Biochimica et Biophysica Acta, 1105 (1992) 125–130

BBAMEM 75557

Calcium ion-mediated regulation of the α-toxin pore of *Staphylococcus aureus*

Hiroko Tokunaga and Taiji Nakae

Department of Cellular Information Sciences, Tokai University School of Medicine, Isehara (Japan)

(Received 22 May 1991)
(Revised manuscript received 4 December 1991)

Key words: α-Toxin monomer; Pore formation; Calcium ion; Liposome; (*S. aureus*)

The water-soluble α-toxin monomers of *Staphylococcus aureus* become hexamers forming the transmembrane pore when exposed to the membranes. This pore is freely permeable to small hydrophilic molecules, e.g. carboxyfluorescein, and becomes less permeable in the presence of calcium ions. Calcium ion-mediated decrease of the carboxyfluorescein leakage could not be eliminated by EDTA added in the medium, but the carboxyfluorescein could be freed by EDTA added in the intraliposomal space. This result suggests that the α-toxin pore changes its conformation as the calcium ion is bound and that the binding site is exposed to the intraliposomal side of the membrane. The interaction between the α-toxin hexamer and 8-anilino-1-naphthalenesulfonic acid (ANS) was monitored by determining the fluorescence in the presence and absence of calcium chloride. The mean distances between the tryptophan residues of the α-toxin hexamer and the bound ANS were calculated to be 1.90 and 1.80 nm in the absence and presence, respectively, of calcium ions. The results showed the calcium ion mediated conformational change of the membrane-embedded α-toxin hexamer.

Introduction

α-Toxin produced by *Staphylococcus aureus* exists as a water-soluble single polypeptide with M_r, 33000 [1]. This α-toxin damages biological membranes [2–5] and artificial lipid bilayers [6–8]. When these proteins are exposed to the membrane bilayer, the toxin molecules enter into the membrane, converting to a hexameric aggregate forming the transmembrane pore or channel [9,10]. The pore diameter was estimated to be 1.0 to 1.2 nm [11,12].

Calcium ion protects the α-toxin-mediated membrane damage in the biological membranes [13,14]. It is conceivable therefore that the calcium ion-mediated protection of membrane damage might be a consequence of the closing or constriction of the α-toxin channel. To test this possibility, we have determined the interaction of 8-anilino-1-naphthalenesulfonic acid (ANS) and the membrane-embedded α-toxin hexamer. We present here that calcium ion causes the conformational change of the membrane embedded α-toxin hexamer narrowing the channel.

Experimental procedures

Chemicals. Egg-yolk phosphatidylcholine (PC) was purchased from Sigma. Carboxyfluorescein (CF) and ANS were obtained from Wako Pure Chemicals (Tokyo). All other chemicals used were the highest purity grade available commercially.

Materials. Staphylococcal α-toxin was purified from culture supernatant of *Staphylococcus aureus* strain wood 46 grown in tryptic soy broth as described elsewhere [15]. Liposome was prepared from egg-yolk PC according to the procedure described earlier [16]. Since the interaction of α-toxin and liposome is largely influenced by the size of the liposome, we prepared the liposome by sonicating the lipid suspension for 5 min by the procedure reported earlier as liposome-5 [16]. The CF-containing liposome was prepared as above in the presence of 0.183 M CF and free CF was separated by gel filtration as reported earlier [7]. The buffer solution used throughout this study contained 10 mM *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid (Hepes) buffer (pH 7.0), 150 mM NaCl and 3 mM sodium azide (Buffer A).

Abbreviations: ANS, 8-anilino-1-naphthalenesulfonic acid; PC, phosphatidyl choline; CF, carboxyfluorescein; Hepes, *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid.

Correspondence: T. Nakae, Department of Cellular Information Sciences, Tokai University School of Medicine, Isehara 259-11, Japan.

Fluorescence measurement. Fluorescence intensity of CF was monitored at 520 nm as described earlier [7]. For the study of α-toxin–ANS interaction, a liposome suspension ($4.4 \cdot 10^{-8}$ mol of phospholipids) and α-toxin were mixed together in 900 μl of buffer A and kept at 23°C for 30 min. Calcium chloride was added to the mixture and kept at 23°C for more than 15 min, if needed. An appropriate concentration of ANS solution (100 μl) was added. Fluorescence emission was monitored at 480 nm at excitation wavelength 380 nm using a Hitachi 650-10S fluorescence spectrophotometer at 22°C at slit length 4 and 6 nm for excitation and emission, respectively, and light path 10 mm.

Results

Effect of calcium ion on the α-toxin-mediated CF-leakage

When α-toxin monomers were mixed with the liposome containing CF, the fluorescence at 520 nm increased immediately (Figs. 1A, arrow 1; 1B, arrow 1), confirming the previous results [7]. Calcium chloride at 8 mM decreased the CF leakage (Fig. 1B, arrow 2). Addition of EDTA in the medium could not reverse the calcium effect (Fig. 1B, arrow 3). The drop in fluorescence intensity at the time EDTA was added is due to dilution. Velocity of the CF leakage was monitored by the rate of fluorescence increment at 520 nm.

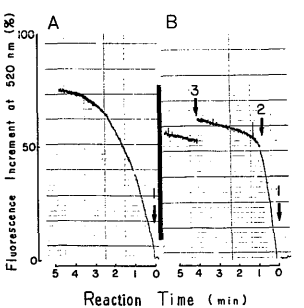

Fig. 1. α-Toxin-mediated CF release and effect of calcium ions. Liposomes were prepared from egg-yolk PC entrapping CF and passed through a sepharose 4B column (0.9×60 cm) as described in Experimental Procedures. Liposomes ($3.2 \cdot 10^{-10}$ mol of phosphorus) were suspended in 1 ml of Buffer A. To this was added 120 μl of α-toxin monomers ($1.08 \cdot 10^{-9}$ mol) and fluorescence at 520 nm was recorded immmediately at an excitation wavelength of 490 nm (A, 1 and B, 1). The cuvette was held at 22°C by circulating temperature-controlled water. Arrows 1, 2 and 3 indicate the addition of α-toxin, 100 μl of 0.1 M calcium chloride and 100 μl of 0.1 M EDTA, respectively.

When $1.35 \cdot 10^{-9}$ mol of α-toxin monomers were added to the suspension of the CF-entrapped liposomes ($3.2 \cdot 10^{-10}$ mol phospholipids entrapping $8.35 \cdot 10^{-12}$ mol of CF), the initial rate of CF release was $1.25 \cdot 10^{-11}$ mol/min as calculated from a tangent of fluorescence curve. The rate of CF release became $5.61 \cdot 10^{-12}$ mol/min after 40 s. This value is equal to 45% of the initial velocity. The initial velocities of CF leakage became 28 and 91% of that of α-toxin alone, as α-toxin was added to the liposomes preincubated with 4.3 mM calcium chloride and 4.3 mM EDTA, respectively (Table IA). Neither EDTA nor calcium chloride alone at 5 mM showed detectable CF leakage. In the next experiment, calcium chloride and/or EDTA was added to

TABLE I

Effect of calcium ion on the α-toxin-mediated CF release

(A) Experimental conditions were similar to those described in the legend to Fig. 2. The listed materials were added in the written order. Fluorescence was recorded at 520 nm as described in Experimental procedures and the legend to Fig. 1. The initial rate of CF release was recorded immediately after the α-toxin addition.
(B) Experimental conditions were similar to those in (A). Calcium chloride was added at 15 s after the α-toxin and the fluorescence recording started at 40 s after the α-toxin. The CF-release rate was calculated from the curve at 40 s after the α-toxin addition. The final concentrations of calcium chloride and EDTA were 2.2 mM.
(C) Liposomes ($2.5 \cdot 10^{-10}$ mol phosphorus) containing 0.15 M CF and 0.1 M EDTA were mixed with $9.3 \cdot 10^{-10}$ mol (C-1) or $5.6 \cdot 10^{-10}$ mol (C-2) of the α-toxin monomers and the rate of CF release was monitored. The rate of CF release was calculated at 90 s after the α-toxin. The final concentration of calcium chloride was 4.7 mM.

Reaction mixture	Rate calculated at time (s) after α-toxin addition	CF release (μmol/min, $\times 10^{-6}$)
(A)		
Liposome	0	0
Liposome + α-toxin	0	12.5
Liposome + 4.3 mM EDTA + α-toxin	0	11.4
Liposome + 4.3 mM $CaCl_2$ + α-toxin	0	3.46
Liposome + 5 mM $CaCl_2$	0	0
Liposome + 5 mM EDTA	0	0
(B)		
Liposome + α-toxin	40	5.61
Liposome + α-toxin + $CaCl_2$	40	1.18
Liposome + α-toxin + $CaCl_2$ + EDTA	40	1.04
(C-1)		
Liposome(EDTA) + α-toxin	90	2.17
Liposome(EDTA) + α-toxin + $CaCl_2$	90	1.78
(C-2)		
Liposome(EDTA) + α-toxin	90	1.34
Liposome(EDTA) + α-toxin + $CaCl_2$	90	1.22

Effect of calcium chloride and EDTA on the diffusion of p-nitrophenyl phosphate through the α-toxin pore

(D) Liposomes were prepared in the presence of 20 units alkaline phosphatase as described in the text and were suspended in the solution containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl and 3 mM sodium azide and 10 mM *p*-nitrophenyl phosphate. The reaction was started by the addition of $4.09 \cdot 10^{-10}$ mol of α-toxin and after 3 min and 6 min calcium chloride and EDTA, respectively, were added. The diffusion rate of *p*-nitrophenyl phosphate was determined spectrophotometrically at 405 nm. The final concentrations of calcium chloride and EDTA were 5 mM.

	Enzyme activity (nmol product/min per μmol phosphatidylcholine)
Experiment I	
Liposome + α-toxin	14.05
Liposome + α-toxin + $CaCl_2$	10.77
Experiment II	
Liposome + α-toxin	16.79
Liposome + α-toxin + $CaCl_2$	11.33
Liposome + α-toxin + $CaCl_2$ + EDTA	10.79
Liposome + EDTA + α-toxin	14.43
Liposome + EDTA + α-toxin + $CaCl_2$	14.07
Liposome	0.43
Total enzyme activity	18.74

the mixture of the liposome and α-toxin, and the velocity of CF leakage was determined at 40 s later. Calcium chloride at 2.4 mM lowered the velocity at 40 s to 21% of toxin alone. While addition of the toxin, calcium chloride and EDTA had no detectable effect compared with the toxin and calcium chloride (Table IB). Since the calcium-mediated decrease of CF release could not be eliminated by the subsequently added EDTA, it is likely that calcium ion bound to the EDTA-inaccessible site of α-toxin hexamer.

In the next experiment, the liposomes entrapping both CF and EDTA were mixed with α-toxin and the fluorescence was measured 90 s later, in the absence and in the presence of 4.7 mM $CaCl_2$ (Table IC). The rate of the CF-release in the presence of calcium chloride was 82 to 91% of that without calcium chloride. This result together with the experiments shown in Table IB, suggests that calcium chloride bound to the α-toxin, where EDTA was only accessible from the intravesicular side (trans) of the membrane. Calcium chloride did not interfere the toxin-hexamer formation, since the EDTA-entrapped liposomes showed 82 to 91% of the CF release by the α-toxin treatment in the presence of calcium chloride (Table IC).

To test the permeability of small solute through the toxin pore in the presence of calcium, the accessibility of *p*-nitrophenyl phosphate to the intraliposomal alkaline phosphatase was determined [17]. As shown in Table ID, *p*-nitrophenyl phosphate was about 70% permeable in the presence of calcium ions compared with the permeability without calcium chloride. This result suggests that the α-toxin pore is constricted in the presence of calcium chloride. When the liposome was incubated with EDTA and the toxin and calcium chloride were subsequently added, the permeability of *p*-nitrophenyl phosphate appeared to be comparable to the liposome incubated with EDTA and α-toxin.

Effect of calcium chloride concentration on the α-toxin-mediated CF release was examined. Calcium chloride at 0.8 mM to 30 mM inhibited CF release about 76% to 84% (Fig. 2).

The state of the calcium chloride-treated α-toxin in the liposome membrane

Since the above experiments suggested that calcium chloride caused the change of the α-toxin pore configuration, we studied the protein conformation in the presence and absence of calcium ions. When ANS was mixed with the liposome suspension, the fluorescence intensity increased 15-times and the emission maximum shifted from 520 nm to 480 nm (Fig. 3B). These results indicate that ANS was entrapped in the hydrophobic environment. The fluorescence intensity of ANS increased further at 480 nm in the liposome containing α-toxin hexamer (Fig. 3A). The fluorescence at 336 nm is the emission from tryptophan residues of the α-toxin molecule (Fig. 3A).

The fluorescence emission shown in Fig. 3A consists of three components. They are fluorescences of free

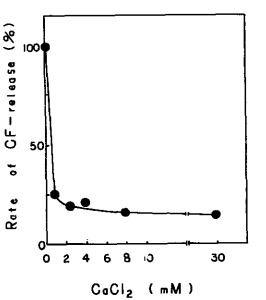

Fig. 2. Effect of calcium chloride on the α-toxin-mediated CF release. Liposomes ($3.2 \cdot 10^{-10}$ mol of phosphorus) were mixed with α-toxin monomers ($1.35 \cdot 10^{-9}$ mol) in 1.15 ml of Buffer A. After tipping α-toxin, the indicated amounts of calcium chloride was added to the mixture as indicated in Table IB. Fluorescence was measured as described in the legend to Fig. 1, 40 s after the α-toxin addition. The initial rate of CF release was expressed relative to that without calcium chloride.

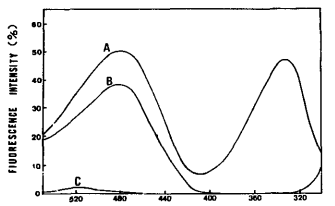

Fig. 3. Fluorescence emission spectra of ANS in the liposome and the liposome containing α-toxin. Buffer A was mixed with the following materials. Curve (A), Liposomes ($5.83 \cdot 10^{-8}$ mol of phosphorus) and α-toxin monomer ($4.4 \cdot 10^{-9}$ mol) were preincubated at 23°C for 15 min, then ANS was added. Curve (B), Liposomes ($5.83 \cdot 10^{-8}$ mol of phosphorus) were mixed with ANS. Curve (C), ANS was added to the buffer. The final volume was adjusted to 1.0 ml at an ANS concentration of 20 μM. The emission spectra were recorded at the fixed excitation wavelength of 284 nm.

ANS, of ANS in the liposome membrane and of ANS bound to the α-toxin in the liposome membrane.

Therefore, the total fluorescence F_{480} may be expressed as

$$F_{480} = F_l + F_p + F_f \quad (1)$$

where F_{480} is the relative fluorescence intensity at 480 nm, and l, p and f refer to the fluorescence intensities of ANS bound to lipid, protein and unbound, respectively. Since the F_f fraction is negligibly small at 480 nm and is included in the F_l, F_{480} can be simplified to

$$F_{480} = F_l + F_p \quad (2)$$

Therefore, the fluorescence intensity due purely to the α-toxin-ANS complex may be calculated by subtracting F_l from F_{480}.

Fig. 4 shows the emission spectra of the ANS in the presence of α-toxin in the membrane. When the increasing amounts of ANS were added to the α-toxin-intercalated liposomes, the fluorescence emission at 480 nm increased, excitation at 284 nm. The tryptophanyl fluorescence at 336 nm was quenched. The fluorescence of free tryptophan or ANS was not influenced in the presence of calcium ions (data not shown). The fluorescence emission at 480 nm in the mixture containing α-toxin-intercalated liposome was determined in the presence and absence of calcium chloride. The extents of fluorescence decrement at 336 nm due to α-toxin were plotted against reciprocals of ANS concentrations (Fig. 4, inset). The results of experiments in the presence and absence of 5 mM calcium chloride gave fairly linear traces. The contribution of unpolymerized monomers (40% of the initially added monomers) to these values were subtracted, by calculating from the amount of the hexamers formed (Fig. 6). The energy transfer efficiencies were 0.606 and 0.526 in the presence and absence of calcium chloride, respectively. The mean apparent separation (R) between the tryptophan residues of α-toxin in the liposome membrane and the bound ANS molecules was calculated tentatively from the energy transfer efficiency according to the equation proposed by Förster [18].

$$E = R^{-6}/R^{-6} + R_0^{-6} \quad (3)$$

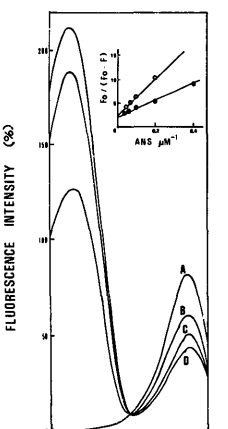

Fig. 4. Fluorescence emission spectra of ANS and α-toxin in the liposome membrane. Liposomes ($5.83 \cdot 10^{-8}$ mol of phosphorus) in 1 ml of Buffer A were mixed with $4.4 \cdot 10^{-10}$ mol of the α-toxin monomers in the presence of 5 mM calcium chloride. ANS was added after keeping the mixture at 23°C for 15 min and the fluorescence emission spectrum was recorded immediately at the fixed excitation wavelength of 284 nm. Curves: A, no ANS; B, 10 μM ANS; C, 20 μM ANS; D, 30 μM ANS. (Inset) The fluorescence emission spectra were recorded in the presence and absence of 5 mM calcium chloride as above. F_0 and F were fluorescence intensities (arbitrary units) of tryptophan residues (336 nm) of the toxin in the absence and presence, respectively, of the added ANS. Excitation wavelength was 284 nm. $F_0/(F_0 - F)$ was plotted against the reciprocal of the ANS concentration. ○——○, Experiments in the absence of calcium chloride. ●——●, Experiments in the presence of calcium chloride.

where R_0 is the distance at which the energy transfer efficiency is 50%.

$$R_0^6 = (8.78 \cdot 10^{-25}) \cdot K^2 Q n^{-4} J \ (\mathrm{cm}^6) \quad (4)$$

where Q is the quantum yield of the donor in the absence of the acceptor, K^2 is the orientation factor of dipole-dipole interaction, n is the refractive index of the protein and J is the overlap integral between the donor emission and the acceptor absorption on the wavenumber scale. J was calculated according to the method of Wright and Takahashi [19].

The J value appeared to be $86.39 \cdot 10^{-16}$ cm^3 M^{-1}. A quantum yield of α-toxin in phospholipid membrane was determined to be 0.067 using 0.78 mol% α-toxin in 58.3 nmol/ml of the egg-yolk PC liposome. A quantum yield of free tryptophan under the same conditions, 0.2 in 10 mM Hepes buffer (pH 7.0) was used for calculation. An n value of 1.6 was adopted from that of a protein [20] and the K^2 value was assumed to be 2/3 in a randomly oriented pair of donor and acceptor [21]. For α-toxin-intercalated liposomes, R_0 was found to be 1.93 nm. By using this value, the mean distances between tryptophan residues in the hexamer and bound ANS molecules were calculated to be 1.9 nm and 1.8 nm in the absence and presence of calcium ions, respectively. These results suggest that the environment of tryptophan residues of α-toxin hexamer was altered in the presence of calcium ions.

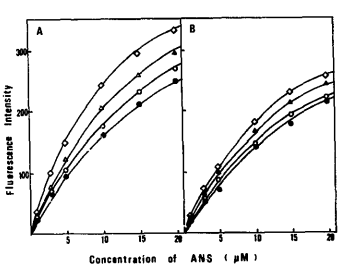

Fig. 5. Effect of ANS concentration and calcium ion on the ANS fluorescence emission in the presence of various concentrations of α-toxin hexamer. Liposomes ($4.4 \cdot 10^{-8}$ mol of phosphorus) and the indicated amount of α-toxin were mixed in 900 μl of Buffer A and incubated at 23°C for 30 min. The mixture was incubated for a further 15 min in the presence (A) or absence (B) of 5 mM calcium chloride and the fluorescence was recorded immediately after the addition of the indicated amounts of ANS. Excitation and emission wavelengths were set to 380 nm and 480 nm, respectively. Protein concentrations were: ○——○, 9.2; △——△, 16.8; and ◇——◇, 30.5 μg/ml. ●——●, Without α-toxin.

Quantitative determination of the ANS and α-toxin interactions

ANS readily interacts with the α-toxin incorporated into the liposome membrane (Fig. 3). However, quantitative determination of the ANS–α-toxin interaction remained to be assessed. We quantified the fluorescence increment of ANS in the liposome containing different amounts of α-toxin. Fluorescence caused by the interaction of ANS and liposome was subtracted from that in the presence of α-toxin. Fluorescence increased 3 to 5%, 16 to 20% and 22 to 29% over the fluorescence of ANS and liposome upon addition of 9.2 μg, 16.8 μg and 30.5 μg α-toxin, respectively (Fig. 5B). In the presence of 5 mM calcium chloride, these values became 8%, 16 to 25%, 32 to 49% at the α-toxin concentrations 9.2 μg, 16.8 μg and 30.5 μg, respectively (Fig. 5A). These results indicate that the α-toxin hexamer in the presence of calcium ions was bound with more ANS molecules than that in the absence of calcium chloride.

Discussion

When water-soluble α-toxin monomers of *Staphylococcus aureus* come into contact with membranes, the toxin enters the membrane forming a hexameric aggregate and a transmembrane aqueous pore [1]. We have reported earlier that the presence of phospholipids with unsaturated fatty acids and the physical states of the membrane are important in the toxin hexamer assembly [5]. The α-toxin pore allows the passage of various small hydrophilic molecules [22].

Millimolar concentration of calcium chloride protected target membranes from the α-toxin action [13,14]. These observations suggest the following three possibilities: (i) calcium ion interferes the binding of α-toxin to the membrane, (ii) calcium ion blocks the α-toxin hexamer formation, (iii) calcium ion blocks the solutes leakage. The last possibility may have two alternative states: (iii-a) calcium ions simply block the pore plugging in the pore interior, and (iii-b) calcium ion alters the α-toxin conformation, changing the fully dilated state of the pore to the constricted state. Possibility (i) was ruled out by the earlier report [13,23]. Possibility (ii) seems unlikely, since the presence of the α-toxin hexamers in the membrane is readily demonstrable by SDS-acrylamide gel electrophoresis (Fig. 6). The amount of the hexamer appeared to be the same in the absence and presence of calcium ion (Fig. 6). Though we can not rule out possibility (iii-a), possibility (iii-b) seems most likely.

Menestrina demonstrated that the ion conductive channels formed by α-toxin on a planner lipid bilayer were inactivated by di- and trivalent cations [12]. We have confirmed this observation with the calcium chloride-mediated decrement of the CF leakage (Figs. 1

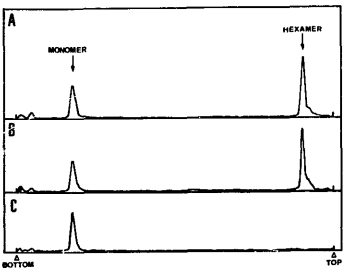

Fig. 6. Densitometric traces of stained electrophoretograms of the reaction mixtures. Liposomes and α-toxin monomers were mixed without (A) or with 5 mM $CaCl_2$ (B) as described in the legend to Fig. 5. The mixture was subjected to SDS-acrylamide gel electrophoresis. The gel was stained with Coomasie brilliant blue. (C) α-Toxin monomer without membrane.

and 2). The permeability of *p*-nitrophenyl phosphate was lowered to 67 to 75% of that without calcium in the presence of calcium ions. Calcium ion-mediated inhibition of CF leakage differs from that of *p*-nitrophenyl phosphate. The inconsistency may be due to the different assay method and the different properties of the test solutes. We prefer to interpret above result as to the conformational change of α-toxin. The α-toxin-mediated leakage of another fluorescent dye, thionin, was tested. The undetectably low leakage of thionin was supposed to be due to the positively charged solute. When ANS was added to the α-toxin hexamer in the liposome membranes, we observed the fluorescence increment at 480 nm. This fluorescence increment was altered in the presence of calcium ions. It was also shown that this calcium effect could be reversed by EDTA added to the intravesicular space, but not by the exogeneously added EDTA (Table IB, IC). Although it is premature to speculate on the calcium binding site, the most likely site would be either interior of the pore distal to the pore mouth, or the trans-side of the hexamer. The binding of calcium to the toxin induces the conformational change of the toxin molecules resulting in constriction of the channel. If the binding affinity of calcium to the toxin is higher than that of EDTA, the above hypothesis may not be valid.

One may argue that the observed fluorescence change at 480 nm is due to the interaction between ANS and the unpolymerized α-toxin monomers. This argument seems less plausible, since incubation of α-toxin monomers and ANS shows little influence on fluorescence at 480 nm. Furthermore the contribution of ANS-monomer complex to the total fluorescence at 480 nm is negligibly small (data not shown). ANS does not cause the hexamer to monomer transition. When the hexamer in the liposome were incubated with ANS, the amount of hexamer was unchanged. Calcium ion-mediated alteration of function of the membrane pore is rare to our knowledge. Recently, it is shown that the pore function of the δ-endotoxin of *Bacillus thuringiensis* is sensitive to calcium ions [24]. A well characterized case is the calcium ion-dependent closing of the gap junction pore [25]. It is of interest, therefore, to study the structural similarity between the α-toxin hexamer and the gap junction hexamer and their conformational change by calcium ions.

References

1 Thelestam, M. and Blomqvist, L. (1988) Toxicon 26, 51–65.
2 Bernheimer, A.W., Kim, K.S., Remsen, C.C., Antanavage, J. and Watson, S.W. (1972) Infect. Immun. 6, 636–642.
3 Füssle, R., Bhakdi, S., Sziegoleit, A., Tranum-Jensen, J., Kranz, T. and Wellensiek, H.-J. (1981) J. Cell. Biol. 91, 83–94.
4 Thelestam, M. (1983) Biochim. Biophys. Acta 762, 481–488.
5 Reichwein, J., Hugo, F., Roth, M., Sinner, A. and Bhakdi, S. (1987) Infect. Immun. 55, 2940–2944.
6 Cassidy, P., Six, H.R. and Harshman, S. (1974) Biochim. Biophys. Acta 332, 413–423.
7 Ikigai, H. and Nakae, T. (1987) J. Biol. Chem. 262, 2156–2160.
8 Belmonte, G., Cescatti, L., Ferrari, B., Nicolussi, T., Ropele, M. and Menestrina, G. (1987) Eur. Biophys. J. 14, 349–358.
9 Tobkes, N., Wallace, B.A., and Bayley, H. (1985) Biochemistry 24, 1915–1920.
10 Rogolsky, M. (1979) Microbiol. Rev. 43, 320–360.
11 Bhakdi, S., Muhly, M. and Füssle, R. (1984) Infect. Immun. 46, 318–323.
12 Menestrina, G. (1986) J. Membr. Biol. 90, 177–190.
13 Harshman, S. and Sugg, N. (1985) Infect. Immun. 47, 37–40.
14 Bashford, C.L., Alder, G.M., Menestrina, G., Micklem, K.J., Murphy, J.J. and Pasternak, C.A. (1986) J. Biol. Chem. 261, 9300–9308.
15 Ikigai, H. and Nakae, T. (1985) Biochem. Biophys. Res. Commun. 130, 175–181.
16 Ikigai, H. and Nakae, T. (1987) J. Biol. Chem. 262, 2150–2155.
17 Tokunaga, M., Tokunaga, H. and Nakae, T. (1979) FEBS Lett. 106, 85–88.
18 Förster, T. (1966) in Modern Quantum Chemistry (Sinanoglu, O., ed.), Section 3B, pp. 93–137, Academic Press, New York.
19 Wright, K. and Takahashi, M. (1977) Biochemistry 16, 1548–1554.
20 McMeekin, T.L., Wilensky, M. and Groves, M.L. (1962) Biochem. Biophys. Res. Commun. 7, 151–156.
21 Stryer, L. (1978) Annu. Rev. Biochem. 47, 819–846.
22 Freer, J.H. and Arbuthnott, J.P. (1986) Toxins of *Staphylococcus aureus*, in: Pharmacology of Bacterial Toxins (Dorner, F. and Drews, J., eds.), pp. 581–633, Pergamon Press, Oxford.
23 Blomqvist, L. and Thelestam, M. (1986) Infect. Immun. 53, 636–640.
24 Knowles, B.H., Blatt, M.R., Tester, M., Horsnell, J.M., Carroll, J., Menestrina, G. and Ellar, D.J. (1989) FEBS Lett. 244, 259–262.
25 Unwin, P.N.T. and Ennis, P.D. (1984) Nature 307, 609–613.