, C1, C2, C3, D, and E) (1) that share numerous biological and biochemical properties. Any of the enterotoxins may be involved in staphylococcal food poisoning; however, enterotoxin A has been involved in a majority of the outbreaks, with enterotoxin D (SED) as the second most important one.

Of the enterotoxins, SED is the most difficult to purify, because it is produced in much smaller amounts than any of the other enterotoxins. The five-step purification procedures now in use to obtain purified SED are long and tedious (2). Chromatofocusing has been used in the partial purification of the other enterotoxins (3, 4) but has not been employed in the purification of SED. The application of chromatofocusing in the purification of SED resulted in the production of SED with a high degree of purity in three steps.

Step 1. The Amberlite CG-50 resin adsorption technique of Reiser et al. (7) was utilized for step 1. Twelve liters of the supernatant fluid (8) from staphylococcal strain 1151m (2) was adjusted to pH 5.6 with 6 N HCl and diluted to 50 liters with distilled water. Packed resin (250 ml, wet volume) equilibrated with 5 mM sodium phosphate buffer (pH 5.6) was added, and the mixture was stirred for 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 (10 by 30 cm) and washed with 500 ml of distilled water. The proteins including SED were eluted from the resin with 0.5 M sodium phosphate buffer containing 0.5 M NaCl (pH 6.2) at a flow rate of 100 ml/h. The eluate was concentrated approximately fourfold by dialysis against Carbowax, with further dialysis against 5 mM Tris buffer (pH 9.4) to prepare it for chromatofocusing.

The SED was approximately 14% pure (2, 5).

Step 2. The chromatofocusing column (Polybuffer Exchanger 94; 1.6 by 26 cm; Pharmacia Fine Chemicals, Piscataway, N.J.) was treated with 5 mM Tris hydrochloride buffer containing 1 M NaCl at pH 5.4 (about 500 ml) and equilibrated with 5 mM Tris (pH 9.4) until the pH of the effluent was 9.4. The dialyzed, concentrated SED from step 1 (approximately 300 to 350 mg of protein) was applied to the column. The column was washed with 500 to 1,000 ml of 5 mM Tris (pH 9.4), followed by the elution of SED with Buffalyte 8-4 (dilution, 1:50; pH 5.4) at a flow rate of 28 ml/h. The pH of the eluate was measured immediately at room temperature to determine the pH gradient. The peak eluted at 345 to 375 ml contained the highest concentration of SED. The pH range for the elution of this peak was from 7.1 to 6.8. Peaks eluted at 143 to 160 ml (pH 7.6 to 7.4), 204 to 210 ml (pH 7.4 to 7.2), 493 to 529 ml (pH 6.4 to 6.2), and 655 to 689 ml (pH 6.3 to 6.1) contained small amounts of SED. The peak containing the highest concentration of SED (345 to 375 ml) was collected and concentrated with Carbowax to approximately 15 mg of protein per ml for gel permeation chromatography. The purity of SED was approximately 31%.

Step 3. A column of Sephacryl S-200 (2.5 by 118 cm) was washed overnight with 0.05 M phosphate buffer (pH 6.8) containing 1 M NaCl at a flow rate of approximately 35 ml/h. The concentrated SED from step 2 (3.5 ml, 13 mg of protein) was applied to the column and eluted with the 0.05 M phosphate buffer at a flow rate of 35 ml/h. Essentially all of the SED was contained in fractions 117 to 130, with a small amount eluted much later after fraction 165. Fractions 117 to 130 were combined, dialyzed against 5 mM phosphate buffer (pH 6.8), and lyophilized.

The purity of the SED was estimated to be at least 98% on the basis that no extraneous bands were visible in disc sodium dodecyl sulfate gel electrophoresis when 50 µg of protein was applied. Immunodiffusion by the optimum sensitivity plate method (9) with concentrations of SED as high as 200 µg showed only one minor line with antiserum produced against crude SED (10). No precipitin lines were observed when the purified SED was reacted with serum prepared against a nonenterotoxigenic staphylococcal strain (FRI-184). The molecular weight of SED was determined to be 28,500 by disc sodium dodecyl sulfate gel electrophoresis (6, 7). The isoelectric point was determined to be 7.6 by isoelectric focusing (8). Immunoblotting clearly demonstrated the specific reactivity of purified SED with antiserum D (8).

Although the percent recovery of SED as highly purified toxin was low (15.5%), the three-step procedure proved to be much shorter and simpler than the original procedure worked out by Chang and Bergdoll (2). The low recovery relates directly to the amount of SED present in the culture filtrate fluids. The properties of the SED purified by this procedure were within experimental error of those reported earlier (2).

Because of the increased interest in many countries in the purification of the enterotoxins, the method reported here for the purification of SED should prove valuable to those attempting its purification.
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LITERATURE CITED


