

Purification and Properties of Two Forms of Staphylococcal α Toxin†

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ABSTRACT: Two stable forms of α toxin have been isolated from broth cultures of Woods 46 strain of *Staphylococcus aureus*. Purification was accomplished by the following steps: (1) concentration of the toxin by ammonium sulfate precipitation; (2) filtration on Sephadex G-50; (3) filtration on Sephadex G-100; and (4) preparative gel electrophoresis at pH 8.9. The combined forms of α toxin represent an overall recovery of 25% of the initial crude activity. The two forms (A and B) are separated electrophoretically. Each form gives a single band in disc gel electrophoresis at pH 8.9 and 4.5 and at pH 8.1 in the presence of sodium dodecyl sulfate. Immunochemical analysis of the two forms by gel diffusion

shows that they are homogeneous and immunochemically identical. Both forms of α toxin have identical specificities and specific activities in hemolytic assays. Neither form of α toxin contains any detectable neutral or amino sugar. However, both forms have the novel property of "binding" Tris, which was identified as the apparent amino sugar detected by gas chromatography. Very similar amino acid compositions are obtained for α -toxins A and B. Terminal amino acid analysis shows that both forms of toxin have alanine as the amino-terminal residue and lysine as the carboxy-terminal amino acid.

In the past decade at least ten different methods have been reported for purifying α toxin. These purification procedures have been summarized and discussed in two recent reviews (Bernheimer, 1968; Arbuthnott, 1970). None has yielded a product which was homogeneous with respect to both charge and molecular size.

Since both the δ - and β -staphylococcal hemolysins have larger molecular weights (Yoshida, 1963; Chesbro *et al.*, 1965) than α toxin and since the Woods 46 strain used in these studies is known to produce small amounts of δ toxin (Bernheimer *et al.*, 1968), an initial purification based on size seemed appropriate. The observation that α toxin was unstable at low salt concentrations (Lominski *et al.*, 1963; Coulter, 1966) suggested that improved recoveries could be obtained by maintaining relatively high salt concentrations during the isolation procedure. The isolation procedure described below reflects these considerations.

Materials and Methods

Reagents. The organism was the Woods 46 strain of *Staphylococcus aureus*. Stock cultures were maintained in vials containing 50% glycerol at -65° . The *Staphylococcus* hemolysin antitoxin was obtained from Lederle Laboratories. Ammonium sulfate, urea, and sucrose were Ultra Pure grade from Schwarz/Mann. Tris was purchased as Trizma base from Sigma and the glycine was ammonia free, Matheson Coleman and Bell. All Sephadex was supplied by Pharmacia Corporation; the resins were swelled and the columns prepared according to their recommendations. Carboxypeptidases A and B were obtained from Worthington Biological Corporation as the diisopropyl fluorophosphate treated enzymes and used without further purification.

Hemolytic Assay. The preparation of erythrocytes, the definition of a hemolytic unit, and the conditions of the hemolytic titrations were those defined by Bernheimer and Schwartz (1963). Unless otherwise stated all titrations were made against rabbit red blood cells. The hemolytic activity of α toxin is known to show a wide range for different individuals within a given species (Cooper *et al.*, 1966). Therefore, the specific hemolytic activity of any given preparation cannot be correlated directly to the degree of purity. For this reason, each preparation of toxin was followed to completion with red blood cells from an individual rabbit. Rabbit erythrocytes were obtained by cardiac puncture of stock laboratory animals and stored at 4° in Alsever's solution. Sheep erythrocytes were obtained as a washed 10% suspension from Baltimore Biological Laboratory. The human erythrocytes were citrated type O, obtained from Vanderbilt blood bank as outdated whole blood.

Production of the Toxin. The preparation of low molecular weight yeast extract media and the procedure for growing the staphylococci are essentially as described by Bernheimer and Schwartz (1963). In brief, 100-ml starter cultures are grown for 24 hr and used to inoculate larger (usually 3 l.) cultures distributed in 500-ml aliquots in 2-l. flasks. After 18 hr of shaking at 35° the growth cultures are cooled to 4° and the staphylococci were removed by centrifugation at 30,000g for 30 min. The supernatant is brought to 90% saturation by addition of solid $(\text{NH}_4)_2\text{SO}_4$. After 24 hr at 4° , the precipitate, containing the hemolytic activity, is collected by centrifugation. This represents the crude starting material.

Analytical Gel Electrophoresis. Polyacrylamide disc electrophoresis was performed at pH 8.9 according to Davis (1964), except that no stacking or spacer gels were used. Samples of 20–50 μ l containing 25–100 μ g of protein were applied directly on the gel in 0.2 M sucrose with 0.003% Bromophenol Blue included as a tracking dye. In all cases, the gels were first preelectrophoresed (Mitchell, 1967) to remove residual persulfate. Electrophoresis at pH 4.5 was conducted as described by Reisfeld *et al.* (1962), with the exceptions that Methyl Green was used as a tracking dye

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and the gels contained 6 M urea. In some cases 0.01 M phthalate buffer, pH 4.3, was used in place of the acetate buffer. For sodium dodecyl sulfate electrophoresis the method of Shapiro *et al.* (1967) was followed. The gels were stained with Coomassie Blue according to the method of Chrambach *et al.* (1967). In some cases the gels were stained with periodate-Schiff reagent (Zacharias *et al.*, 1969).

Preparative Gel Electrophoresis. Preparative gel electrophoresis was performed on a Buchler Polyprep instrument (Buchler Instruments). The gel and buffer compositions were those described for pH 8.9 analytical gel electrophoresis. Samples of from 20 to 100 mg in 4–10 ml were layered directly on the resolving gel which was about 4 cm in length. The elution buffer, 0.10 M Tris-HCl, pH 8.1, was pumped at a constant rate of 0.5 ml/min and fractions were collected at 7-min intervals. The samples were electrophoresed at a constant current of 50 mA. Both the electrophoresis chamber and the elution fractions were maintained at 6°.

Amino Acid. Amino acid analyses were performed using a Technicon amino acid analyzer equipped with type C₂ resin. Samples were hydrolyzed in 6 N HCl in sealed evacuated tubes at 110°, for 20, 40, and 70 hr. Methionine and cysteine residues were determined after oxidation as described by Moore (1963). The tryptophan content was estimated by the spectrophotometric procedure of Goodwin and Morton (1946).

Carbohydrate Analysis. The procedure used was that of Lehnhardt and Winzler (1968), and analyses were performed on a Microtek MT 220 gas chromatography unit. Samples were run with and without the incorporation of an internal standard (Arabinitol). Samples taken for analysis usually contained 1–3 mg of protein.

Carboxy-Terminal Analysis. Samples of the toxins were dissolved in 8 M urea and then freed of urea by dialysis against water. The protein was then lyophilized and dissolved in 0.1 M NaCl–0.02 M Tris-HCl, pH 7.5. Carboxypeptidase B was added at 2% of the toxin concentration. The incubation was carried out at room temperature, and at 0, 1, 2, and 20 hr aliquots equivalent to 20–30 nmol of toxin were removed. The reaction was terminated by lowering the pH to 2.0 by the addition of HCl. The samples were clarified by centrifugation and the supernatant was run directly on the amino acid analyzer. Carboxypeptidase A digestions were run under the same conditions, except that the enzyme was dissolved in 10% LiCl before addition to the reaction mixture.

Amino-Terminal Analysis. Samples of the toxins were prepared as described above, except that no NaCl was present and the pH of the Tris was 8.3. Leucine aminopeptidase was activated in 0.05 M MgCl₂–0.02 M Tris, pH 8.3, at 37° for 2 hr. The enzyme was then added to the toxin at one-tenth the volume to yield a final molarity of 5×10^{-3} M Mg²⁺. Aliquots were removed as described above.

Results

Purification Procedure. CHROMATOGRAPHY ON SEPHADEX G-50. The crude preparation of α toxin, obtained as an ammonium sulfate pellet from the growth medium (see Materials and Methods), was dissolved in 3 vol of 0.05 M sodium acetate buffer, pH 5.0. Insoluble denatured material was removed by centrifugation at 5000g for 5 min. The supernatant, containing 85–90% of the original hemolytic activity, was chromatographed on Sephadex G-50. The column (5 × 20 cm) was pre-equilibrated and eluted with 1 M ammonium sulfate. The hemolytic activity, which was eluted in the void volume, was

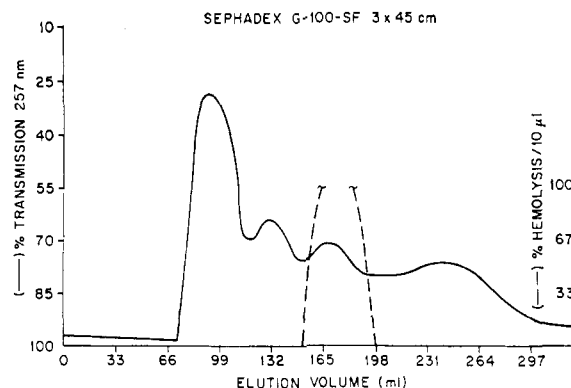


FIGURE 1: Sephadex chromatography of crude staphylococcal α toxin. α toxin obtained from 1 l. of culture fluid was chromatographed as described in the text. The per cent transmission was recorded continuously with a UVicord monitor (LKB Produktor). Aliquots of each fraction were diluted 1:100 in 0.15 M NaCl–0.02 M phosphate buffer, pH 7.2. Aliquots (10 μ l) of each dilution were added to 2.0 ml of rabbit erythrocytes. Hemolysis was then measured as described under Materials and Methods.

pooled, precipitated by the addition of ammonium sulfate to 90% saturation, and collected by centrifugation. This step effectively removes low molecular weight contaminants which mostly come from the medium. Eighty-five per cent of the input activity is routinely recovered which corresponds to 70–75% of the original hemolytic activity.

CHROMATOGRAPHY ON SEPHADEX G-100. The ammonium sulfate pellet of toxin obtained after chromatography on Sephadex G-50 was dissolved in 3 vol of acetate buffer as above and chromatographed on Sephadex G-100-SF again using 1 M ammonium sulfate to elute the column. A 3 × 45 cm column was used and the yield of toxin from 3 l. of growth medium was chromatographed in two equal batches. Both larger and smaller contaminants were removed and the α -toxin hemolytic activity was eluted as a single symmetrical peak. A typical pattern is shown in Figure 1. The α toxin was pooled, precipitated with ammonium sulfate, and collected by centrifugation as described above. The overall recovery of hemolytic activity at this stage is approximately 50%. Amino acid analysis of the material from the Sephadex G-100 column corresponds closely to that reported by others for α toxin (Coulter, 1966; Bernheimer and Schwartz, 1963). However, electrophoretic analysis in disc gels showed that the product contained two major bands and eight minor bands of protein. Moreover, although the toxin was stable for several weeks when stored under ammonium sulfate, extensive dialysis to remove the salt not only led to a loss of hemolytic activity, but also to a continuous loss of amino acids. The instability is due to a contaminant protease, which apparently is inhibited by ammonium sulfate. The presence of proteolytic activity was confirmed by the liquification of gelatin and the release of ninhydrin-positive material from methylated albumin.

PREPARATIVE GEL ELECTROPHORESIS. Aliquots from the Sephadex G-100-SF material, containing 20–100 mg of protein, were collected by centrifugation and dissolved in 5 ml of Tris-glycine buffer, pH 8.9. The solution was dialyzed against 100 ml of the buffer for 3–4 hr and applied to the gel. These particular conditions were chosen to minimize the protease activity and yet reduce the salt concentration enough to permit efficient electrophoretic fractionation. Under these conditions, two major peaks of hemolytic activity are obtained. The more acidic protein has been termed α -toxin A and the

TABLE I: Purification and Recovery of Staphylococcal α Toxin.

Preparation	Total Vol (ml)	Total Protein (mg) ^a	Total Hemolytic Units	Hemolytic Units/mg	Total Recovery (%)
Culture fluid	1000		2.0×10^6		100
Sephadex G-50	20 ^b	750	1.5×10^6	2.0×10^3	75
Sephadex G-100	10 ^b	62	1.0×10^6	1.6×10^4	50
Preparative electrophoresis					
A form	10 ^c	5	1.0×10^5	2.0×10^4	5
B form	10 ^c	20	4.0×10^5	2.0×10^4	20

^a Estimated by ultraviolet absorption at 280 nm. ^b After concentration with ammonium sulfate. ^c After concentration by preevaporation.

more basic α -toxin B. A typical pattern is shown in Figure 2. The ratio of A to B forms of the toxin has been consistently 1:4 through 20 separate preparations. The hemolytic activity of the combined toxin fractions represents a final recovery of 25% of the original activity. Thus, from a typical preparative electrophoresis about 5 mg of A and 20 mg of B are obtained. The fractions may be conveniently concentrated by pressure dialysis or by preevaporation followed by dialysis and ammonium sulfate precipitation.

The purification and recovery of α toxin are illustrated in Table I. The absolute extent of purification cannot be conveniently calculated because of the high peptide content and pigmentation of the growth medium. The major purification involves removal of the culture medium peptides which is achieved in large part by the initial ammonium sulfate precipitation and the subsequent two Sephadex steps. Thus, the product after the Sephadex G-100 step is approximately 80% pure toxin. The final preparative gel electrophoresis not only resolves the α toxin into the two forms A and B, but effectively separates the remaining contaminants including the protease activity. Based on the final hemolytic specific activity and the per cent recovery it can be calculated that under these growth conditions the Woods 46 strain produces 100 mg of α toxin/l. of growth medium.

Properties of Purified α -Toxins A and B. STABILITY. The purified toxins are unstable to lyophilization and remain stable for only a few days at 4° in physiological salt solutions. They can be stored as a suspension in 90% saturated ammonium sulfate at 4° for months. The preparations are free of detectable protease activity.

DISC GEL ANALYSIS. Analysis of each form of toxin by disc gel electrophoresis gave a single band for each at pH 8.9 and 4.5. Each form of toxin also gave single bands when electrophoresed in sodium dodecyl sulfate gel. These results demonstrate the purity of the preparations and indicate that each toxin contains only one kind of peptide chain (Figure 3). The observed mobilities of each form of toxin in sodium dodecyl sulfate gel suggest that α -toxins A and B have very similar molecular weights. A more detailed study of the molecular weight which suggests a single peptide chain per molecule is the subject of a separate report (Six and Harshman, 1973).

IMMUNOCHEMICAL STUDIES. Immunochemical homogeneity was demonstrated using commercial antisera prepared against α toxin. Double diffusion in gel (Ouchterlony, 1949) demonstrates a number of impurities in the material after chromatography on Sephadex G-100-SF which are not seen in the purified α -toxin A and B preparations. The absence of spur

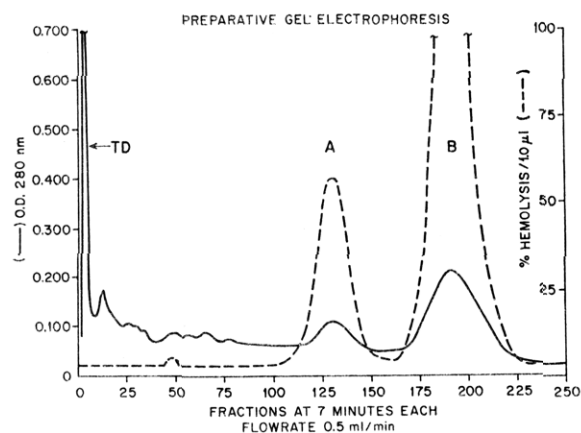


FIGURE 2: Preparative gel electrophoresis of partially purified α toxin. Partially purified α toxin (50 mg) was electrophoresed at pH 8.9. A 7.5% acrylamide gel 3.9 cm in length was used. The voltage was maintained at a constant 280 V throughout the run. The voltage was maintained at a constant 280 V throughout the run. The fractions were monitored for optical density, on a Zeiss PM Q II spectrophotometer, and for hemolytic activity, as described under Materials and Methods; TD = tracking dye, A = toxin A form, B = toxin B form.

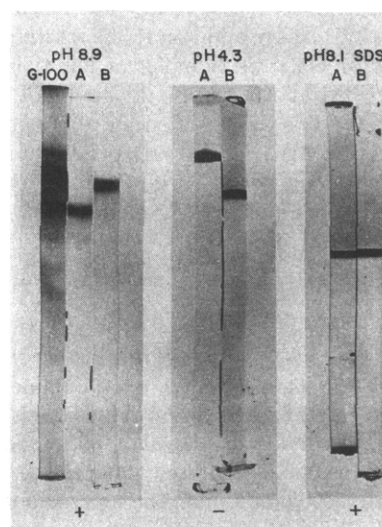


FIGURE 3: Electrophoretic pattern of α -toxins A and B: (A) electrophoretically purified α -toxin A; (B) electrophoretically purified α -toxin B; (1) pH 8.9; (2) pH 4.5; (3) pH 8.1 in 0.1% sodium dodecyl sulfate.

TABLE II: Amino Acid Compositions of α Toxin.

Residue	Residues per Molecule ^a					
	A Form		B Form		Bernheimer and Schwartz (1963) ^b	Coulter (1966) ^b
	Extrap or Av Value ^c	Nearest Integer	Extrap or Av Value ^c	Nearest Integer		
Aspartic acid	39.8	40	43.2	43	44	50
Threonine	21.5	22	23.1	23	23	24
Serine	19.0	19	18.7	19	22	20
Glutamic acid	18.9	19	20.2	20	21	22
Proline	7.5	8	9.0	9	7	10
Glycine	19.7	20	23.7	24	23	24
Alanine	10.9	11	11.4	11	12	14
Valine	12.5	13	14.1	14	12	16
Cystine	0.0	0	0.0	0	0	1 ^d
Methionine	5.6	6	5.9	6	10 ^e	4 ^e
Isoleucine	12.6	13	13.9	14	13	16
Leucine	12.6	13	14.0	14	15	14
Tyrosine	8.9	9	10.1	10	9	10
Phenylalanine	7.9	8	8.4	8	10	10
Lysine	21.3	21	22.7	23	23	26
Histidine	4.0	4	4.0	4	4	4
Arginine	8.0	8	8.2	8	10	8
Tryptophan	3.6	4	4.0	4	NR ^f	NR ^f

^a Residues were calculated by assigning histidine a value of four residues. ^b Recalculated from the original report by assigning histidine as four residues. ^c Threonine and serine are zero-time values; valine, isoleucine, and leucine are the maximum values obtained; methionine was determined as the sulfone after performic oxidation; tryptophan was determined spectrophotometrically.

^d Recovered as cysteic acid. ^e Summation of methionine, methionine sulfoxides, and methionine sulfone. ^f NR, none reported.

formation between the A and B forms indicates that they are immunochemically identical (Figure 4).

HEMOLYTIC ACTIVITY. Analysis of the hemolytic activity of both α -toxins A and B showed that each had the same specific activity when tested with red blood cells from a given rabbit. Depending on the blood cells used the activities ranged from 14,000 to 32,000 hemolytic units/mg of protein. The usual value obtained was about 20,000 hemolytic units/mg. These values are comparable to those reported by Bernheimer and Schwartz (1963). Both forms of the toxin showed the expected specificity for red cells of different species (Cooper *et al.*, 1966). Thus, sheep RBC were hemolyzed with one-twentieth the efficiency of rabbit RBC and human RBC with only about one-tenth the efficiency of sheep RBC.

Chemical Compositions. **CARBOHYDRATE ANALYSIS.** The observation that the bands of α -toxins A and B in disc gel analysis could be stained with periodate-Schiff reagent raised the possibility that the toxins contained carbohydrate. Accordingly, 1–3-mg samples of each toxin were exhaustively dialyzed against water and analyzed for the presence of neutral sugar by the method of Lehnhardt and Winzler (1968). A single peak was detected, which was found to correspond to Tris, a component of the electrophoresis buffer. This was confirmed by cochromatography with authentic acetylated Tris and by mass spectral analysis. The unusual capacity of α toxins to “bind” 25–30 molecules of Tris/molecule of protein is unexplained and is currently being studied. No neutral sugars were detected in either α -toxin A or B.

AMINO ACID ANALYSIS. Aliquots of each toxin were hydrolyzed for 20, 40, and 70 hr in 6 N HCl under vacuum at 100°. The values for serine, threonine, and tyrosine were obtained by extrapolation to zero time hydrolysis. The 70-hr hydrolysis

values were used to calculate valine and isoleucine. The absence of cysteine and the value for methionine, as the sulfone, were determined after performic oxidation (Moore, 1963). Although the analysis was very similar for α -toxins A and B, slight differences in residue numbers for certain amino acids such as aspartic acid and glycine were obtained consistently through several preparations (Table II). The significance of

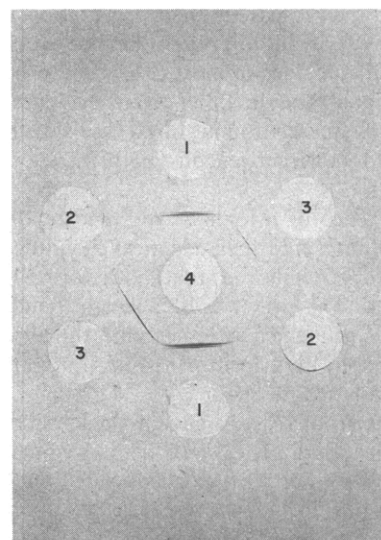


FIGURE 4: Immunodiffusion of the α toxins. Immunodiffusion was carried out at 1.2% ion agar in 0.15 M NaCl. The distance between the wells was 8 mm: (1) partially purified α toxin after Sephadex chromatography; (2) electrophoretically purified α -toxin A, 100 μ l/ml; (3) electrophoretically purified α -toxin B, 300 μ l/ml; (4) commercial antitoxin.

these differences must await more detailed sequence data. Two laboratories have reported amino acid composition data for α toxin (Bernheimer and Schwartz, 1963; Coulter, 1966) and their results have been included for comparative purposes.

AMINO-TERMINAL ANALYSIS. To establish the amino-terminal amino acid, 7-mg samples of each form of α toxin were submitted to Edman degradation (Harshman *et al.*, 1969). The released phenylhydantoin and its silyl derivative were identified by gas chromatography on a Beckman GC-45 instrument (The Beckman Protein Peptide Sequencer Instruction Manual, 1969). With both α -toxins A and B a single amino acid alanine was obtained as the amino-terminal amino acid in approximately 65% yield. The phenylhydantoins were analyzed by thin layer chromatography (Inagami and Murakami, 1972) and again only alanine was obtained from each form of toxin. Alanine was also indicated by its release with leucine aminopeptidase; after 2 hr of incubation 0.5 residue of alanine was released per 28,000 mol wt. No other amino acids were released.

CARBOXY-TERMINAL ANALYSIS. Exposure of both forms of α toxin to carboxypeptidase B (see Materials and Methods) released 1 mol of lysine/mol of toxin assuming a mol wt of 28,000. The release of lysine from both forms of toxin was complete in 1 hr and incubation for an additional 19 hr failed to release any other amino acids. The addition to carboxypeptidase A after the release of the lysine failed to release any amino acids. This result suggests that the amino acid sequence immediately adjacent to the C-terminal lysine is similar in both forms of α toxin.

Discussion

The procedure described here permits the isolation of two forms of α toxin each of which is homogeneous as judged by a variety of procedures. These include disc gel electrophoresis at pH 8.9 and 4.5, sodium dodecyl sulfate gel electrophoresis, immunologic double diffusion analysis, and terminal amino acid analysis. Since neither α -toxin A nor B contains detectable carbohydrate it is probable that the inactivation of α toxin by amylases reported by Goshi *et al.* (1963) was due to the presence of the protease in their preparations. A comparison of α -toxins A and B to preparations of α toxin reported by others is difficult since in most cases rigid proof of purity was not presented and in other cases evidence of heterogeneity was subsequently found. In no case were the preparations shown to be homogeneous both in size and electrophoretic mobility.

The coemergence of α -toxin A and α -toxin B from Sephadex G-100 columns and their identical mobilities in sodium dodecyl sulfate gel indicates that both forms have similar molecular sizes. The presence of a single band in sodium dodecyl sulfate gel, taken together with the absence of cysteine, suggests that each form of α toxin is composed of a single kind of polypeptide chain.

Since each form of α toxin gave a single band in disc gel electrophoresis at both pH 8.9 and 4.5, it is concluded that α -toxins A and B do not represent associated and dissociated forms of toxin nor do they represent readily interconvertible conformers.

Both α -toxins A and B are homogeneous by immunologic analysis in gel diffusion. The reaction of identity between the two forms indicates that both have similar antigenic determinants. Immunologic identity of electrophoretically separated forms of α toxin has been reported by others (Bern-

heimer and Schwartz, 1963; McNiven *et al.*, 1972). Analysis of the hemolytic potency of α -toxins A and B against rabbit, sheep, and human red blood cells also showed the two forms to be identical.

The similarities in amino acid compositions of α -toxins A and B are striking and in close agreement with those reported for α toxin by Bernheimer and Schwartz (1963) and by Coulter (1966). The similarities in amino acid composition cannot, however, be taken as evidence of purity. Thus, amino acid analysis of α toxin after the G-100 Sephadex step is also equivalent to that obtained with pure α -toxins A and B even though the Sephadex G-100 material is demonstrably heterogeneous by both immunologic and disc gel analyses. Although analyses of α -toxins A and B are very similar, differences in composition of certain amino acids, particularly glycine and aspartic acid, have been obtained consistently. These differences are slight and their significance is uncertain at present.

End group analyses give identical results with both α -toxins A and B. In each case alanine is obtained as the amino-terminal residue and lysine as the carboxy terminus. These results do not agree with Coulter (1966) who reported the amino terminus to be arginine or with Wiseman and Caird (1970) who reported it to be histidine. The basis for this discrepancy is not known. It is possible that mild proteolysis during the isolation procedure could account for the differences, but such degradation would be expected to generate multiple end groups and this was not observed in our preparations. The finding of single terminal amino acids for α -toxins A and B not only suggests that they are not generated by the action of an endogenous protease but adds additional support to the view that each is composed of a single kind of polypeptide chain.

Both forms of α toxin exhibit the unusual capacity to bind Tris. The present data do not permit distinction between direct binding and occlusion of Tris within the molecule. The observation that denatured α toxin does not contain bound Tris, although compatible with an occlusion hypothesis, does not rule out the possibility that the native configuration is required for binding. Although Tris binding has been shown to alter the electrophoretic mobility of at least one protein, lactalbumin (Rawitch and Gleason, 1971), it is unlikely that it accounts for the separation of the two forms of α toxin, since both forms bind equal amounts of Tris.

The obvious difference in the two forms of α toxin is clearly in the charge of the proteins. Although this could be due to a simple difference in amide content, the constant ratio of A form to B form and the failure to observe interconversion of the two forms argue against this interpretation. The basis of the difference between the two forms must await more detailed structural and sequence studies.

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Physical and Chemical Studies on Staphylococcal α -Toxins A and B†

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ABSTRACT: Physical studies on purified A and B forms of staphylococcal α toxin show that the forms have similar minimal and functional molecular weights but differ from each other in charge. By isoelectric focusing, the A form is more acidic, pI 7.2, than the B form, pI 8.4. A comparison of mobilities of the two forms at pH 8.9 in analytical gel electrophoresis with varying gel concentrations gave parallel lines, indicating that the two forms are charge isomers, rather than size isomers. Both forms of the toxin gave identical mobilities in sodium dodecyl sulfate gel electrophoresis corresponding to a minimal mol wt of 27,500. Sedimentation equilibrium studies of the B form of α toxin in 8 M urea and in 6 M guanidine-HCl gave extrapolated values for the minimal mol wt of 28,700 and 31,000, respectively. Sedimentation velocity studies gave a value of 3.0 S from which a functional mol wt of 26,100 was calculated. Peptide mapping studies of the two forms gave 27

detectable peptides for the A form and 23 for the B form, 20 of which appear to be identical in both forms, indicating a large degree of homology in the amino acid sequences. The number of peptides obtained is in good agreement with a minimal mol wt of about 28,000. Automated N-terminal analysis of the A and B forms of staphylococcal α toxin permitted the unambiguous ordering of the first ten amino acids. Identical amino acid sequences were obtained for the two forms of toxin. Fractionation of the CNBr peptides from α -toxin A by Sephadex chromatography, following maleylation of the peptides, yielded seven peptides which eluted in positions identical with those obtained from the B form of toxin. Amino acid analysis showed each of the peptides to be very similar in composition to its B form counterpart. The absence of homoserine established peptide VII as the C terminus in both forms of the toxin.

Despite general agreement that staphylococcal α toxin in velocity centrifugation analysis has an $s_{20,w}$ of 2.8–3.0 S (Bernheimer and Schwartz, 1963; Lominski *et al.*, 1963; Coulter, 1966), other methods give divergent values for the molecular weight. Various laboratories using centrifugation and sodium dodecyl sulfate gel methods have reported values

of 22,000 (Coulter, 1966), 36,000 (McNiven *et al.*, 1972), and 44,000 (Bernheimer and Schwartz, 1963) for purified α toxin. It has been suggested that variation in observed molecular weights is due to a mixture of molecules present in even highly purified preparations of α toxin (Coulter, 1966; Bernheimer, 1968). The mixture has been assumed to be caused by varying degrees of molecular association of α toxin (Arbuthnott, 1970). However, the possibility that high molecular weight aggregates of α toxin do not consist exclusively of α -toxin molecules cannot be excluded at present (Bernheimer, 1968).

Purified α toxin has also been shown to be heterogeneous in

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