Isolation and Identification of Two Hemolytic Forms of Streptolysin-O

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Streptolysin-O was isolated from culture supernatants of group-A β-hemolytic streptococci (Richards strain) by ammonium sulfate and polyethylene glycol precipitation, DEAE-ion exchange chromatography, preparative isoelectric focusing, and chromatography on Sephacryl S-300. Two forms of the toxin possessing similar hemolytic capacity were identified. The native toxin was a single polypeptide chain devoid of amino sugars with a sedimentation coefficient of 3.9S and a molecular weight of 69,000, and was isoelectric at pH 6.0 to 6.4. Partial degradation of the native toxin occurred during the isolation procedure, yielding a hemolytically active polypeptide with a molecular weight of 57,000 and a pI of 7.0 to 7.5. Both forms of the toxin generated the typical, heterogeneous, open and closed ring-structured channels in erythrocyte membranes. Structural considerations indicated that between 25 and 100 monomer toxin molecules constituted the individual ultrastructurally recognizable channels. Hemolytic titrations indicated that the presence of 70 to 125 toxin molecules per erythrocyte was required to generate an average of one functional lesion per cell. The data are consistent with the concept that one or very few streptolysin-O channels will cause hemolysis.

Streptolysin-O (SLO) is the prototype of sulfhydryl-activated bacterial cytolysins, a group comprising at least 15 exotoxins that share several properties in common (1, 3, 4, 21; J. E. Alouf and C. Geoffroy, in J. E. Alouf and J. Jeljaszewicz, ed., Bacterial Protein Toxins, in press). All are reversibly inactivated through oxidation; reactivation occurs in the presence of reducing reagents such as dithiothreitol. The toxins interact with membrane cholesterol and exert cytolytic-cytotoxic effects on a broad spectrum of mammalian cells. In many cases, cytolysis has been shown to be accompanied by the occurrence of circular and semicircular structures on the target membranes (3, 8, 10, 21). Until recently, it was thought that membrane damage ensued as the result of cholesterol rearrangement and disruption of the bilayer and that the (semi)circular structures viewed in the electron microscope represented complexes of the toxin with tightly bound cholesterol (1, 3, 10). However, after the isolation of extensively delipidated SLO ring structures and their reconstitution into bilayers of pure lecithin, it became apparent that cholesterol, although representing the primary toxin acceptor, could not itself substantially contribute to formation of the ring structures, which were identified as supramolecular polymers of the toxin (5). These results, moreover, indicated that the SLO polymers generated very large transmembrane channels and allowed SLO to be classified as a member of the channel-forming proteins (S. Bhakdi and J. Tranum-Jensen, Philos. Trans. Roy. Soc. Lond. B. Biol. Sci., in press). Studies by Duncan and Buckingham stand in excellent agreement with this view (6, 9). These authors reported that membranes damaged by SLO harbored extremely large functional pores (effective diameter, >15 nm), consistent with the diameter of SLO "lesions" observed by electron microscopy (30 to 40 nm) (6). Hemoglobin would directly escape through such channels, and this would explain why SLO-dependent hemolysis does not follow the characteristics of osmotic lysis (9), as found for complement (11, 19) and staphylococcal α-toxin-induced hemolysis.

Although the membrane form of SLO can be easily purified (5), the isolation of native toxin still poses serious problems. Many publications have described isolation protocols for the toxin (e.g., 1, 2, 7, 12, 16, 20, 25, 26), but a full consensus regarding such basic parameters as molecular weight, isoelectric point, and hemolytic capacity of the toxin is wanting. There are also no definitive reports on the amino acid composition of the toxin. Alouf (1) has excellently reviewed the present state of knowledge in this area. In brief, the majority of investigators place the M r of SLO in the range of either 60,000 to 70,000 (1, 20, 25) or 53,000 to 58,000 (2, 7), and the pI is placed around 6.0 to 6.4 (2, 20, 22) or 7.0 to 7.5 (2, 12, 20, 22; Alouf and Geoffroy, in press) or both. The sedimentation coefficient has been reported to be 3.7S (25). The possible presence of two distinct forms of the toxin that could account for these findings has been anticipated by Alouf (1), but certainty regarding this point is lacking because of the difficulties encountered in the isolation of the SLO stemming from the small quantities produced in culture on the one hand and from the proteolytic degradation occurring during the isolation procedures on the other.

In this paper, we present a method for SLO isolation from bacterial culture supernatants by classical techniques of protein purification. We identify and characterize two hemolytically active forms of the toxin and show that one form derives from partial proteolytic degradation of the native toxin. Hemolytic assays support the contention that the generation of a single toxin channel suffices to cause lysis of a target erythrocyte.

MATERIALS AND METHODS

Bacterial culture. Four liters of brain heart infusion broth were inoculated with 50 to 100 ml of an overnight culture of group-A streptococci, Richards strain. After 3 h of incubation at 37°C, the pH of the culture and toxin titers were determined hourly. When the pH dropped to ~6.0, the broth was supplemented with 1% glucose, and the pH was adjusted to 7.0 to 7.2 with 1 N NaOH every hour. Peak hemolytic titers were obtained after a total of 7 to 10 h of incubation, and the cultures were then placed overnight in a cold room (4°C). Bacteria were subsequently sedimented in a Sorvall
GS-3 centrifuge (rotor HG-4; 4-liter capacity, 7,000 × g for 30 min, 4°C), and the culture supernatants were concentrated to 400 to 500 ml through continuous ultrafiltration in a filtration chamber, model SM 17112 from Sartorius (Gottingen, Federal Republic of Germany) by using a type SM 16566 membrane (exclusion limit, molecular weight of 20,000) at 4°C. This procedure required ca. 4 h. The samples were then centrifuged again (Sorvall centrifuge RC 2B, rotor GSA; 17,000 × g for 30 min) to remove residual cells.

Toxin isolation. For inhibition of proteases, the culture supernatant was made 1 mM in phenylmethylsulfonyl fluoride and 10 mM in EDTA. Solid ammonium sulfate (53 g) was then added per 100 ml of solution, and the samples were stirred in the cold for 60 to 90 min. The precipitate was collected by centrifugation (Sorvall RC 2B centrifuge, rotor GSA; 17,000 × g for 30 min), and the supernatant was discarded. The precipitate was dissolved in distilled water (final volume, 60 to 80 ml) and dialyzed overnight against 5 liters of 50 mM NaCl-4 mM EDTA (pH 7.0) in the cold.

PEG precipitation. Solid PEG 4000 (E. Merck AG, Darmstadt, Federal Republic of Germany) was added to the toxin preparation (25 g/100 ml) and stirred at 4°C for 30 min. After centrifugation (Sorvall RC 2B centrifuge, rotor SS 34; 30,000 × g for 60 min), the supernatant was discarded, and the PEG precipitate was dissolved in ca. 10 ml of 25 mM veronal buffer (pH 7.0) containing 50 mM NaCl and 3 mM NaN3.

DEAE-ion exchange chromatography. The toxin preparation was next passed rapidly through a 70-ml DEAE-Sephasel (Pharmacia, Uppsala, Sweden) column (2.6 by 14 cm; flow rate, 50 ml/h; 4°C) equilibrated in the above described veronal-NaCl buffer. The bulk of the contaminating substances adhered to the column, whereas SLO passed the column and was collected in a total of 120 ml after a column wash with 100 to 110 ml of buffer. The now clear and colorless solution was concentrated over a PM 10 Amicon ultrafiltration membrane (200-ml chamber) to ca. 10 ml, dialyzed overnight at 4°C against 1 liter of 10 mM Tris-hydrochloride (pH 9.0)—10 mM NaCl-3 mM NaN3 and applied to a 50-ml DEAE-Sephasel column (2.6 by 10 cm) equilibrated in the same buffer. The column was washed with 50 ml of buffer and eluted with 300 ml of a 50 to 200 mM NaCl gradient at pH 9. Fractions containing SLO as assayed by hemolytic titrations were pooled and dialyzed for 6 to 16 h against 0.1% (vol/vol) glycerol at 4°C for subsequent isoelectric focusing.

Preparative isoelectric focusing. This was performed in a 110-ml LKB preparative isoelectric focusing column in 10 to 50% (wt/vol) sucrose density gradients with pH 3.5 to 10.0 Ampholines (LKB, Uppsala, Sweden). The SLO sample was admixed to both sucrose solutions, and the gradients were prepared with the LKB gradient mixer. Isoelectric focusing was performed at 4°C for 16 to 20 h under a constant field strength of 3 W. The first 15 ml of the gradient was then discarded, and 2-ml fractions were collected.

Sepharcl gel chromatography. The SLO-containing fractions obtained after isoelectric focusing were pooled, diluted with 5 volumes of 20 mM Tris-hydrochloride (pH 8.2)—100 mM NaCl—3 mM NaN3, and concentrated by ultrafiltration to 1 to 1.5 ml. They were then applied to a Sephacryl S-300 column (Pharmacia; 1 by 60 cm), and fractions were collected at a flow rate of 4 ml/h. This step removed most of the remaining contaminants and the Ampholines. Toxin-containing fractions were pooled, given 1 mM phenylmethylsulfonyl fluoride and 10 mM EDTA, and stored at −20°C, where they remained stable over periods of at least 4 to 8 weeks.

Ultracentrifugal analyses. Analyses were performed in a model E apparatus equipped with a UV-Vis monochromator, scanner, and multiplexer for use of the multicell rotor, type AN-F Ti, with double-sector cells (Beckman Instruments, Inc., Fullerton, Calif.). Sedimentation velocity experiments were run for estimation of homogeneity of the preparations by the band-forming method of Kegeles (14) and Vinograd et al. (27). For high-speed performance (up to 60,000 rpm) aluminum double-sector cells were modified and experimental conditions were standardized as described by Kranz and Schmidt (15). Molecular weight runs were performed at 12,000 rpm and at 20°C by the method of Yphantis (28). Both ultracentrifugal experiments were recorded and evaluated by on-line computer coupling (type HP 1000; Hewlett-Packard, Palo Alto, Calif.).

Amino acid analyses. Amino acid analyses were performed with an automatic analyzer (Chromakon 500; Kontron, Zurich, Switzerland) equipped with an Anacomp 220 computer (Kontron).

Hemolytic titrations. All titrations were performed with suspensions of 2.5 × 10⁸ rabbit erythrocytes per ml of phosphate-buffered saline (pH 7.4). For rapid assays used to monitor the toxin isolations, titrations were done in phosphate-buffered saline, and the titers were defined by visual reading of the last dilution wells (Titertek microtiter plates) yielding 100% hemolysis. Before dilution, toxin preparations were activated with 10 mM diethiothreitol for 5 min at 22°C. It was of essence that the microcassette tips were regularly renewed after every sixth well to avoid artifactual overestimates of the hemolytic titers. Titrations were performed by using 50 μl of serially diluted toxin incubated with 50 μl of erythrocyte suspensions for 60 min at 37°C. For precise determinations of the hemolytic capacity of purified toxin samples, similar titrations were performed in 10% (wt/vol) bovine serum albumin-phosphate-buffered saline solution, and titers were read by determining the points of ca. 60% hemolysis. Hemolytic titers were always appreciably higher in the presence of albumin.

Immunization. Antibodies were raised by injecting rabbits intracutaneously with 50 μg of purified toxin admixed with an equal volume of Freund incomplete adjuvant (GIBCO Laboratories, Grand Island, N.Y.) by the immunization schedule of Harboe and Ingild (13). The membrane-derived form of the toxin was used as antigen. For this, the membrane-bound toxin was isolated from detergent-solubilized membranes by ultracentrifugation in sucrose density gradients as described (5). The first two booster injections were administered at 2-week intervals, and satisfactory antisera were obtained after the first booster. Thereafter, rabbits were given booster injections every 4 to 10 weeks and bled regularly.

SDS-PAGE and immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting (23) were performed as described previously (2a). Blots were developed with the use of peroxidase-labeled second antibody and stained with 3-amino 9-ethylcarbazole.

Electron microscopy. Negative stainings and electron microscopy were performed as previously described (24).

RESULTS

Toxin purification. As pointed out by earlier investigators (for review, see reference 1), bacterial cultures should be terminated when peak hemolytic titers are reached, and the pH should not be allowed to drop below 6.0, since titers will otherwise decrease due to toxin degradation. Passage of the ammonium sulfate- and PEG-precipitated material through a
DEAE-ion exchange column at pH 7.0 removes the bulk of contaminating proteases and thus decisively facilitates toxin isolation. Upon a second passage over DEAE at pH 9.0, some toxin passes the column, and these fractions should be discarded (Fig. 1). Two toxin pools were formed from the DEAE column fractions, i.e., one comprising fractions appearing during the column wash (designated pool II; Fig. 1) and one eluting after commencement of the salt gradient (pool I). The major component of pool II was the proteolyzed form of SLO, designated SLOₙ, whereas pool I contained primarily the native toxin (SLOₐ) and some SLOₙ. Preparative isoelectric focusing resolved pool I into the two components (Fig. 2). SLOₐ (native toxin) was isoelectric at pH 6.0 to 6.4, and SLOₙ (proteolyzed form) was isoelectric at pH 7.0 to 7.5 (Fig. 2A). Pool I yielded only the latter toxin peak (Fig. 2B). It is known that isoelectric focusing separates both toxin forms from contaminating NADase (22). When the respective fractions from the electrofocusing column were pooled and chromatographed over Sephacryl S-300, the two toxin forms were recovered in a satisfactorily pure state. There usually were some contaminants present, accounting for ~10% of the Coomassie-stainable material (Fig. 3, lanes a and b), but we occasionally obtained very pure preparations (Fig. 3, lanes c and d). Preparations of the quality shown in Fig. 3, lanes c and d, were used for the ultracentrifugal analyses, for determination of the amino acid composition, and for hemolytic titrations. Estimates for yields based on hemolytic activity indicated overall recoveries (sum of both toxin forms) in the order of 10% (see Table 2). The toxin preparations were stable for at least 4 to 8 weeks at 4°C or −20°C when stored in the presence of 2 mM phenylmethylsulfonyl fluoride and 10 mM EDTA. Trace protease contaminations were usually present, since slow toxin degradation generally occurred despite the presence of the inhibitors.

**Molecular properties of SLO.** In the ultracentrifuge, both forms of SLO exhibited a sedimentation coefficient of 3.9S. Symmetrically sedimenting peaks were obtained, and the toxin did not spontaneously aggregate at concentrations of 0.6 mg/ml. Molecular weight determinations were performed

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**FIG. 1.** DEAE-ion exchange chromatography of SLO at pH 9.0. Hemolytic activity (closed circles, toxin titer) was detected in pregradient fractions and in a peak eluting after application of the NaCl gradient. The first toxin-containing fractions (7 and 8) contained large amounts of contaminants and were discarded (for details, see the text). Other fractions containing hemolytic activity were pooled as depicted and subjected to preparative isoelectric focusing. Fraction volume: 7.5 ml; ms; conductivity in millisiemens (dashed line).

**FIG. 2.** Preparative isoelectric focusing of pool I and pool II recovered from the DEAE-ion-exchange column of Fig. 1. Pool I was resolved into two hemolytic peaks in the pH region of 6.0 to 6.4 (SLOₐ; pool A) and 7.0 to 7.5 (SLOₙ). Pool II yielded one hemolytic peak corresponding to SLOₙ (pool B).
at sedimentation equilibrium. With this method, the $M_r$ of SLO$_a$ was determined to be $69,000 \pm 3,000$ and that of SLO$_b$ to be $57,000 \pm 5,000$.

The amino acid composition of SLO$_a$ is given in Table 1. The composition of SLO$_b$ did not exhibit clear differences. In analyses of five toxin preparations, we failed to detect tyrosine. The toxin also contained very low amounts of cysteine, proline, and histidine. On the basis of the quantitative amino acid analyses, the $E_{280}$ nm for a 1-mg/ml solution of SLO$_a$ was determined to be 1.8, and for SLO$_b$ it was determined to be 1.9. The toxin contained no amino sugars.

**Hemolytic capacity.** SLO$_a$ or SLO$_b$ at a concentration of 2 to 3 ng/ml, corresponding to $1.7 \times 10^{10}$ to $3.1 \times 10^{10}$ molecules, lysed ca. 60% of an erythrocyte suspension containing $2.5 \times 10^8$ cells per ml. The observed activity corresponded to ca. 800,000 hemolytic units per mg of protein, according to the definition proposed by Alouf (1). According to hemolysis statistics (18), the 63% hemolysis point indicates the presence of one functional lesion per cell. From this, it can be concluded that the presence of 70 to 125 molecules of SLO per cell generates one functional lesion per cell.

**Ultrastructural appearance of SLO lesions.** Figure 4 shows electron micrographs of erythrocyte membranes lysed with the two forms of SLO. Both generated the same typical structures corresponding to SLO polymers. When target membranes were solubilized and centrifuged through sucrose density gradients, both forms sedimented similarly over broad molecular regions corresponding to 18 to 40S, and the delipidated toxin containing little or no cholesterol exhibited the structures identical to those described previously (5). By electron microscopy, the volumes of the SLO polymers were calculated to be in the range of 2,000 to 6,000 nm$^3$, corresponding to a molecular mass ranging from $1.5 \times 10^6$ to $5.0 \times 10^6$ daltons. From the known molecular weight of the monomer toxin, we thus estimate that SLO polymers contain between 25 and 100 toxin molecules.

**Immunological cross-reactions between SLO$_a$ and SLO$_b$.** By SDS-PAGE immunoblotting, cross-reactivity between the two toxin forms was apparent. An example is shown in Fig. 5, which depicts an immunoblot developed with anti-SLO$_b$. Culture supernatants obtained after various incubation periods were analyzed (left gel). In this culture, native SLO (SLO$_a$) appeared after 6 h of cultivation. No degradation of the toxin occurred either over the period of culture (10 h) or after a further 15 h at 4°C (right gel, lane A). The specificity of the antisera is apparent; by silver staining, there were at least 40 polypeptides present in these culture supernatants (data not shown). Lanes B through I show the blots obtained upon analysis of various samples taken at different stages of the isolation procedure. It can be seen that SLO degradation commences during concentration of the culture supernatant, and many lower-molecular-weight polypeptides carrying SLO epitopes are formed. SLO$_b$ represents the major degradation product and persists throughout the isolation procedure. The immunoblotting experiments thus clearly identify SLO$_a$ as the true native form of the toxin and SLO$_b$ as its major hemolytically active degradation product.

Both antisera to SLO$_a$ and SLO$_b$ exhibited high titer when tested in the classical toxin neutralization tests.

**DISCUSSION**

Streptolysin-O is considered an important pathogenic factor of ß-hemolytic group-A streptococci and represents the prototype of sulfhydryl-activated toxins that damage membrane probably through generation of very large transmembrane channels. Although the isolation of membrane-bound toxin can be easily achieved, purification of the native toxin has presented problems in the past. We described a method that is reasonably simple and reproducible. Two forms of the toxin have been identified. Native toxin is a polypeptide with an $M_r$ of 69,000. This value agrees excellently with those given by Shany et al. (20) and Alouf (1) earlier. The isoelectric point of 6.0 to 6.4 agrees with the data of several investigators (2, 20, 22), and the sedimentation coefficient of 3.9S is in good accord with the value of 3.7S found by van Epps and Andersen (25). During the isolation procedure, some toxin is degraded, and a major, hemolytically active polypeptide with an $M_r$ of 57,000 and an isoelectric point of 7.0 to 7.5 is generated. In retrospect, the present identification of these two hemolytic forms reconcile many divergent reports on the molecular weight and isoelectric point of the toxin appearing in the past. In particular, they corroborate and extend the finding made by several groups that two

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**TABLE 1. Amino acid composition of streptolysin-O**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Residues/100 residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>15.2</td>
</tr>
<tr>
<td>Thr</td>
<td>7.1</td>
</tr>
<tr>
<td>Ser</td>
<td>7.3</td>
</tr>
<tr>
<td>Gin</td>
<td>12.5</td>
</tr>
<tr>
<td>Pro</td>
<td>1.0</td>
</tr>
<tr>
<td>Gly</td>
<td>8.4</td>
</tr>
<tr>
<td>Ala</td>
<td>9.0</td>
</tr>
<tr>
<td>Cys</td>
<td>0.7</td>
</tr>
<tr>
<td>Val</td>
<td>7.1</td>
</tr>
<tr>
<td>Met</td>
<td>3.0</td>
</tr>
<tr>
<td>Ile</td>
<td>5.2</td>
</tr>
<tr>
<td>Leu</td>
<td>5.9</td>
</tr>
<tr>
<td>Tyr</td>
<td>2.5</td>
</tr>
<tr>
<td>Phe</td>
<td>0.4</td>
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<tr>
<td>His</td>
<td>1.2</td>
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<tr>
<td>Lys</td>
<td>3.3</td>
</tr>
<tr>
<td>Arg</td>
<td>ND*</td>
</tr>
</tbody>
</table>

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* ND, Not determined.
forms of the toxin indeed exist that display similar hemolytic capacity (1, 2, 20, 22). It is not clear which biological function, if any, is carried on the small fragment that is cleaved from the native toxin, or whether the two toxin forms exhibit any differences with regard to biological properties in the organism. Both generate micromorphologically identical, circular and semicircular polymers in the lipid bilayer and exhibit antigenic cross-reactivity. Structural considerations indicate that the heterogeneous polymer structures contain between 25 and 100 toxin monomers. Hemolytic titrations indeed indicate that the presence of 70 to 125 monomers per cell suffice to generate one functional lesion per cell. It is clear that such titrations can only give an indication of the true number of lesions required to lyse a cell, particularly since detailed knowledge of the binding process is lacking. If toxin-binding primarily occurs without exception to cholesterol, polymer formation must necessarily be a consequence of lateral toxin diffusion in the membrane plane. Alternatively, it is possible that conformational changes take place in a given SLO molecule after it becomes bound to cholesterol, triggering an autocatalytic reaction that leads to preferential binding of other monomers to the initial SLO-cholesterol complex. In any event, it is conceivable that “small” polymers form on target membranes, particularly at low toxin doses that may be neither lytic nor visible in the electron microscope. Additionally, many toxin molecules might remain bound in monomeric form. These considerations underline the fact that the calculated number of SLO molecules required to induce hemolysis is indeed low, and they would be very compatible with the contention that one or very few SLO oligomers will lyse an erythrocyte.

In closing, it is of interest to remark on a principle

FIG. 4. Human erythrocytes (10^6 cells per ml) lysed with SLOa (A and B) and SLOb (C and D) at final toxin concentrations of 40 μg/ml. After lysis, the membranes were washed and fixed in OsO₄ before negative staining with sodium silicotungstate. Both forms of the toxin generated apparently identical open and closed ring-shaped lesions. Bars, 50 nm.
difference existing between the hemolytic capacity of channel formers such as SLO, which bind with high affinity to membrane acceptor molecules (cholesterol), and that of staphylococcal α-toxin, for which a specific membrane binder is probably lacking. In the former case, binding is probably very effective at low toxin molarity. In contrast, the binding of the latter (receptorless) class of toxins is very ineffective (2a). For this reason, much higher concentrations of α-toxin are required to induce hemolysis, and marked variations in sensitivity of cells are noted (17). In contrast, SLO lyses erythrocytes of different mammalian species with fairly uniform efficacy (1) and is hemolytically active at much lower concentrations than α-toxin (1, 17, 21). The availability of SLO in highly purified form and the definition of its physicochemical parameters will facilitate investigations into the biological properties and the pathogenic mechanisms underlying the action of this interesting bacterial toxin in the host organism.

ACKNOWLEDGMENTS

We are most grateful to M. Wiesner (Max-Planck Institute of Immunobiology, Freiburg, Federal Republic of Germany) for kindly performing the amino acid analyses and to K. H. Schmidt (Behringwerke, Marburg, Federal Republic of Germany) for performing the ultracentrifugal analyses. This work was supported by the Deutsche Forschungsgemeinschaft (Bh 2/1-5).

TABLE 2. Purification of streptolysin-Oa

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Vol (ml)</th>
<th>Activity (U/ml)</th>
<th>Total activity (U)</th>
<th>Yield (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture supernatant</td>
<td>4,000</td>
<td>64</td>
<td>250,000</td>
<td></td>
</tr>
<tr>
<td>Ammonium sulphate precipitate</td>
<td>60</td>
<td>4,000</td>
<td>240,000</td>
<td>9</td>
</tr>
<tr>
<td>DEAE (pH 7.0)</td>
<td>10</td>
<td>16,000</td>
<td>160,000</td>
<td>64</td>
</tr>
<tr>
<td>DEAE (pH 9.0)</td>
<td>80</td>
<td>2,000</td>
<td>160,000</td>
<td>64</td>
</tr>
<tr>
<td>IEFb (SLOa + SLOβ)</td>
<td>28</td>
<td>2,000</td>
<td>56,000</td>
<td>22</td>
</tr>
<tr>
<td>Sephacryl S-300 (SLOa + SLOβ)</td>
<td>12</td>
<td>2,000</td>
<td>24,000</td>
<td>10</td>
</tr>
</tbody>
</table>

a One unit of hemolytic activity is defined as the reciprocal hemolytic titer (read at 100% hemolysis).

ADDITIONAL INFORMATION

M. Kehoe and K. N. Timmis (Infect. Immun. 43:804–810, 1984) have obtained data through molecular genetic studies indicating the existence of two SLO gene products with $M_\text{s}$ of 68,000 and 61,000. Their results are in excellent accord with those of the present study, which identifies the lower-molecular-weight moiety as the major, hemolytically active degradation product of the native toxin.

LITERATURE CITED