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BIOLOGICAL PROPERTIES OF STAPHYLOCOCCAL α-TOXIN

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SUMMARY

The biological properties of staphylococcal α-toxin were examined.

1. We have confirmed that α-toxin, at a concentration of 1 μg/ml, induced spastic paralysis of isolated mammalian smooth muscle, which was not released by isoproteranol and was not responsive to added Ca^{2+} or K^+.

2. α-Toxin had no effect at high concentrations (20–100 μg/ml) on membrane ATPases, proton translocation in mitochondria, or *Mycoplasma* plasma membrane integrity.

3. Artificial lipid spherules (liposomes) containing egg or beef lecithin or total extracted lipid from erythrocytes were disrupted by α-toxin at 15–30 μg/ml. No selectivity of toxin action was demonstrated for liposomes composed of rabbit erythrocyte lipid. α-Toxin released low-molecular-weight internal marker, and, at higher concentrations, it also released macromolecular marker from liposomes.

4. It is suggested that α-toxin's action toward membranes may not be fully explained by a postulated interaction with membrane lipid.

INTRODUCTION

Staphylococcus aureus is known to produce a number of extracellular toxic proteins [1]. One of these, staphylococcal α-toxin, has recently been isolated in homogeneous form [2], and the physical properties [3] and binding kinetics to red cell membranes [4] reported. Despite the recognized problem of impurities and contamination of various published preparations of α-toxin [5,6], most workers are in agreement that α-toxin is a specific protein possessing multiple biological properties. Thus, α-toxin is partially defined by its capacity to lyse rabbit erythrocytes, which it does preferentially relative to sheep, rat or human erythrocytes. α-Toxin is reported, also, to be cytotoxic for a variety of cultured mamalian cells [7] and lethal for laboratory animals [6]. The capacity of alpha toxin to induce contraction and paralysis of smooth muscle, which has been termed "spastic paralysis", has been demonstrated in a number of laboratories [8, 9]. In contrast to these properties, many of the reported biological activities of alpha toxin such as lysis of bacterial spheroplasts [10], lysis of mycoplasma [11], enhancement of Mg^{2+}-dependent

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mitochondrial ATPase [12,13] have been challenged as being due to the presence of staphylococcal δ-toxin which was a contaminant of the α-toxin preparations used [14,15]. Likewise, the lysis of artifical lipid spherules, which was proposed as a useful model for the study of α-toxin-induced membrane disruption [16], was later considered uncertain also due to the detection of δ-toxin in the α-toxin preparations [17]. Finally, α-toxin has been reported to inhibit ($Na^+ + K^+$)-activated mitochondrial ATPase in various tissues of a variety of animals [18].

Since many of the biological properties attributed to α-toxin may be due to contaminants in the various preparations of the toxin [14,15,17] and since we now have a procedure that permits the isolation of α-toxin in pure form [2] it was of interest to reexamine the biological properties of purified staphylococcal α-toxin preparation. The results of this study are presented below.

METHODS AND MATERIALS

Staphylococcal α-toxin

Staphylococcal α-toxin was prepared by the method of Six and Harshman [2]. Form B was used exclusively, and typically had a specific hemolytic activity of 20000 hemolytic units/mg of protein when assayed on rabbit erythrocytes by the method of Bernheimer [5].

Reagents

Purified egg lecithin and beef lecithin were obtained from Sylvania Laboratories, Millburn, N.J. Cholesterol was obtained from Sigma Chemical Co., St. Louis, Mo., and dicetyl phosphate was obtained from K and K Laboratories, Plainview, N.J. Total membrane lipids were obtained from freshly drawn rabbit and human erythrocytes by Bligh-Dyer extraction as modified by Kinsky et al. [19] and were dissolved and stored in chloroform at 4 °C, under nitrogen.

Assay of smooth muscle contraction

The procedure followed was that of Hurwitz and Joiner [20]. Isolated smooth muscle of the guinea pig ileum was prepared and 3-cm-long segments were affixed to a standard kymograph under approximately 0.35 g of tension. The muscle was bathed in 10 ml physiological salt solution at 32 °C. The physiological salt solution in mM consisted of: NaCl, 125; KCl, 2.7; $CaCl_2$, 1.8; glucose, 11; and Tris buffer 23.8. The pH was adjusted to 7.5 and bathing solution saturated with oxygen.

Assay of ($Na^+ + K^+$)-dependent ATPase

Guinea pig kidney cortex cell membranes were prepared and assays of ATPase activity were performed as described by Hegyvary and Post [21]. Assays contained 1 ml of incubation medium (100 μmoles NaCl, 25 μmoles KCl, 4 μmoles $MgCl_2$, 4 μmoles ATP, and 26 μg membrane protein in 30 mM imidazole glycylglycine, pH 7.4). A similar medium contained 0.5 μmole of ouabain in place of NaCl and KCl to estimate ($Na^+ + K^+$)-insensitive ATPase activity. α-Toxin was added at final concentrations of 0–100 μg/ml and the tubes were incubated for 20 min at 37 °C. The reaction was terminated and samples assayed for inorganic phosphate on a Technicon AutoAnalyzer as described by Hegyvary and Post [21].

Assay of Ca^{2+} uptake and ATPase activity in sarcoplasmic reticulum

Isolated vesicles were prepared from the sarcoplasmic reticulum of rabbit skeletal muscle and Ca^{2+} uptake and total ATPase activity were assayed as described by Meissner and Fleischer [22]. α-Toxin at concentrations of 0–20 μg/ml was preincubated with the vesicles for 5 min at 32 °C in 2 ml of a mixture containing 0.1 M KCl, 5 mM $MgCl_2$, 0.1 mM $CaCl_2$, and 10 mM histidine (pH 7.3) and then assayed for Ca^{2+} uptake and total ATPase activity

Assay of respiratory driven proton translocation

Rat liver mitochondria were prepared by the zonal centrifugation method for the preparation of sub-cellular organelles as described by Smigel and Fleischer [23]. The assay medium was 1 mM PIPES buffer, pH 6.5, 0.25 M sucrose, 0.04 M KCl, 0.02 M $MgCl_2$, 0.01 M $MnCl_2$, 2 mM disodium succinate and 6.7 μg/ml oligomycin. The assay was performed by the method of Mitchell [24]. In each case, the mitochondria at 4 mg protein per ml were preincubated for 10 min at 37 °C either with no reagent, with α-toxin (B), 44 μg/ml, with phospholipase-C, 33 μg/ml, or with carbonylcyanide *m*-chlorophenylhydrazone (CCCP), 0.7 μg/ml. The change of pH of the suspension on introduction of O_2 was continuously recorded.

Mycoplasma growth conditions and incubation with α-toxin(B)

Mycoplasma strain Y (Goat Y) (25) was the kind gift of Dr Alan Rodwell. The organism was grown in Brain Heart Infusion Medium (1.9 BHI powder per 40 ml water, 2.5 ml Gibco Yeast Extract, 7.5 ml serum). Either horse or rabbit serum were used in separate cultures. A 25-ml culture of the organism was incubated for three days at 37 °C. The cells were centrifuged at 30000 × *g* for 20 min and the pellets were washed three times with 10 ml 0.15 M NaCl. The cells were finally suspended in 0.15 M NaCl (0.05 M potassium phosphate, pH 7.2) containing 1 mg/ml bovine serum albumin to give a final absorbance at 450 nm of 0.300. The suspensions were incubated at 25 °C with α-toxin(B) in spectrophotometer cuvettes and the decrease in absorbance at 450 nm was used as a measure of cell lysis.

Liposome preparation and assay of internal marker release

Liposomes were prepared and marker release was assayed essentially by the methods described by Kinsky et al. [19] and Katoaka et al. [26]. Either 4 μmoles of egg or of beef lecithin, 3 μmoles cholesterol and 0.4 μmole dicetyl phosphate were dissolved in chloroform and liposomes were prepared by the method of Kinsky [19]. Glucose was used as the internal marker but in some cases hexokinase was also incorporated together with glucose, as described by Katoaka et al. [26]. The swelled liposomes were then freed of untrapped marker by passage through a 1.5 cm × 25 cm Sephadex-G-200 column in 0.15 M NaCl (0.05 M Tris buffer, pH 7.5). In some cases, lipids extracted from rabbit or human erythrocytes were used to prepare liposomes and in those cases 4 μmoles of phospholipid as judged by total phosphorus assays were used without any further added sterol or other lipid for the preparation of liposomes. Final concentration of phospholipid in the liposome preparations after combination of peak fractions from the Sephadex-G-200 column was approximately 1 mM. The assay for marker release by α-toxin(B) was carried out by preparing a series of test tubes containing α-toxin(B) dissolved in 0.15 M NaCl,

20 mM Tris, pH 7.5 (Tris-buffered saline) and adding 100 μl of the liposome suspension. Before adding the liposome suspension, the volume of each tube was adjusted with Tris-buffered saline to give a final reaction volume of 160 μl. Spontaneous glucose release was measured by incubation of 100 μl liposome suspension with 60 μl of Tris-buffered saline. In control tubes, 100% glucose release was affected either by the addition of 10 μl of 10% (v/v) Triton X-100 in water and heating to 100 °C for 30 s or by incubation with 50 μg/ml phospholipase C. After an incubation of 30 min at 25 °C, marker release was assayed in the hexokinase–glucose-6-phosphate dehydrogenase coupled system as described by Kataoka et al. [26]. In all assays, values for internal marker release were corrected for the small amount of spontaneous marker release which occurred during the incubation period.

Phospholipid assay

Phospholipid concentration was obtained by $HClO_4$ hydrolysis and assay for total phosphorous by the method of Gerlach and Denticke [27].

RESULTS

Staphylococcal α-toxin has been found to exist in two molecular forms having identical amino and carboxyl terminals [2, 3]. We have already demonstrated [4] that both molecular forms of the toxin are 20-fold less hemolytic for sheep erythrocytes and 100-fold less hemolytic for human erythrocytes relative to rabbit cells in the hemolytic assay. Since the two forms have identical hemolytic activity and specificity and since form B naturally occurs as 80% of the α-toxin isolated from the culture medium [2], form B (α-toxin(B)) was used throughout the following experiments.

Contraction of mammalian smooth muscle

The contractile effect of α-toxin on smooth muscle has been reported by a number of laboratories [6], most recently by Brown and Quillian [8] and Wurzel et al. [9]. The type of contraction of isolated smooth muscle preparations is known as "spastic paralysis", a slow irreversible contraction. Using a preparation of guinea pig smooth muscle responsive to acetylcholine, we find that α-toxin (B) at a concentration of 1 μg/ml causes a slow partial contraction which is maintained. The muscle retains responsiveness to acetylcholine, although a new baseline of contraction has been established. Additional α-toxin (10 μg/ml final concentration) induces an 80% of maximum contraction that is maintained even upon addition of isoproteranol, a powerful muscle relaxant. It was also observed that after treatment with α-toxin, the preparation remains viable, as indicated by its further contraction with acetylcholine, although its responsiveness to Ca^{2+} or K^+ addition has been abolished.

α-Toxin effect on membrane-bound ($Na^+ + k^+$)-dependent ATPase

Electrophoretically homogeneous α-toxin(B) was examined for its effect on the ($Na^+ + K^+$)-dependent ATPase in membranes from guinea pig kidney cortex. As shown in Table I, α-toxin(B) at a concentration of 100 μg/ml, which is a 4-fold excess of toxin to total membrane protein, has no significant effect on the ($Na^+ + K^+$)-dependent ATPase activity of this membrane.

TABLE I

EFFECT OF α-TOXIN(B) ON Na^+/K^+-DEPENDENT ATPase ACTIVITY OF GUINEA PIG KIDNEY MEMBRANE

Na^+/K^+-dependent ATPase activity was measured as described by Hegyvary and Post [21].

α-Toxin(B) (μg/ml)	Membrane protein (μg/ml)	μg α-toxin(B)/ μg membrane protein	ATPase activity*	Specific activity**
0	25.9	–	0.052	2.0
10	24.7	0.38	0.050	2.0
25	27.5	1.01	0.043	1.6
50	27.5	1.81	0.048	1.7
100	23.5	4.20	0.043	1.8

* μmoles ATP hydrolysed/ml per min.
** μmoles ATP hydrolysed/mg membrane protein per min.

α-Toxin effect on Ca^{2+} uptake and Ca^{2+}-dependent ATPase

The sarcoplasmic reticulum regulates the contraction–relaxation cycle of skeletal muscle by releasing and accumulating calcium. Since α-toxin is known to induce spastic paralysis in muscle, it was of importance to test the action of α-toxin directly on the enzymes involved in Ca^{2+} transport. Isolated sarcoplasmic reticulum vesicles energized by ATP are capable of accumulating Ca^{2+} from the medium [22]. It can be seen in Table II that α-toxin has no effect on energized Ca^{2+} uptake or on Ca^{2+}-stimulated ATPase activity even at equal weights of α-toxin to sarcoplasmic protein.

TABLE II

EFFECT OF α-TOXIN(B) ON Ca^{2+} UPTAKE AND ATPase ACTIVITY OF SARCOPLASMIC RETICULUM OF RABBIT SKELETAL MUSCLE

Ca^{2+} uptake (in the presence of 5 mM oxalate) and total ATPase activity were measured as described by Meissner and Fleischer [22].

α-Toxin(B)		Ca^{2+} uptake (μmoles Ca^{2+}/mg protein)	ATPase activity (μmoles P_i/mg protein per min)
(μg/ml)	(μg α-toxin(B)/μg SR protein)		
–	–	3.9	1.0
5	0.33	3.8	0.9
10	0.67	3.9	1.05
20	1.33	3.7	1.2

α-Toxin effect on mitochondrial membrane function

In the past, a number of laboratories have reported that α-toxin has disruptive effects on functions of the mitochondrial membrane. One of the most delicate assays of mitochondrial performance is respiratory-driven proton translocation. Mitchell [24] has shown that mitochondrial electron transport drives proton trans-

location across the membrane from the matrix side to the outside medium. If rat liver mitochondria are suspended in an anaerobic assay mixture containing substrate (succinate), and electron transport is initiated by pulsing a known amount of oxygen into the system, there is a rapid lowering of the pH of the system concomitant with electron transport. When electron transport stops, there is a gradual return to the original pH, due to passive proton permeability of the mitochondrial membrane. These two processes are presented in Fig. 1, panel A. As can be seen the extent of pH shift is proportional to the amount of oxygen admitted to the system. Preincubation for 10 min at 37 °C with α-toxin (Fig. 1, panel B) has no effect on proton translocation or on passive proton permeability, and it can be inferred that the toxin also has no effect on electron transport or the integrity of the membrane compartment in mitochondria. As controls, incubation with phospholipase C or the uncoupler, carbonylcyanide *m*-chlorophenylhydrazone, Fig. 1, panels C and D, completely interrupt respiratory-driven proton transport. Similar experiments, not shown, with submitochondrial vesicle preparations also indicate no interaction with α-toxin.

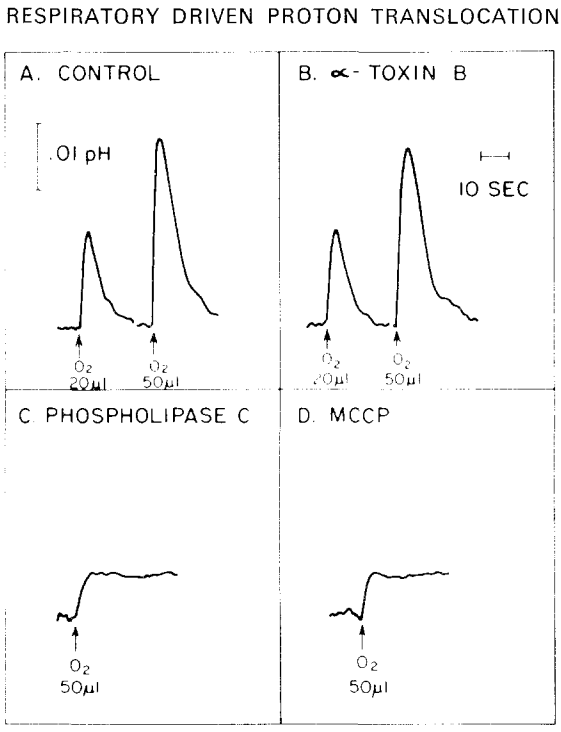

Fig. 1. Mitochondrial suspensions were pre-incubated anaerobicly for 10 min at 37 °C with the respective reagents and proton translocation was initiated by adding aliquots of water saturated with atmospheric O_2 at 37 °C. The vertical scale indicates decreasing pH of the incubation medium. (A) No reagent; (B) α-toxin (B), 44 µg/ml; (C) phospholipase C, 33 µg/ml; (D) carbonylcyanide *m*-chlorophenylhydrazone, 0.7 µg/ml (CCCP). CCCP was synthesized by P. G. Haytler [37] and generously supplied to S. Fleischer. Total volume of the reaction mixture was 1.5 ml. For further details, see text.

α-Toxin effect on mycoplasma plasma membrane

Due to a previous report [1] that α-toxin disrupts *Mycoplasma*, *Mycoplasma* strain Y, a derivative of *M. mycoides* which cannot synthesize fatty acids but must incorporate them from the growth medium [25], was tested for its susceptibility to the toxin. At a concentration of 150 μg/ml, α-toxin was found to have no lytic effect on suspensions of *Mycoplasma* strain Y, over a 180-min incubation at 25 °C, as judged by absorbance measurements of the suspensions at 450 nm. Identical results were obtained when the organism was grown in media containing either horse or rabbit serum as the source of fatty acid. Fig. 2.

α-Toxin effect on liposomes

Since the observation by Weissmann et al. [16] that high concentrations of a partially purified α-toxin preparation released trapped marker anions or glucose from artificial lipid spherules, a number of laboratories have used this phenomenon as a model for studying the interaction of α-toxin with biomembranes. However, it has remained unclear whether or not α-toxin or a contaminant is responsible for marker release [17].

To resolve this question electrophoretically homogeneous α-toxin(B) was incubated for 30 min at 25 °C with liposomes composed of purified egg lecithin, cholesterol, and dicetyl phosphate (2:1.5:0.2 molar ratio). Fig. 3 shows that relatively high concentrations of α-toxin, 20–40 μg, induce marker (glucose) release. In comparison experiments, heat treatment of α-toxin(B) at 100 °C for 3 min, which inactivates the toxin, or preincubation of the toxin with anti-α-toxin (B) rabbit serum reduced marker release significantly. 22 μ of α-toxin preheated at 100 °C released 2% of the glucose, while 22 and 44 μg of toxin treated with specific antisera released only 10 and 15% of the glucose respectively. Control preincubation of the toxin with normal rabbit serum did not affect α-toxin activity, which at these concentrations of toxin releases approximately 80% of the available glucose.

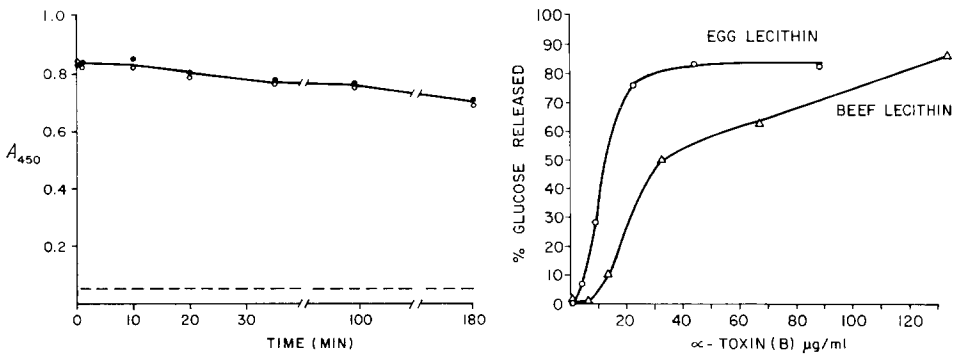

Fig. 2. α-Toxin (B) was added at 0 min and incubated at 25 °C with *Mycoplasma* stain Y. ○, *Mycoplasma* no addition; ●, *Mycoplasma* + 150 μg/ml α-toxin(B); – – – –, *Mycoplasma* + 0.05% sodium dodecylsulfate.

Fig. 3. Liposomes were prepared as described in Methods, and the final concentration of phospholipid in the incubation mixture was 0.6 mM. Liposomes were incubated at 25 °C and assayed for internal glucose release.

To investigate further the specificity of α-toxin action of liposomes, lipid spherules prepared with egg lecithin were compared with those made with beef lecithin. 50% of the internal marker, glucose, was released from egg lecithin liposomes when the toxin concentration was 15 μg/ml and from beef lecithin liposomes at a toxin concentration of 30 μg/ml (Fig. 3). Since staphylococcal α-toxin has been historically identified by the relative sensitivity of rabbit erythrocytes to toxin-induced hemolysis, it was of interest to compare the sensitivity to α-toxin of liposomes prepared from lipid extracts of erythrocytes of different species. As seen in Fig. 4, liposomes prepared from total lipid extracts of rabbit and human erythrocytes are equally sensitive to toxin-induced marker release. Indeed, the sensitivity to α-toxin of liposomes prepared from lipids obtained from membrane extracts appears to be very similar to that of artificial egg lecithin liposomes in that they all display 50% marker release at about 15 μg/ml α-toxin (B).

Recently, Kataoka et al. [26] have used enzymes as macromolecular internal markers in studying antibody–complement-dependent damage to liposomal model membranes. In order to determine whether or not α-toxin-induced liposomal damage released a macromolecular marker, α-toxin(B) was incubated with liposomes containing both glucose and hexokinase (mol. wt 102000). Although α-toxin released both internal markers, Fig. 5, it did not release them in parallel fashion. While 50% release of the hexokinase marker required a concentration of 40–50 μg/ml α-toxin (B), 50% of the glucose was released at a concentration of 15 μg/ml of toxin.

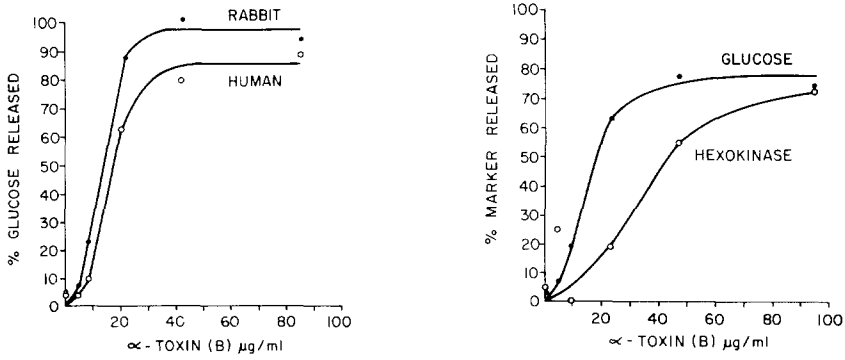

Fig. 4. Erythrocyte membrane lipids were extracted and liposomes were prepared as described in Methods. Final concentration of phospholipid in each case was 0.6 mM. Assay was as in Fig. 3.

Fig. 5. Liposomes were prepared by swelling rabbit erythrocyte membrane lipid containing 4 μmoles of phospholipid in 0.4 ml of 0.3 M glucose which contained 350 μg/ml hexokinase. Liposomes were freed of untrapped markers and incubations with α-toxin (B) were performed as in Methods.

DISCUSSION

The "spastic paralysis" of mamalian smooth muscle by staphylococcal α-toxin has been reexamined using a toxin preparation of demonstrated homogeneity. Our results essentially confirm those reported by Wurzel et al. [9]. In addition it is of interest to note that the α-toxin-contracted muscle does not respond upon addition of relatively high concentrations of K^+ or Ca^{2+} but does contract to

maximum upon addition of acetylcholine. The toxin-treated muscle is paralysed in that even the addition of isoproteranol does not relieve the contraction. The molecular mechanism underlying this phenomenon remains unknown.

The guinea pig kidney cortex membrane system of Hegyvary and Post [21] was chosen to investigate the effect of α-toxin on membrane-bound $(Na^+ + K^+)$-dependent ATPase for several reasons. First, the isolated membranes have a high level of ATPase activity. Second, it had been reported that α-toxin has cytopathic effects on rabbit kidney cells growing in monolayer culture [28]. And finally, it had been reported that α-toxin on injection into the whole animal, has an inhibitory effect on the $(Na^+ + K^+)$-dependent ATPase activity in guinea pig kidney [18]. Our results indicate that, at least in this tissue, purified α-toxin(B) does not interact with the plasma membrane in a manner affecting $(Na^+ + K^+)$-dependent ATPase activity and we suggest that such activities reported in the literature are due to contaminants in the α-toxin preparations used in the experiments.

We have extended the observations of α-toxin on membrane bound ATPases to show that α-toxin(B) has no effect on the Ca^{2+}-dependent ATPase of isolated vesicles of rabbit skeletal muscle sarcoplasmic reticulum. It is of interest that the capacity of such vesicles to transport Ca^{2+} was also unaffected by α-toxin even at concentrations of α-toxin that were equal to the total μg of sarcoplasmic reticulum protein in the assay.

A number of reports have appeared demonstrating various effects of α-toxin on isolated mitochondria [13], and on oxidative phosphorylation and ion transport in isolated tissue preparations [18,29–31]. Recently, Rahal [15] has reported, in a comparison of the effects of purified α-toxin and δ-toxin, that many of the previously reported disruptions of mitochondrial membrane function could be explained on the basis of δ-toxin contaminant in α-toxin preparations. Indeed, in many cases, proof of homogeneity of α-toxin preparations has not been presented. We have confirmed Rahal's conclusion that purified α-toxin does not interact with the mitochondrial membrane even when measured by the very sensitive respiratory-driven proton translocation assay of Mitchell [24].

The observation that α-toxin(B) fails to lyse *Mycoplasma* is in agreement with recent literature [17]. Using strain Y, which is metabolically defective, such that the organism must utilize lipid from the medium for membrane formation [25], we were unable to detect any lytic effect of α-toxin. This was true even when the cells were grown in rabbit serum, the species of choice for hemolytic assays.

Although accumulating evidence suggests that lipid may not be the primary membrane binding site for α-toxin, our results show that α-toxin(B) can interact with lipid spherules (liposomes) composed of lecithin, cholesterol, and dicetyl phosphate and with liposomes composed of lipid extracted from erythrocyte membranes. The interaction was measured by the release of both small molecular markers and macromolecular markers. However, despite the known 100-fold difference in sensitivity to α-toxin of rabbit erythrocytes as compared to human erythrocytes, no difference in sensitivity to the toxin was observed between liposomes composed of lipids extracted from rabbit or human blood cells. In contrast to our results, a number of investigators [32] have successfully employed the liposome experimental system to correlate the selective action of toxins or antibiotics on biomembranes with the presence of specific lipids in susceptible membrane types. Thus, Hsu Chen

and Feingold [33] observed that the antibiotic polymixin B, which has a selective disruptive effect on membranes of bacteria and little effect on membranes of mammalian origin, specifically disrupts liposomes composed of lipid extracted from *Escherichia coli* membranes. The source of this selective action was shown to be due to the presence of phosphatidylethanolamine in susceptible membranes. Our observation that no analogous selectivity exists for α-toxin action on liposomes containing rabbit or human erythrocyte lipid indicates that interaction with membrane neutral or phospholipid [34] is not the source of selective α-toxin action.

Buckelew and Colaccio [35] have suggested that the selectivity of α-toxin toward erythrocytes of different species may be based on electrostatic interactions of the toxin with negatively charged moieties on the surface of erythrocytes such as sialic acid. In their view, such an interaction would prevent lytic activity by preventing penetration on the toxin molecule into the lipid environment of the membrane. It has been shown by use of radiolabeled α-toxin, however, that the total binding of α-toxin to erythrocytes of different species is correlated with its lytic activity [4]. Also, an 8-fold difference in erythrocyte membrane sialic acid between rabbit and human erythrocytes [36] may not be sufficient to explain a 100-fold difference in sensitivity to α-toxin. Further, we have observed (Cassidy, P., unpublished) that treatment of rabbit and human erythrocytes with trypsin (1 mg/ml) at 37 °C for 60 min, or influenza virus PR-8 which removes 60 and 49% respectively, of membrane sialic acid from human erythrocytes, does not alter α-toxin activity or selectivity toward the cells.

The liposomal experimental system in the study of staphylococcal α-toxin has been employed by a number of investigators since 1966 [6]. In describing the original observation, Weissmann et al. [16] stated that "The direct action of α-toxin upon synthetic lipid membranes is sufficient to explain its effects on erythrocytes..." Later investigators of α-toxin's interaction with liposomes have continued, in general, to support this assumption. In view of our observation that the liposomal system has not proven to be a useful model in studying α-toxin selectivity for biomembranes, we suggest that while an interaction of α-toxin with membrane lipid may be necessary, it may not be sufficient to explain the toxin's activity.

The data presented have shown that staphylococcal α-toxin is much more specific in its interaction with certain biomembranes than was originally believed. This narrowing of the effects of α-toxin suggests that it may serve as a very specific probe for the presence of specific receptors [4] in biomembranes or for the presence of specific structuring of membrane components. Investigations to test this possibility are in progress.

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