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Elapid Neurotoxins. Purification, Characterization, and Immunochemical Studies of α-Bungarotoxin†

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ABSTRACT: The purification to homogeneity of α-bungarotoxin (α-Bgt) from the venom of *Bungarus multicintus* by starch electrophoresis followed by gradient elution on carboxymethylcellulose is described. Characterization of the purified toxin was carried out with respect to its amino acid composition, isoelectric point (p*I*), minimal molecular weight, N-terminal amino acid, and its electrophysiological characteristics. The amino acid composition of α-Bgt was found to correspond to its general basic nature (p*I* = 9.19 for iodinated [^{125}I]α-Bgt). Minimal molecular weight determination from the amino acid composition data gave a value of 7904, in agreement with a value of 8000 found by sodium dodecyl sulfate disc gel electrophoresis. Quantitative determination of the N terminus as isoleucine allowed estimation of the purity of this preparation of α-Bgt to be at least 99%. Electrophysiological characterization of α-Bgt and its purified iodinated analog showed both preparations to be effective in blocking the acetylcholine response of frog rectus abdominus muscle. Rabbit antibodies against purified α-Bgt were purified by removal of macroglobulin on Sephadex G-200. Subsequently the remaining immunoglobin G was coupled to cyanogen bromide activated Sepharose 2B. The binding capacity and conditions for eluting bound toxin from the toxin specific immunosorbent were investigated.

The recent interest (Changeux *et al.*, 1970; Miledi *et al.*, 1971; Cooper *et al.*, 1971)[1] in low molecular weight protein neurotoxins for the study of neurochemical processes has necessitated their fractionation, purification, and characterization. Of the elapid neurotoxins, only the purification and immunochemical characteristics of cobrotoxin from *Naja naja atra* have been fully described (Yang, 1964; Chang and Yang, 1969). Among the elapid neurotoxins investigated by Lee (1970) are those from *Bungarus multicintus*, for which he reports the amino acid composition of two (α and β) of this venom's many components. However, the purification of α-bungarotoxin (α-Bgt),[2] which blocks neuromuscular transmission by acting at the postsynaptic membrane of the neuromuscular junction (Lee, 1970), has yet to be described in full (Changeux *et al.*, 1970; Lee, 1970), although its amino acid sequence has been reported (Mebs *et al.*, 1971). We report here a purification procedure and some chemical properties of α-Bgt as well as the preparation of toxin-specific antibodies.

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[1] See companion papers in this issue.

[2] Abbreviations used are: Bgv, venom from *Bungarus multicintus;* α-Bgt, α-bungarotoxin; IgG, immunoglobin G; IgM, macroglobin; AcCh, acetylcholine; AcChE, acetylcholine esterase; AcChR, acetylcholine receptor; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); SDS, sodium dodecyl sulfate.

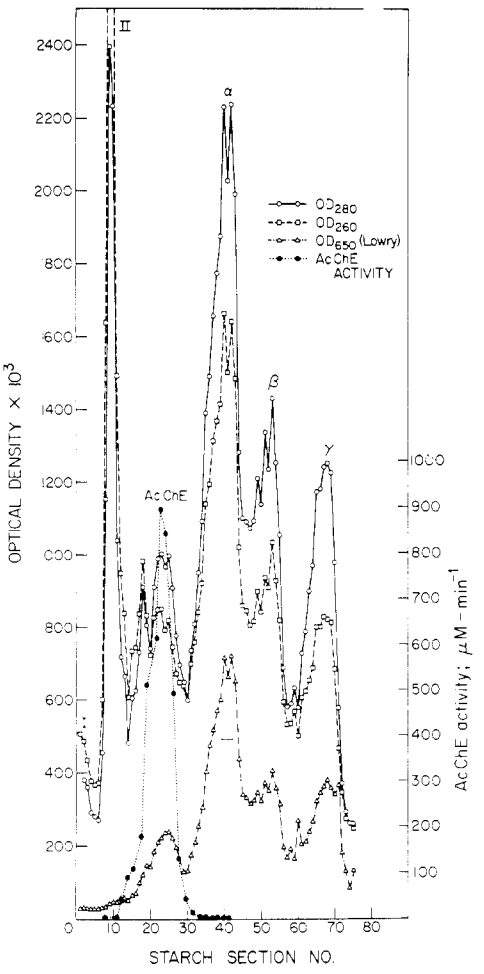

FIGURE 1: Starch electrophoresis of crude Bgv at constant voltage at 4° for 40 hr. The sample was introduced at fraction five and the polarity was such that fraction 80 represents the cathode. Following completion of electrophoresis, the starch was removed in 0.5-cm sections and eluted with water, and absorbance at 260 and 280 nm was determined. In addition, Lowry determinations were made for each fraction. The designations of peaks α, β, and γ are those of Chang and Lee (1963), while peaks I and II are components which gave negative Lowry tests.

Experimental Section

Materials

Crude venom of *Bungarus multicintus* (Bgv) was obtained from two sources: Sigma Chemical Co., lots 406-2170 and 49B-0830, and Miami Serpentarium Laboratories, lot BM 12345 F. The carboxymethylcellulose used was Whatman CM-52. Sepharose 2B and Sephadex gels used were from Sigma, and the potato starch used was "Swan" brand, from Stein-Hall and Co., New York. Reagent grade chemicals were used throughout. Male rabbits were obtained from Hill Top Labs, Chatsworth, Calif., Freunds Adjuvant (complete) from Difco, and 0.9% nonpyrogenic saline was obtained from McGraw Laboratories. ^{125}Iodide, carrier free, was obtained from New England Nuclear and all samples were counted in a dioxane-based scintillator on a Packard Tri-Carb Model 3375 liquid scintillation counter. Ampholine, pH range 7–10, was an LKB product.

Methods

Electrophoresis on Potato Starch. Resolution of the components of Bgv by starch electrophoresis was performed in a 3.5 × 42 cm hemicylindrical glass trough as described by Chang and Lee (1963). Constant-voltage electrophoresis (300 V) was conducted at 4° for 40 hr. At the completion of the run the starch was divided into and removed in sections of 0.5 cm, each of which was washed with 3 ml of water. Protein concentration in the supernatant was determined by the method of Lowry *et al.* (1951) and the extinctions of each fraction at 260 and 280 nm were determined. These data are summarized in Figure 1. Various fractions were tested for their effectiveness in blocking the response to AcCh in frog rectus abdominis muscle, and their AcChE activity was determined by the method of Ellman *et al.* (1961). The desired starch fractions were each washed twice more with water, pooled, concentrated by pressure dialysis using an Amicon UM-2 membrane, and further purified by gradient elution chromatography as described below.

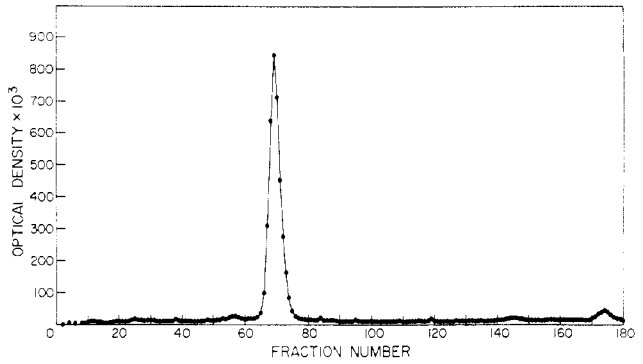

FIGURE 2: Gradient elution chromatography on carboxymethylcellulose of fractions using a column 2.5 × 20 cm from starch electrophoresis (Figure 1) and an ammonium acetate gradient from 0.05 (pH 5.0) to 1.0 M (pH 6.8). The minor peaks are due to contaminants from Bgv remaining after starch electphoresis and subsequent chromatography on Sephadex G-50.

Chromatography on Carboxymethylcellulose. Various components from potato starch electrophoresis were pooled, desalted on columns (2.5 × 50 cm) of Sephadex G-50, lyophilized, and dissolved in a minimal volume of 0.05 M NH_4OAc (pH 5.0) and applied to a 2.5 × 20 cm column of Whatman CM-52, equilibrated with the same buffer. Elution was carried out using a linear 1-l. gradient of NH_4OAc from 0.05 (pH 5.0) to 1.0 M (pH 6.8). Fractions of 5 ml each were collected. The protein concentrations of fractions were determined by the Lowry *et al.* (1951) method and their optical densities at 260 and 280 nm were recorded as shown in Figure 2 for a sample of α-Bgt. Various fractions were tested for (a) acetylcholine receptor (AcChR) blocking activity using the frog rectus abdominis preparation as described in a later section and (b) acetylcholineesterase (AcChE) activity (Ellman *et al.*, 1961).

As an alternative to preliminary starch electrophoresis, followed by CM-52 chromatography, a sample of 100 mg of crude Bgv was dissolved in a minimum volume of 0.05 M NH_4OAc (pH 5.0) and subjected to gradient elution chromatography as described above. The elution behavior of this chromatography is shown in Figure 3.

Fractions either from electrophoretic or CM-52 preparations of Bgv were rechromatographed using CM-52 equilibrated at 0.1 M NH_4OAc (pH 5.5) by application in the same buffer and elution with a 1.2-l. gradient of NH_4OAc (pH 5.5) from 0.1 to 0.4 M.

Lastly, after iodination of α-Bgt by the method of Pressman and Eisin (1950), [^{125}I]α-Bgt was separated from unreacted

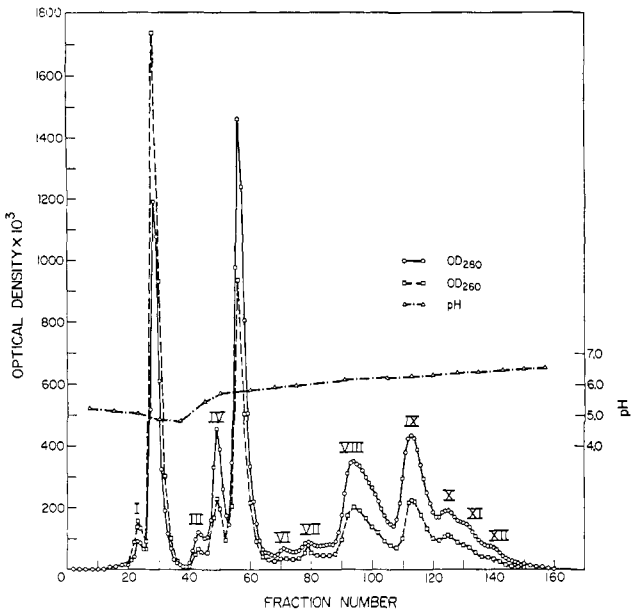

FIGURE 3: Gradient elution chromatography of crude Bgv on carboxymethylcellulose using a column 2.5 × 20 cm and an ammonium acetate gradient as described in Figure 2. Peak V was found to be α-Bgt.

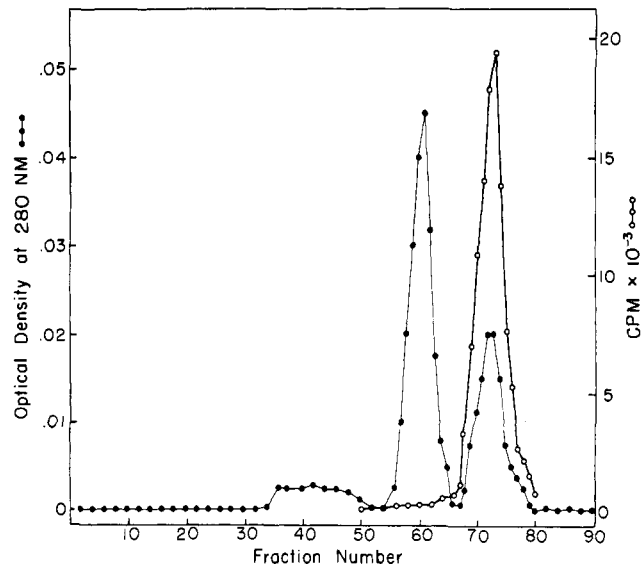

FIGURE 4: Gradient elution chromatography on carboxymethylcellulose of the reaction products from the iodination of α-Bgt. Elution was carried out on a 2.5 × 30 cm column using a 1.2-l. pH 5.8 gradient from 0.1 to 0.4 M ammonium acetate. Fractions of 6.6 ml were taken, absorbance of the fractions at 280 nm was determined, and 10-μl aliquots of the appropriate fractions were taken for the determination of radioactivity.

native toxin on Whatman CM-52. Desalted, lyophilized iodinated toxin was dissolved in 0.1 M NH_4OAc (pH 5.8) and applied to a CM-52 column (2.5 × 30 cm) equilibrated in the same buffer. A 1.2-l. NH_4OAc gradient (0.1 (pH 5.8) to 0.4 M (pH 5.8)) was run and 6.6-ml fractions were collected. Optical density at 280 nm of the fractions was recorded and radioactivity of 10-μl aliquots of the individual fractions was determined. The resulting elution profile is shown in Figure 4.

Characterization of Purified α-Bgt. The purified protein (5.9 mg) was taken for end-group analysis following the method of Stark and Smyth (1963) for amino terminii. The only modifications were (a) the use of 5 M guanidine hydrochloride as the denaturing agent and (b) determination of the presence of hydantoins by monitoring the effluent from Dowex 1 columns at 230 nm instead of taking the suggested volume cuts.

Amino acid analysis of the alkaline hydrolysis products of the recovered hydantoins were done according to the method of Spackman *et al.* (1958). Purified α-Bgt buffered at pH 8.0 was tested for the presence of free sulfhydryl groups by the method of Ellman (1959) using DTNB.

Electrofocusing of [^{125}I]*α-Bgt.* Purified, salt-free, [^{125}I]Bgt (2 μl; specific activity 42 × 10^6 cpm/mg) was applied to a 110-ml electrofocusing column (LKB 8101) and electrofocused at 6° according to the method of Vesterberg and Svensson (1966). The pH range of ampholine used was pH 7–10, at a final ampholine concentration of 1%, w/v. Voltage was maintained at 400 V. At the end of 20 hr, the current was steady, and 0.6-ml fractions were collected. Every other fraction was analyzed for radioactivity and the pH of every fifth fraction was measured in a Radiometer pH meter 26 at 6°.

Preparation and Purification of Antisera to α-Bgt. Purified toxin (0.1 mg) in Freund's adjuvant (complete) was injected weekly into male rabbits. Immunization of the animals was begun by injecting the toxin dissolved in nonpyrogenic 0.9% saline subcutaneously along the backs of the animals; subsequently intramuscular injections were made weekly into the thighs of the rabbits for a period of 3 months. Rabbits exhibited appreciable titer, as demonstrated by the precipitin test, 1 week after initiation of the intramuscular injections and were thereafter bled weekly 6 days following the intramuscular injection.

Immunoglobin G was precipitated from pooled rabbit sera by the addition of saturated ammonium sulfate to 40% saturation. The resulting precipitate was dissolved in 0.15 M NaCl, dialyzed against 0.15 M NaCl, and after clarification of the dialysate by centrifugation was reprecipitated by addition of saturated ammonium sulfate to 33% saturation. The immunoglobin thus obtained was either (a) dialyzed against 0.15 M NaCl and used without further purification or (b) was dialyzed against 0.10 M Tris·HCl (pH 8.0) containing 0.15 M NaCl. The material as obtained in step b was then chromatographed on G-200 in 0.10 M Tris·HCl (pH 8.0) containing 0.15 M NaCl. Fractions were pooled as indicated (Figure 5) and only the second pool (fractions 35–53) was used in further studies.

Antiserum specific to α-Bgt was isolated by precipitation of the sera by iodinated [^{125}I]α-Bgt. Anti-Bgt sera (5 ml; 4.40 mg/ml), which had been purified by ammonium sulfate fractionation and chromatography on Sephadex G-200, was precipitated by the addition of 178 μg of [^{125}I]α-Bgt (specific activity 25.8 × 10^6 cpm/mg). The toxin–antibody solution was incubated at 37° for 8 hr. At the end of this time, the solution was allowed to stand at 4° overnight, then the precipitate was centrifuged, washed twice with cold 0.15 M NaCl, and the washed precipitate was dissolved in 2 M deionized urea containing 0.55 M formic acid. The dissolved antigen–antibody complex was chromatographed on Sephadex G-200 (2.5 × 45 cm), equilibrated in the above buffer, resulting in the elution profile shown in Figure 6. The amount of toxin bound to immunoglobin was determined by ^{125}I counts while protein concentration was determined, after pooling the appropriate fractions and dialyzing against 0.15 M NaCl, by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

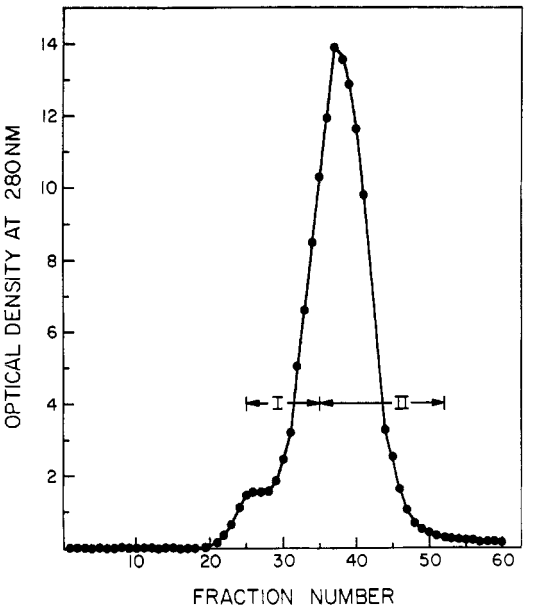

FIGURE 5: Chromatography of twice ammonium sulfate fractionated anti-α-Bgt sera on a Sephadex G-200 column (2.5 × 70 cm) using 0.10 M Tris·HCl (pH 8.0) containing 0.15 M NaCl. Fractions of 4.5 ml were taken. Protein was pooled as indicated; the second pool being essentially free of macroglobulin (IgM).

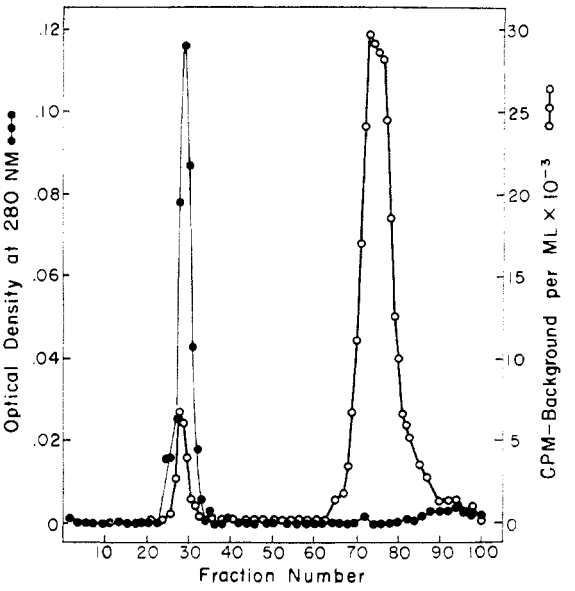

FIGURE 6: Partial separation of [^{125}I]α-Bgt from anti-α-Bgt sera which had been ammonium sulfate fractionated and chromatographed on Sephadex G-200. Chromatography at 4° was carried out on Sephadex G-200 using a 2.5 × 45 cm column eluting with 2 M urea–0.55 M formic acid. Fractions of 2.0 ml were taken.

Preparation of α-Bgt Immunosorbent. Coupling of α-Bgt antiserum to Sepharose 2B was performed according to the method of Givol *et al.* (1970). Sepharose containing 25 or 77 mg of antiserum protein per ml of packed gel was used to determine [^{125}I]α-Bgt binding capacity and conditions for removal from the immunosorbent. Immunosorbent (0.5 ml) was packed in a Pasteur pipet plugged with glass wool at the tip, equilibrated with buffer in 1% Triton X-100, and an aliquot of [^{125}I]bungarotoxin was added. Buffer (32 column volumes) was then washed through the column at room temperature, finishing with a buffer containing 0.1% Triton X-100. The toxin was then eluted from the immunosorbent column using conditions of low pH, high salt, or both. Regeneration of the gel after such treatment was measured by its ability to again adsorb toxin. The conditions and results of these experiments are shown in Table I.

Electrophysiological Characterization of Purified Toxin. Purified α-bungarotoxin and [^{125}I]α-bungarotoxin were assayed for biological activity on the frog rectus abdominis. The muscle was dissected out and immersed immediately in frog Ringer's solution (0.11 M NaCl–1.9 mM KCl–1.2 mM $CaCl_2$–2.4 mM $NaHCO_3$). One end of the muscle was pinned to a beeswax-filled cup and the other end hooked with a Nichrome wire to an FT03 force transducer. Solutions of acetylcholine freshly prepared in Ringer's were pipetted onto the dorsal side of the muscle, and the muscle contractions were recorded with a Grass polygraph. In each assay, the responses of the muscle to 10^{-6}, 10^{-5}, and 10^{-4} g per ml of acetylcholine were first recorded. After thorough rinising in Ringer's, the muscle was then immersed in the toxin and allowed to soak for 30 min. After this time, the toxin was removed and the response of the muscle to the acetylcholine solutions was determined. In those cases where the degree of reversibility of Bgt blocking was tested, the muscle was rinsed in Ringer's dripping at a constant rate from a separatory funnel into the cup.

The effect of α-bungarotoxin antibody on toxin binding to the frog rectus adbominus muscle was tested in two ways. In one series of experiments the antibody was added to the muscle–toxin preparation after the muscle had soaked 30 min in the toxin. In a second series, antibody was added to the toxin solution and the mixture was allowed to stand 10 min before immersing the muscle for 30 min.

Results and Discussion

Purification of α-Bungarotoxin. Purification of crude Bgv by starch electrophoresis has been shown by Chang and Lee (1963) to separate the crude venom into components designated α, β, and γ, as well as an AcChE component. The separation of crude Bgv by starch electrophoresis in our hands gave an additional two components designated I and II (Figure 1). Components I and II were not Lowry positive and were presumably not detected by Chang and Lee. Assays for AcChR blocking activity showed that only the component labeled α was effective against frog rectus abdominis muscle as described in a later section. Chromatography of the combined supernatants from the center fractions of this peak on Sephadex G-50 resulted in a single major peak and a small component chromatographing in the void volume which exhibited a high level of AcChE activity. The major peak from Sephadex G-50 chromoatgraphy was slightly asymmetric, and chromatography of this peak on CM-52 (Figure 2), using gradient elution, revealed the presence of approximately five minor contaminants in addition to the major fraction.

Fractionation of crude Bgv on CM-52 (Figure 3) by gradient elution gave eight well-defined components and four or five minor fractions as determined by their absorbance at 260 and 280 nm. Lowry analysis for protein in the effluent agreed with these observations except in the case of components I and II which were not detected; components I and II also gave higher extinction at 260 nm than at 280 nm. Peak V in this fractionation of crude Bgv corresponds to α-Bgt as previously isolated by starch electrophoresis followed by gradient elution on CM-52 columns. On testing for AcChR block-

TABLE I: Binding Capacity, Conditions of Toxin Elution, and Regeneration Characteristics of Anti-α-Bgt Immunosorbent.

Immunosorbent	Toxin (μg)	% of Toxin Bound	Eluent[a]	Percentage of Bound Counts Eluted under Condns 1–9	Deg of Regeneration (%)
77 mg of antibody/ml of Sepharose 2B	177	80	1	100	
	277	80	1	100	50
	540	66	1	100	65
	750	80	1	100	
25 mg of antibody/ml of Sepharose 2B	17	82	1	100	90
	17	95[b]	2	66	100
	17	97[b]	3	78	100
	17	95[b,c]	4	81	100
	17	85	5	6	
	17	97[b]	6	58	
	17	86	7	50	
	17	77	8	100	20
	17	98[b]	9	3	
	17	95[c]	4	75–81	100

[a] Eluents: 1, 0.55 M formic acid–0.15 M NaCl–0.1% Triton; 2, 0.50 M acetic acid–Ringers solution (Karlin, 1967)– 0.1% Triton; 3, 1.0 M acetic acid–Ringers solution (Karlin, 1967)–0.1% Triton; 4, 2.0 M acetic acid–Ringers solution (Karlin, 1967)–0.1% Triton; 5, 0.10 M $(NH_4)_2CO_3$ (pH 10.0)–0.1% Triton; 6, 0.90 M NH_4OH–0.15 M NaCl–0.1% Triton; 7, 3 M guanidine·HCl (pH 6.0)–0.1% Triton; 8, 6 M guanidine·HCl (pH 6.0)–0.1% Triton; and 9, 8 M urea (deionized)–0.1% Triton. [b] Rate of toxin load onto immunosorbent column decreased by a factor of 3, to 17 μg of toxin/ml per min. [c] Resin prewashed with 8 M deionized urea, reequilibrated in 1% Triton–Ringers.

ing activity, maximum effect was found in component V (Figure 3) with partial activity in component IV. AcChE assays revealed two activity maxima, one before and one after peak V.

Gradient elution chromatography of iodinated α-Bgt on CM-52 (Figure 4) clearly separated [^{125}I]α-Bgt from native toxin and toxin decomposition products produced during the course of the iodination reaction. The elution profile, as determined by the optical desnity of the eluent fractions at 280 nm, revealed that the yield of [^{125}I]α-Bgt was, in this instance (Figure 4), 35% of the toxin reacted. Indeed, chromatography of several different lots of iodinated toxin has demonstrated variability of the yield of iodinated toxin between 10 and 35%.

Chemical Characterization. An amino acid analysis of a 24-hr 6 N HCl hydrolysis of 30 nmoles of α-Bgt purified by starch electrophoresis followed by gradient elution on CM-52 is shown in Table II. The results agree well with those of Lee (1970) with the exception of the values for valine and possibly tryptophan. Within experimental error, no free sulfhydryls could be detected with the Ellman reagent (DTNB); the cysteine content of the toxin is listed in Table II as half-cystine. The minimum molecular weight calculated from these data is 7904 and agrees well with that known for other elapid neurotoxins (Lee, 1970) and with an appropriate molecular weight of 8000 obtained by electrophoresis of purified α-Bgt in 12.5% polyacrylamide gels (10% cross-linked, containing 0.1% SDS and 8 M urea) (Swank and Munkres, 1971). Additionally, the basic nature of purified α-Bgt was demonstrated by the amino acid content and agrees well with a p*I* of 9.19 obtained from electrofocusing experimenta on [^{125}I]α-Bgt.

Determination of the N terminus of the purified α-Bgt by the cyanate method of Stark and Smyth (1963) resulted in release of equimolar quantities of isoleucine and *allo*-isoleucine. The amino terminus of α-Bgt is therefore isoleucine in agreement with Lee (1970). We estimate on the quantitative basis of this result that there can be no more than 1% impurity present in α-Bgt prepared by starch electrophoresis followed by chromatography on CM-52.

Toxin-Specific Antibody. Following ammonium sulfate fractionation of pooled anti-α-Bgt sera, remaining macroglobulin was separated from the lower molecular weight immunoglobin G (IgG) on Sephadex G-200. Pool II (Figure 5) was used for subsequent studies. Isolation of antibody specific for [^{125}I]α-Bgt from the purified anti-α-Bgt sera was attempted for the purposes of (a) demonstrating the antigenicity of [^{125}I]α-Bgt toward anti-α-Bgt antibody and (b) establishing the approximate fraction of toxin-specific IgG in the total igG pool.

Figure 6 illustrates the partial separation of the toxin-specific antibody–antigen complex into free and high molecular weight bound [^{125}I]α-Bgt, and IgG. Previous studies by Chang and Yang (1969) on antibodies made against purified cobrotoxin showed that, of the immunoglobins, only IgG was produced against that protein toxin. The fact that in this study macroglobulins had also been removed prior to precipitation of antibody by antigen leads to the conclusion that the radioactive peak of [^{125}I]α-Bgt chromatographing with the principal protein peak, as monitored by optical desntiy at 280 nm, must represent a solubilized toxin–IgG complex which was not dissociated in 2 M urea–0.55 M formic acid.

The principal protein peak in Figure 6 as monitored by optical density at 280 nm represents 1.2 mg of IgG as determined by the method of Lowry *et al.* (1951). This quantity of toxin specific antibody represents 5% of the total IgG pool.

Electrophysiology. Purified bungarotoxin in concentrations of 10^{-5} and 10^{-6} g per ml completely abolished the muscle re-

TABLE II: Amino Acid Composition of α-Bgt.

Amino Acid	nmoles Found		Mole Ratio Based on Phe or Lys[a] (Nearest Integer)	
	A	B[a]	A	N[a]
Asp	133.5		4	
Thr	208.4		7	
Ser	181.7		6	
Glu	166.2		5	
Pro	275.5		8	
Gly	133.6		4	
Ala	159.8		5	
Cys	337.1		10	
Val	130.9		4	
Met	31.1		1	
Ile	48.0		2	
Leu	65.3		2	
Tyr	65.0		2	
Phe	34.1		1	
His	59.7	38.4	2	2
Lys	195.0	133.8	6	6
Arg	99.1		3	
Trp		43.8		2
Calculated molecular weight[b]			7904	

[a] Calculated by hydrolyzing in the presence of mercaptoacetic acid and comparing to lysine. [b] Exclusive of amide NH_3.

sponse to all concentrations of acetylcholine used. Rinsing with 1 l. of Ringer's for 2 hr did not restore the muscle response. [^{125}I]α-Bungarotoxin (10^{-5} g/ml) also completely and irreversibly abolished all muscle response, while 10^{-6} g/ml of [^{125}I]bungarotoxin was not sufficient to completely block the muscle response to 10^{-4} g/ml of acetylcholine.

About 10^{-3} g/ml of specific anti-α-bungarotoxin antibody added to muscle which had incubated 30 min in 10^{-6} and 10^{-5} g/ml of [^{125}I]α-bungarotoxin was unable to reverse the acetylcholine response inhibition by the toxin. Similarly, 3.25×10^{-3} g/ml of specific antibody did not reverse blockade produced by 10^{-5} g/ml of [^{125}I]α-Bgt or α-Bgt. After preincubation for 10 min with specific antibody in concentrations up to 3×10^{-3} g/ml, 10^{-5} g/ml of α-bungarotoxin was minimally effective in blocking the acetylcholine response. The results from both types of experiment indicate that at least some of the antigenic sites on α-bungarotoxin probably do not overlap with the acetylcholine receptor specific sites of the toxin or that receptor toxin binding is considerably firmer than is toxin antibody binding.

Toxin-Specific Immunosorbent. Immunsorbent was successfully produced from twice ammonium sulfate fractionated IgG after chromatography on Sephadex G-200 (Figure 5); coupling to Sepharose 2B was accomplished in yields of 95–99%.

Conditions for eluting bound toxin from the immunosorbent (Table I) indicate the necessity of low pH or high concentrations of selected salts. Previous studies on immunosorbents (Givol *et al.*, 1970; Melchers and Messer, 1970) have used 8 M urea, 20% formic acid, guanidine hydrochloride, or 0.8 M ammonium hydroxide to elute bound antigen. Our experience with anti-α-Bgt immunosorbent indicates that conditions of high salt are not as useful as low pH conditions. Conditions of low pH such as pH 1.8 (0.55 M formic acid) allow quantitative elution of toxin while at pH 2.5 (1–2 M acetic acid) toxin was eluted almost quantitatively. Both conditions leave immunosorbent fully regeneratable.

Because of the demonstrated ability of toxin-specific Sepharose immunosorbent to bind[125]I-labeled toxin under specific conditions and to further release it upon changes in eluting solvent, it seems worthwhile to investigate the possibility of isolating acetylcholine receptors employing this approach.

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