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SURFACE PROPERTIES OF MEMBRANE SYSTEMS

TRANSPORT OF STAPHYLOCOCCAL δ-TOXIN FROM AQUEOUS TO MEMBRANE PHASE

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Summary

Hemolytic δ-toxin from *Staphylococcus aureus* was soluble in either water, methanol or chloroform/methanol (2 : 1, v/v). The toxin spread readily from distilled water into films with pressures (π) of 10 dynes/cm on water and 30 dynes/cm on 6 M urea; from chloroform/methanol it produced 40 dynes/cm pressure on distilled water. The toxin adsorbed barely from water (π = 1 dyne/cm) but it did rapidly from 6 M urea (π = 35 dynes/cm). The protein films had unusually high surface potentials, which increased with the film pressure and decreased with increasing both pH and urea concentration in the aqueous phase. The fluorescence of 1-aniline 8-naphthalene sulfonate with δ-toxin was much greater than that with RNAase and dipalmitoyl phosphatidylcholine itself, indicating probably a marked lipid-binding character of the toxin. By circular dichroism the α-helix content of δ-toxin was 42% in water, 45% in methanol, 24% in 6 M urea. Infrared spectroscopy showed predominant α-helix in both ^2H_2O and deuterated chloroform/methanol as well as in films spread from either solvent on ^2H_2O. In spreading from 6 M [^{2}H]urea, in which the major infrared absorption was that of [^{2}H]urea with peaks at 1600 and 1480 cm^{-1}, the δ-toxin film showed prevalently non-α-helix structures with major peak intensities at 1633 cm^{-1} > 1680 cm^{-1}, indicating the appearance of new β-aggregated and β-antiparallel pleated sheet structures in the film. The data prove that (1) high pressure protein films can consist of α-helix as well as non-α-helix structures and, differently from another cytolytic protein, melittin, δ-toxin does not resume the α-helix conformation in going into the film phase from the extended chain in 6 M urea; (2) conformational changes are important in the transport of proteins from aqueous to lipid or membrane phase; (3) δ-

* Abbreviation: ANS, 1-aniline 8-naphthalene sulfonate.

toxin is by far more versatile in structural dynamics and more surface active than α-toxin.

Introduction

Surface activity and penetration of cytolytic exotoxins of *Staphylococcus aureus* in plasma membranes and in other lipid-protein systems may be relevant to the study of bacterial infections of mammalian lung. We have already described some surface properties of α-toxin from *S. aureus*. In the ability to form a film at the air/water interface the α-toxin paralleled such lipid-bound proteins as the apoprotein of high density serum lipoprotein and the mitochondrial structural protein, and it was far superior to ordinary globular proteins such as ribonuclease or lysozyme [1,2]. Another potent toxin, melittin, from bee venom, also penetrated lipid/water interfaces [3]. Toxins of relatively small molecular weight and well defined amino acid sequences could serve as models in studies of lipid-protein interactions in membranes [4] as well as for the study of the mechanism of the toxins' membranolytic action.

Although it can be appreciated that the presence of protein alongside the lipid in the main architecture of the natural membrane requires that the protein possesses a very high surface activity, it was soon recognized that the protein which is isolated in various forms from the biological membrane and is presented to the air/water or to the lipid/water interface in the laboratory experiment may have undergone various conformational changes and thus may not express the surface activity otherwise expected from its orientation in the membrane. Accordingly it became important to compare the surface activity of the membrane proteins under different conditions of pH, urea and protein concentrations, organic solvent, temperature [4–6].

Contrary to previous reports [7,8], our staphylococcal α-toxin [1] did not lyse spheroplasts or protoplasts. Bernheimer [9] later suggested that some of the lytic and surface activities of α-toxin could be due to δ-toxin contamination in α-toxin preparations. δ-toxin indeed lyses spheroplasts and protoplasts, so did also some preparations of α-toxin [7,8], which, for reasons that will be discussed below, may have been contaminated with δ-toxin. In order to obtain some understanding of the similarities and differences between these toxins, it was thus important to examine the surface behavior and some related properties of staphylococcal δ-toxin.

Materials and Methods

Bacterial δ-toxin was obtained from cultures of *S. aureus* by the procedure described by Kreger et al. [10]. The protein appears to have a molecular weight of 103 000 in the absence of detergents; however, subunits of 20 000 and 5000 daltons were observed in non-ionic detergent and in guanidinium chloride, respectively [9,11].

Melittin from bee venon (Lot No. 15C-0022), synthetic dipalmitoyl phos-

phatidylcholine and salt-free RNAase were obtained from Sigma, St. Louis, Mo. Crystallized serum albumin was purchased from Miles Lab., Kankakee, Ill. Ultrapure urea (Lot No. EE 1565) was a Schwartz/Mann (Orangeburg, N.Y.) product. The sodium salt of ANS (1-aniline 8-naphthalene sulfonate) was obtained from Eastman Organic Chemicals (Rochester, N.Y.). Deuterium oxide (Lot No. 0746), [$^2H_4$]urea (Lot No. 7402), [^{2}H]chloroform (Lot No. 505) and [$^2H_4$]methanol (Lot No. 7022) were obtained from Stohler Isotope Chemicals, Rutherford, N.J.

Monolayer techniques. Organic solvents were spectral grade, and water was distilled twice, once over alkaline permanganate. Electrolyte solutions were foamed to remove surface active materials ordinarily present in commercial salts [12].

Determinations of surface pressure and surface potentials were made at the air/water interface by known techniques [12]. A sandblasted platinum blade measured the surface tension of the water without film (γ_0) and with film (γ); the surface pressure (π) was calculated as the difference between such values. A radioactive ^{266}Ra air electrode was used to measure the electrical potential V_0 and V across the clean water surface and the film covered surfaces, respectively; surface potential (ΔV) is the difference between such V values.

Films were formed by known techniques [5] in a circular trough at constant area either by spreading the protein from aqueous and organic solvents or by adsorption of the protein from its solutions in the aqueous subphase. The trough consisted of a crystallizing dish of Pyrex glass measuring a constant circular area of 58 cm^2.

Circular Dichroism (CD). The CD measurements were performed at 25°C, using a Cary Model 60 spectropolarimeter with a 6001 CD attachment. The degree of ellipticity in degree cm^2/dmol was calculated by the equation $[\theta] = \theta_{obs}$ (MRW)/10lc; l is path length in cm, c is concentration of protein in g/ml and mean residue weight (MRW), 113 g [13]. The percent helix was calculated by computer analysis according to established methods [13,14] and personal advice from Dr. I. Listowsky, Department of Biochemistry.

Infrared spectra. A Perkin-Elmer Model 21 Infrared Spectrophotometer was equipped with an Attenuated Total Reflectance attachment. A saturated film of δ-toxin was made on ^2H_2O by spreading from ^2H_2O, deuterated urea solution or deuterated chloroform/methanol (2 : 1, v/v). One single film was picked up on a KRS-5 thallium iodide-thallium bromide crystal, and infrared absorption was measured by multiple reflectance.

To remove the interference of the H-O absorbing frequencies, all solvents were deuterated. The protein was dissolved at a concentration of 1 mg/ml in ^2H_2O and exhaustively dialyzed against ^2H_2O. After removal of ^2H_2O by evaporation under a N_2 stream, the protein was redissolved in $C^2HCl_3/C^2H_3O^2H$ (2 : 1, v/v).

Fluorescence spectra. The fluorescence spectra were recorded from an Aminco Bowman spectrophotofluorimeter equipped with Xenon arc lamp and photomultiplier microphotometer. The excitation and emission wavelengths were 380 and 470 nm, respectively. The protein (1 mg/ml) was dissolved in 5 mM Tris · HCl buffer of given pH, 25°C. Under these conditions the fluorescence of ANS in the buffer was negligible [15].

Results

Monolayer experiments

Spread films. The toxin formed unusually stable, lipid-like films, with an equilibrium or collapse pressure of 40 dynes/cm when the protein was spread from organic solvent (chloroform/methanol (2 : 1, v/v)). In contrast, it exhibited a negligible surface activity when spread from either distilled water or ^2H_2O (Fig. 1). In the last two cases, the protein films had low surface pressures but relatively high surface potentials.

Adsorbed films. From a solution of 1 μg/ml in distilled water, δ-toxin adsorbed exceedingly slowly into films that, after 20 min had 1 dyne/cm pressure and 100 mV surface potential. Both π and ΔV values rose uniformly though slightly during that time. However, when the hypophase contained 6 M urea, with 1 μg/ml toxin, a saturation pressure of 35 dynes/cm was reached in the first 2 min and a pressure of 30 dynes/cm instantaneously, whereas the surface potential decreased linearly from 380 to 360 mV during 20 min (Fig. 2).

Influence of pH. Since pH is known to influence protein conformation [16], and since conformational changes may be important in the transport of proteins in and out of membranes [4,5], the effect of pH on the surface activity of δ-toxin was determined. With two quantities of protein applied on the aqueous phase, 0.35 and 0.68 μg/cm^2, both surface pressure and surface potential of the spread films of δ-toxin decreased linearly as the pH of the aqueous phase increased between 2 and 12 (Fig. 3). Both surface pressure and surface potential were higher at the greater surface concentrations of protein.

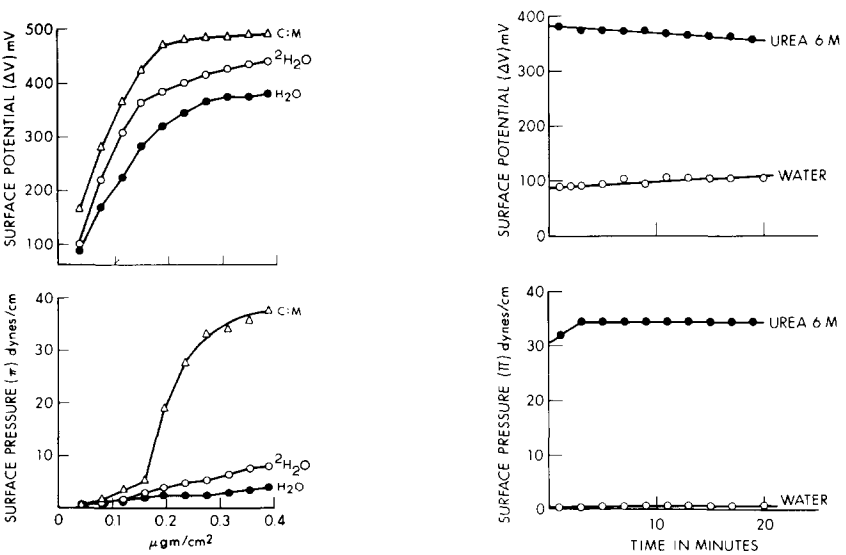

Fig. 1. Surface pressure and surface potential of δ-toxin spread films as a function of the quantity of toxin applied onto the water surface in μg/cm^2. Subphase, distilled water, 25°C. Spreading media: H_2O, ^2H_2O or chloroform/methanol (C : M) (2 : 1, v/v).

Fig. 2. Kinetic curves of surface pressure and surface potential of toxin films adsorbed from either distilled water or 6 M urea at 25°C.

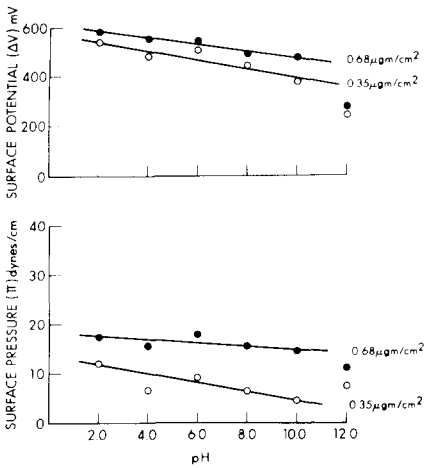

Fig. 3. Influence of hypophase pH on the surface pressure and surface potential of spread films of δ-toxin. The protein at a concentration of 1 mg/ml in distilled water was applied onto the surface of distilled water which contained various concentrations of HCl or NaOH at 25°C. The measurements were made at two surface concentrations of applied toxin: 0.35 and 0.68 $\mu g/cm^2$.

Influence of urea. Since urea is a modifier of protein conformation [16], and protein conformations may be important in the transport of protein from bulk phase to films [2,4—6] the effect of different urea concentrations was studied. The toxin film was spread from distilled water on a subphase containing various concentrations of urea. It appears that 2 M urea was as effective as 6 M urea, for they produced similar saturation values of surface pressure (30 dynes/cm) and surface potential (450 mV with 2 M urea and 410 mV with 6 M urea); however, less protein was required to reach the π and ΔV saturation values with 6 M than with 2 M urea. With 10 M urea in the subphase, the saturation pressure was the same, 30 dynes/cm, but the potential was lower than with less urea, and both π and ΔV curves showed signs of marked phase changes (Fig. 4).

Fluorescence spectra. The binding of fluorescent dyes to lipids and proteins has been used as a criterion of the hydrophobic character of lipid, protein and membrane structures. The intensity of fluorescence of ANS decreased with increasing pH, and at a given pH, e.g. 7.4, it varied: δ-toxin > dipalmitoyl phosphatidylcholine > RNAase (Fig. 5). The fluorescence of ANS with serum albumin (not shown) was many fold that of δ-toxin and followed a similar pH dependence.

Circular dichroism spectra. Knowledge of the toxin's conformation in various media is fundamental to understanding the mechanism of transport and structuring of the protein in membranes. Circular dichroism is one of several necessary and complementary approaches. The degree of ellipticity was related to wavelength in nm (Fig. 6); the percent α-helix was calculated at 222 nm. As expected of protein conformations in media of different dielectric constant [17], the α-helix content of toxin in bulk phase decreased with increasing dielectric constant in the order methanol-cyclohexane > methanol > water >> 6 M urea > 10 M urea. The high α-helix content of the toxin in distilled water (42%) was not too different from 45% in methanol. Expected also was the loss

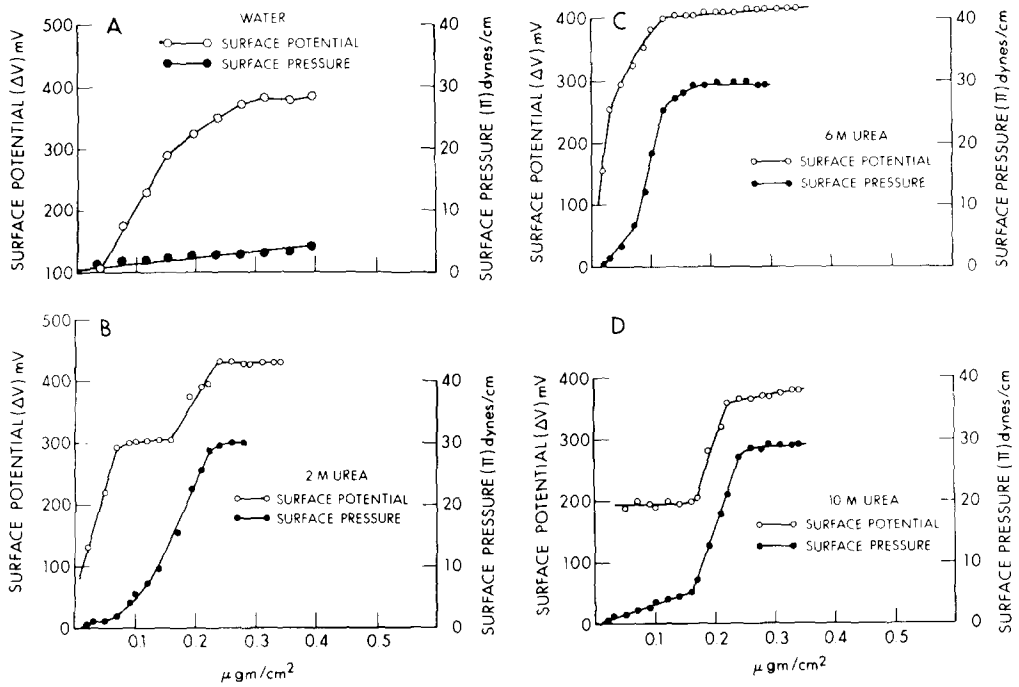

Fig. 4. Influence of urea concentration in the hypophase at 25° C on surface pressure and surface potential of spread films of δ-toxin. At different urea concentrations, the π and ΔV values are related to the quantity of protein applied per cm^2.

of α-helix in 6 M urea, and 10 M urea, although 24% of it was still left in 6 M urea.

Infrared spectroscopy. Optical rotatory dispersion and circular dichroism cannot distinguish between α- and β-protein conformations, but infrared spec-

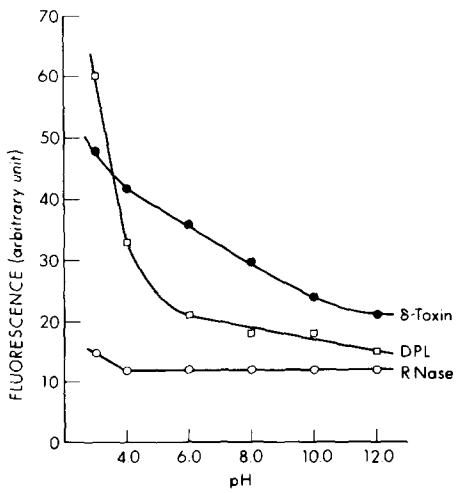

Fig. 5. Intensity of fluorescence of RNAase, dipalmitoyl phosphatidylcholine (DPL) and δ-toxin at 10 μg/ml each in 5 mM tris buffer, 25° C. Dipalmitoyl phosphatidylcholine was sonicated in distilled water for 5 h in ice bath; the lipid dispersion was clear, transparent and stable for several days. ANS, 5 μg/ml.

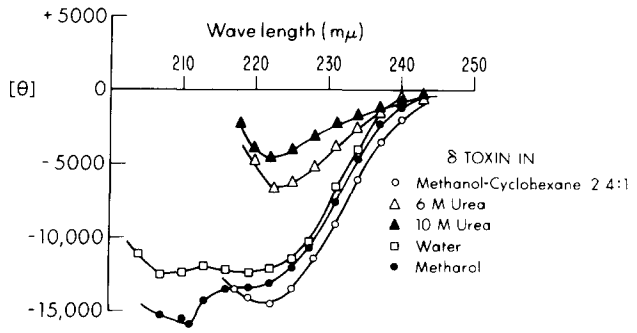

Fig. 6. Circular dichroism spectra of δ-toxin (0.1 mg/ml) in various solvents at 25°C.

troscopy can [18]. In the upper panels (A, B, C) of Fig. 7, a toxin solution containing 1 mg/ml protein in deuterated solvents (^2H_2O, deuterated urea and deuterated chloroform/methanol, respectively) was used. Beside the shifted amide II at about 1460 cm^{-1}, the predominant absorbance was that of the α-helix at 1650 cm^{-1} (amide I) in both distilled water (A) and chloroform/methanol (C).

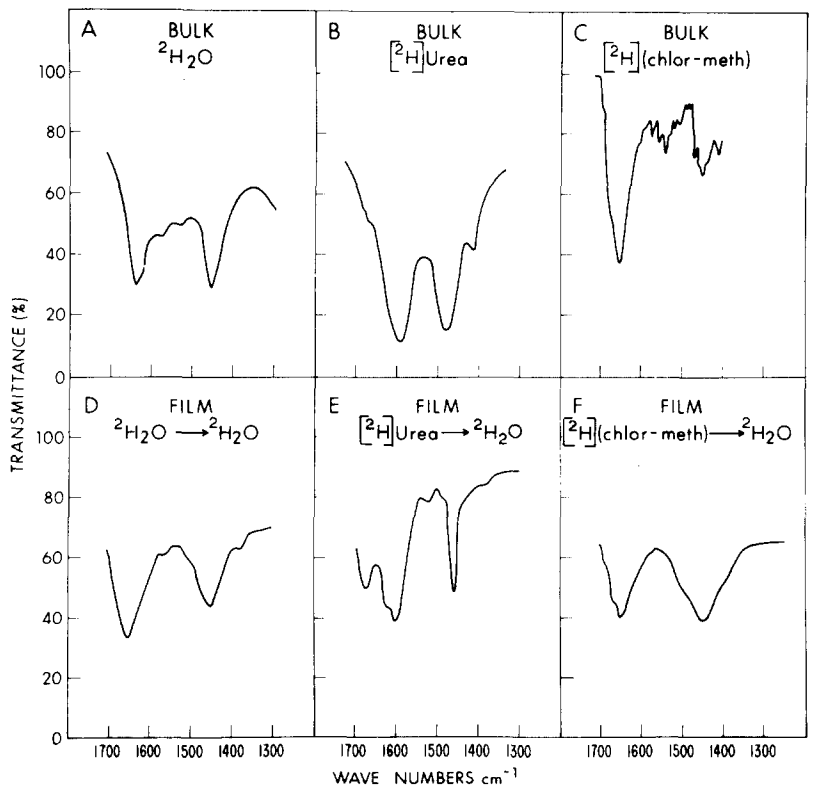

Fig. 7. Infrared spectra of δ-toxin at 25°C (A,B,C) bulk solution, 1 mg/ml, against KRS-5 crystal window. (D,E,F) films picked from the monolayer on the KRS-5 crystal. The spectrum in B is that of 6 M [^{2}H]urea, thus masking the absorption spectrum of the protein. In panels D,E and F, films were spread (D) from ^2H_2O on ^2H_2O; (E) from [^{2}H]urea on ^2H_2O; (F) from [^{2}H]chloroform/[^{2}H]methanol on ^2H_2O.

In contrast, the prevalent absorption peaks of the protein solution in 6 M [^{2}H]-urea were at 1600 cm^{-1}, and 1480 cm^{-1}, which are those of 6 M [^{2}H]urea. A quantity of α-helix, which was still revealed in 6 M urea by circular dichroism, could be masked in the broad peak of 6 M [^{2}H]urea at 1600 cm^{-1}.

In panels D, E, F, a saturated film of δ-toxin was made on ^2H_2O by spreading the protein from ^2H_2O, deuterated urea and deuterated chloroform/methanol (2 : 1, v/v), respectively, and picked up in one single pass on KRS-5 thallium iodide-thallium bromide crystal. The films spread from ^2H_2O and from deuterated chloroform/methanol show preservation of the α-helix at 1650 cm^{-1} as the prevalent film structure. In contrast, in addition to the strong 1600 cm^{-1} band of [^{2}H]urea in bulk, the films spread from 6 M urea, showed absorption at 1633 and 1680 cm^{-1} for β-aggregated and β-antiparallel pleated sheet structures, respectively [19]. One cannot exclude that some α-helix absorption be masked by other peaks. Notice two striking phenomena. One is the enrichment in the 1650 cm^{-1} peak and a loss of 1460 cm^{-1} absorption in the transport of the protein from ^2H_2O to film (Panels A and D); the other is the appreciable loss of 1650 cm^{-1} peak intensity with corresponding gain in the 1460 cm^{-1} absorption after transport of the toxin from organic solvent to the film which is in contact with ^2H_2O (Panels C and F). We have no explanation for the sharpness of the peak at 1460 cm^{-1} in Fig. 7E. The H-^{2}H exchange may be ruled out since the atmosphere was saturated with N_2 gas.

For a comparison, in separate experiments, we studied the infrared spectra of melittin films spread from 6 M urea. Solutions of melittin and δ-toxin in 6 M [^{2}H]urea had similar unresolvable absorption bands at 1600 cm^{-1} and 1480 cm^{-1} typical of 6 M [^{2}H]urea but unlike δ-toxin, melittin reacquired fully the α-helix when spread into a film from 6 M [^{2}H]urea, namely the peak at 1600 cm^{-1} in 6 M [^{2}H]urea was shifted to 1650 cm^{-1} in the film (unpublished data).

Discussion

Knowledge of the parameters accompanying the transport of toxin from an aqueous to a membrane phase is important in order to postulate some mechanisms for cytolytic action of the toxin, and to add to our understanding of the surface behavior of membrane and membranophilic proteins.

Surface activity

The unusual property of δ-toxin to dissolve in both organic and aqueous media and to form high pressure films is portentous of conformational versatility and is reminiscent of the amphiphilic lipid character attributed to membrane proteins [4]. In its solubility, the toxin behaved indeed like the more polar phospholipids, e.g. unsaturated lecithins. These are readily dispersable in water, in which they form large particles [20], and more readily soluble in organic solvents.

With regard to its ability to form a film at the air/water interface, δ-toxin behaved both as phospholipid and as protein. It resembled a phospholipid when it readily formed high pressure films (40 dynes/cm) by spreading from chloroform/methanol (2 : 1, v/v) (Fig. 1) and by spreading or adsorbing very poorly from water (Fig. 2) [5,21]. It behaved as a protein when its ability to spread as

a film from water decreased with increasing pH between 2 and 10 (Fig. 3) and when it adsorbed readily, almost instantaneously, from 6 M urea to form a film (Fig. 4) whose high pressure (35 dynes/cm) resembles that of a lipid. In conclusion, in organic solvent and in a membrane phase, δ-toxin exhibits a lipid-like behavior, which was attributed to membrane structural proteins and which must correspond to the presence of lipid-like protein subunits inserted in the membrane. Although it was suggested that hydrophobic α-helix terminating in hydrophilic amino acid residues had to be the lipid-like structure responsible for the surface activity (π) of membrane proteins in situ [4,6], infrared spectra showed that δ-toxin can make high pressure films with either α-helix or β-structures (Figs. 4 and 7). However, since we cannot exclude the presence of α-helix in those films showing abundance of β-structures, we also cannot distinguish whether the observed film pressures are determined strictly by asymmetric α-helices oriented perpendicularly to the interface [4] or if and how the β-helices contribute to film pressure. The uncertainty relates also to the question as to whether β-forms are intrinsic or extrinsic membrane structures, or they can be both.

Surface potential

Because a complete picture of the molecular correlates of surface potential is not available [22—25], we shall limit our discussion to some empirical relationships between chemical structure and ΔV values and to a comparison of different systems. In principle, the appearances of $\pm$ and $\mp$ oriented dipoles at the air/water interface of the film cause, respectively, positive and negative contributions to the surface potential [23,26]. Experience also shows that compact (liquid condensed and solid) lipid films such as distearoyl and dipalmitoyl phosphatidylcholines and phosphatidylethanolamines have higher surface potentials than those of liquid expanded films such as dimyristoyl lecithin [25,27,28]. Also, the appearance of fixed charges in the Gouy diffused electrical double layer of the film causes positive contributions from cationic species such as amine, guanidine, choline groups, and a negative contribution from anionic species such as carboxylate, phosphate, sulfate groups [23,26,29]. Furthermore, according to the mathematical expression of the potential of the parallel plate capacitor ($V = 4\pi nel/AD$), low and high dielectric constants of the film would produce positive and negative contributions to the surface potential (ΔV), respectively.

Staphylococcal δ-toxin formed films that were very stable over the entire pressure range, from the very low to the very high π values, and the surface potential readings were also stable under those conditions. Except on subphase containing urea, the trends of the surface potential paralleled those of the surface pressure. The toxin produced an unusually high surface potential reminiscent of that of saturated lecithins, such as distearoyl and dipalmitoyl phosphatidylcholines, and saturated phosphatidylethanolamines [25,27,28]. The high surface potentials may be interpreted to mean either (a) a net positive charge at pH 5.7 on the water surface of δ-toxin, whose principal isoelectric point is 9.5 [10,30], (b) a dense packing of hydrophobic lipid-like structures, or both. The high surface potentials of polyalanine and several other uncharged polypeptides [31,32] could be due to the packing and orientation of lipid-like protein struc-

tures, such as α-helices perpendicular to the interface [4,32,33].

When the protein was spread from water on subphases containing urea, the surface potential was lower than on water, and it decreased with increasing urea concentrations. Though high (30 dynes/cm), the surface pressure of the film spread from water on 6 M urea was quite lower than that of a film spread from chloroform/methanol on water (40 dynes/cm). Accordingly, a surface potential of 400 mV on 6 M urea as opposed to 500 mV on water could be accounted for by the abundance of β-conformations in the δ-toxin film on 6 M urea and larger quantity of low surface potential water [4,24]. Relevant to the structure of toxin films on urea is collagen, which is noted for its β-conformations, low surface potentials and high surface viscosity [24,34]. Reasons and mechanisms for lower surface potentials were discussed above and elsewhere [24]. In brief, more hydration and less compactness of the protein film on urea and on high pH should cause a higher permittivity and thus a lower surface potential.

Relation of chemical structure to surface behavior

Staphylococcal δ-toxin seems to possess the structural characteristics of a membranophilic protein that can behave simultaneously as a lipid and as a protein and can readily insert itself into hydrophobic membrane structures. This surfactant property could be related to the high content of hydrophobic amino acids (50%) and to the absence of disulfide bridges [9,10]. The latter would confer flexibility and versatility of conformations to the polypeptide chains, thus facilitating changes in protein conformations that are probably required in processes of transport of the protein across membranes.

Although a tentative correlation was once made between solubility of a protein in organic solvent, its surface activity and its lipid-binding character, at the same time we hastened to point out that the correlation is not a simple one, and several experimental approaches are required to explain the multiplicity of observations and the interweaving of various phenomena [4,24].

For instance, lysozyme does not spread into a film from its aqueous solution [4], and serum albumin does modestly (unpublished observation) unless one uses high salt concentrations, 1—4 M, in the hypophase [35]. However, lysozyme dissolved readily in chloroform/methanol/water mixtures, from which it spread instantaneously into films with 20 dynes/cm pressure [4]. Albumin instead precipitated out from the organic solvent. In contrast, above and beyond the behavior of any known protein, δ-toxin dissolves in organic solvent from which it spreads to form films with 40 dynes/cm pressure on water, as opposed to the highest pressure of 20 and 22 dynes/cm that are possible with lysozyme and serum albumin, respectively, under the same conditions [4].

The fluorescence spectra of ANS with δ-toxin and serum albumin seem to accentuate the complexities. For instance, the fluorescence of ANS in a protein or a membrane has been taken to indicate the presence of the amphiphilic dye in a hydrophobic medium [36,37] and thus a lipid-binding character. Albumin, with much less surface activity, produced an intensity of fluorescence which was many fold greater than that of δ-toxin, whose surface activity is at least double that of serum albumin. Therefore, correlations between protein conformations, lipid binding and surface activity do not appear to be direct and simple.

Effect of urea and organic solvent. The positive influence of urea on the surface activity of δ-toxin is in line with previous observations in this laboratory with α-toxin [2] and other proteins (ref. 4 and unpublished data). The similar values of both π and ΔV on 2 and 6 M urea confirm the earlier suggestion [2] that probably a complete denaturation of the protein is not necessary in order for the protein to reach the full surface activity. However, as before, the kinetics were different at the two urea concentrations.

With regard to the structure vs. function relationship, the dielectric constant of the medium, film or bulk, is likely to affect the surface potential and the ion-ion as well as the dipole-ion and dipole-dipole interactions [4,6,17,24,38, 39] that lead to conformational changes of the protein at the interface. However, δ-toxin formed films of comparably high pressures when spread from either a low or a high dielectric constant medium, chloroform/methanol (2 : 1, v/v) or water, respectively. But, certain structures of the film protein were different in the two cases (Figs. 7A, C, D and F). This means that in the same protein the mechanism of film formation may take two different routes depending on the solvent. The dilemma was shown also by a comparison of infrared spectra and circular dichroism spectra of δ-toxin and melittin in aqueous and film phases. Indeed, in 6 M [^{2}H]urea, the infrared spectra of melittin and δ-toxin, could not be distinguished since the absorption peaks of the protein were masked by the intense absorption of 6 M [^{2}H]urea. However, in film spread from [^{2}H]urea, δ-toxin acquired non-α-conformations (Fig. 7E), whereas melittin reverted from the extended polypeptide chain in 6 M urea (as seen by circular dichroism) the the α-helix or/and random coil in the film (1650 cm^{-1}).

In conclusion, the two proteins (δ-toxin and melittin) may follow different mechanisms in the transport from bulk to film phases, a partially irreversible one for δ-toxin and a fully reversible one for melittin. Since by infrared spectroscopy we cannot distinguish between δ-toxin and melittin in 6 M [^{2}H]urea, our conclusion is based on a comparison of the circular dichroism data of the proteins in bulk and the infrared spectra of the proteins in the film.

Perspectives and biological significance

At present it is not quite possible to account for the detailed mechanisms and structures that are at the basis of either behavior, δ-toxin or melittin. The easy obeyance of the denaturation-renaturation mechanisms [4] may be effective with melittin because of its small molecular weight, 3000, which may confer greater mobility and flexibility for the melittin molecules to perform the reversible α-helix ⇌ unfolded coil transition. We do not know what prevents the δ-toxin from achieving the α-helix in the transport from urea to the film; larger molecular weight and intermolecular assembly of δ-toxin's subunits [9—11] could be a cause. The process α-helix → unfolded chain → β-structures is a feature offered by δ-toxin in going from water to urea and to film phase.

β-Structures have also been found in films of β-protein having relatively low pressures [40]. In all the cases it is not established whether the high pressure values are determined by the insertion of lipid-like α-helices exclusively [4] or also β-forms and if either structures must be inserted perpendicularly or horizontally to the interface. Information from polarization of ultraviolet and

infrared spectra [41] of film protein and from the protein's amino acid sequences [4] should provide the basis for the knowledge of interrelations between protein conformations, film pressure and orientation of amphiphilic subunits at membrane interfaces.

In the ability to form a film and produce a surface pressure (π) by either spreading or adsorption, δ-toxin and α-toxin differ markedly. For instance, the highest saturation pressures were 16 dynes/cm and above 22 dynes/cm for α-toxin spread from buffer and 6 M urea, respectively [1,2], and 30 and 40 dynes/cm for δ-toxin spread from 6 M urea and chloroform/methanol, respectively. The noted differences are not so simple; they are significant when related to the behavior of the two proteins in various solvents. Qualitatively and quantitatively, δ-toxin, soluble in organic solvents, is much more surface active than α-toxin, which is precipitated by such solvents. This observation may explain the absence of δ-toxin in α-toxin preparations that were obtained by ethanol precipitation. Such α-toxin preparations did not lyse spheroplasts and protoplasts (unpublished work), which are normally lysed by δ-toxin [9,10].

Since α-toxin and δ-toxin have cytolytic preferences, they may also have an expression of interfacial behavior that relates to the extent and specificity of their cytolytic action. Where δ-toxin, and not α-toxin, lyses a given cell membrane, some specific molecular mechanism may differentiate the two. Correlation of differences in surface activity and interfacial protein structures with the noted biological preferences must await further studies.

Acknowledgements

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