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Fusogenic activity of δ-haemolysin from *Staphylococcus aureus* in phospholipid vesicles in the liquid-crystalline phase

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A study has been conducted of the interaction of the lytic toxin δ-haemolysin with vesicles of phospholipid, using electron microscopy, fluorescence depolarisation and excimer fluorescence. The peptide is shown to be a fusogen towards phosphatidylcholine vesicles in fluid phases. In the presence of gel phase lipid, fusion between fluid and gel phases is not seen. Fluid phase lipid vesicles are fused together to form large multilamellar structures, and initial vesicle size does not appear to be important since small unilamellar vesicles and large unilamellar vesicles are similarly affected. Fusogenic activity of δ-haemolysin is compared to that of melittin. The former is a progressive fusogen for fluid phase lipid, while the latter causes vesicle fusion in a manner related to occurrence of a lipid phase transition.

Introduction

The bacterium *Staphylococcus aureus* secretes a variety of peptides of various molecular weights and having diverse physiological effects [1]. Amongst these are the heat stable hydrophobic peptides known as δ-toxins, and the most studied of these is the lytic peptide δ-haemolysin, isolated from the *S. aureus* 186X strain [2]. δ-Haemolysin is a 26-residue peptide with clearly defined regions of a hydrophobic and of a hydrophilic nature. It is stable to organic solvents, and can be isolated by solvent extraction from culture medium. The purified toxin has a single tryptophan and no other fluorescent residues, and this serves as an intrinsic fluorescence probe to monitor the conformation and interactions of the peptide. The toxin is surface active, and exists in solution as aggregates of high molecular weight under some conditions of ionic strength and pH [3].

The properties of δ-haemolysin have invited comparison with those of the peptide melittin, found in bee venom in high concentration [4]. Melittin is heat stable, soluble in organic solvents and is a very potent lytic toxin which shows strong surface activity. The lytic effects of melittin do not appear to be a simple consequence of surface activity, since subfragments which are not lytic to cells can show similar surface activity to the native toxin [5]. Melittin, like δ-haemolysin, has 26 residues with clearly defined hydrophobic and hydrophilic regions, though it differs from the δ-toxin in that no negatively charged residues are present. Melittin has a single tryptophan residue, which has served as an intrinsic fluorescent probe [6]. The aggregation equilibria of melittin in solution also depend on ionic strength and pH, but unlike the δ-toxin only monomer and tetramer have been observed [7]. In a recent study of β,γ-dipalmitoyl-

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L-α-phosphatidylcholine (DPPC) in the gel phase, we have compared the lytic activity of melittin and δ-haemolysin in their ability to cause release of trapped marker from vesicles [8]. Melittin was found to cause loss of marker when present either as monomer (low-phosphate buffer), or as tetramer (high-phosphate buffer), though the leakage kinetics were sensitive to buffer concentration. In contrast, the δ-haemolysin was found to cause loss of vesicle contents in low phosphate concentrations, but to lose all such activity in high-phosphate buffer. We demonstrated by fluorescence methods that this effect was not due to lack of peptide binding at high ionic strength, and showed that the δ-toxin penetrated into the lipid bilayer at all phosphate buffer concentrations tested [8]. The δ-toxin also caused a slow, temperature dependent and highly non-linear increase in light scattering for fluid phase DPPC vesicles in high- and low-phosphate buffer.

In dispersions of neutral DPPC vesicles, melittin induces rapid vesicle aggregation with eventual precipitation if added below the phase transition temperature of the lipid. On heating, rapid vesicle fusion occurs [9,10]. It was therefore of interest to determine whether δ-haemolysin might have similar effects. In this paper, we show that δ-haemolysin causes fusion of phospholipid vesicles under appropriate circumstances, and we compare the fusogenic effects of the toxin with that of melittin.

Materials and Methods

δ-Haemolysin was isolated and purified as previously described [2] and stored as solid at −20°C. Samples were dissolved immediately before use. All other chemicals were of 'Analar' grade or equivalent. All phospholipids were purchased from Sigma Ltd., and were used without further purification. Small unilamellar phospholipid vesicles were prepared by the ethanol injection method described elsewhere [11]. Large unilamellar vesicles (LUV) of egg-yolk phosphatidylcholine (egg PC) were prepared by deoxycholate removal [12]. LUV of DPPC were prepared by a modified deoxycholate procedure, as previously described [8]. The release of calcein from LUV was monitored fluorimetrically at 520 nm, exciting at 470 nm, as previously described [8]. Fluorescence and light scattering measurements were made using a Schoeffel RRS 1000 spectrofluorimeter as previously described [9].

The fluorescent probe DPH-PC was prepared and purified as previously described [9]. Diphenylhexatriene (DPH) was purchased from Sigma Ltd. Vesicles containing fluorescent probe were prepared by mixing phospholipid and probe in ethanol at an appropriate molar ratio prior to vesicle formation. The probe/phospholipid mixture was injected into vortexing buffer solution at a temperature above the phase transition of the phospholipid used. The sample was then incubated for approx. 30 min at the appropriate phase transition temperature in the dark before use in order to allow equilibration of trapped ethanol with the bulk medium. The final ethanol concentration in solution did not exceed 1% by volume. The fluorescent probe PyPC (β-(pyren-1-yl)decanoyl-γ-palmitoyl-L-α-phosphatidylcholine) was obtained from Sigma. Lipid mixing between fluorescently labelled and unlabelled vesicles was monitored using PyPC. Measurement of excimer emission at 470 nm for PyPC was ratioed to monomer emission at 395 nm, excitating at 335 nm, to measure local concentration of fluorescent probe. The excimer/monomer emission ratio corresponding to random mixing of probe with unlabelled phospholipid was measured independently under conditions appropriate to individual experiments. Fluorescence polarisation measurements were made using a 'T-format' apparatus similar to that described previously [9]. The apparatus was modified for computer control both of data acquisition and of sample heating and cooling rates (using a Peltier effect semiconductor heat pump). A BBC microcomputer fitted with a commercial analogue/digital interface (3-D Ltd) acted as device controller and data plotter.

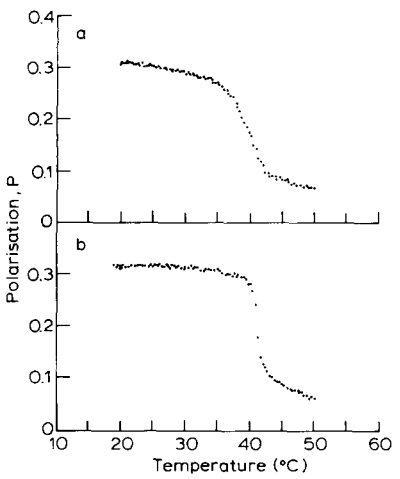
Transmission electron microscopy was performed on samples negatively stained with uranyl acetate, as previously described [13].

Results and Discussion

Phospholipid vesicles formed by the ethanol injection method are of the so-called 'small unilamellar' type. Such small vesicles typically

show less cooperativity in their bilayer dynamics than do larger vesicles, where the ratio of numbers of lipid molecules in the inner and outer bilayer leaflets tends to unity. Synthetic lipids such as DPPC show highly cooperative phase transitions centred at well defined temperatures. The transition temperature and half-width for a given lipid are sensitive to the bilayer cooperativity, with small vesicles showing broader transitions centred at lower temperatures than large unilamellar or multilamellar vesicles. Measurement of fluorescence polarisation of the probe DPH within a lipid bilayer is a convenient method to monitor bilayer dynamics and phase behaviour [14]. The temperature/fluorescence polarisation profile of DPH in DPPC vesicles is shown in Fig. 1. A relatively broad transition centred at about 39°C is evident. Also shown in Fig. 1 is the effect of δ-haemolysin on this lipid. Toxin was added to the lipid at 50°C to a final concentration of 2.7 μM and the sample was cooled after standing for 5 min to equilibrate. The polarisation profile shown in the figure was obtained on reheating this sample. The transition is seen to be sharpened and to occur at a higher temperature than before δ-toxin was added. Such a transition is normally seen for larger vesicles showing higher cooperativity. However, this in itself is not definitive, since it is possible that the toxin itself might in some way influence the motion of the DPH probe or increase the transition temperature by directly interacting with the lipid acyl chains. At the concentration of toxin used, there is only one molecule of δ-haemolysin for every hundred lipid molecules assuming complete binding, so that a direct interaction is somewhat unlikely. If lipid vesicles are somehow becoming larger, then either the effect must be vesicle-vesicle fusion, or else lipid must be exchanged through solution. The latter effect might perhaps be mediated by the known surface activity of the peptide [3]. In order to distinguish between these possibilities further experiments were performed.

Fig. 2a shows the temperature/polarisation



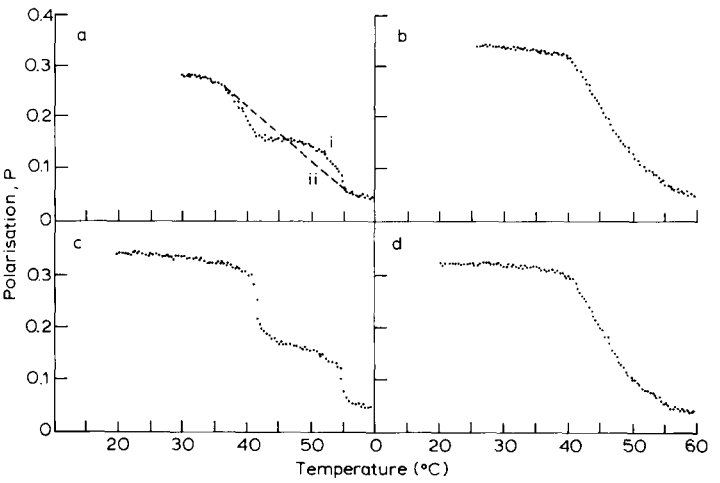


Fig. 1. (Left). Fluorescence polarisation vs. temperature profiles for DPH (1 : 200, mole ratio) in DPPC vesicles (0.2 mg/ml in 50 mM sodium phosphate buffer, pH 8). Trace (a), small unilamellar DPPC vesicles without toxin. Trace (b), after addition of δ-toxin (2.7 μM, 1 : 100 mol relative to DPPC). Toxin was added at 50°C and the data were measured after cooling. Both traces were recorded heating at 2 K/min.

Fig. 2. (Right). (a) Fluorescence polarisation vs. temperature profile for DPH (1 : 200 mol relative to total phospholipid) in (i) a mixture of vesicles of DPPC with vesicles of DSPC (equimolar amounts: final concentration was $1.35 \cdot 10^{-4}$ M for each lipid); (ii) vesicles ($2.7 \cdot 10^{-4}$ M) formed from an equimolar mixture of DPPC with DSPC. (b) Effect of δ-toxin (2.7 μM) on DSPC/DPPC vesicle mixture as in Figure (2a), trace (i). δ-Toxin was added at 60°C and the sample cooled after 5 minutes and reheated. (c) Addition of δ-toxin (2.7 μM) to a mixture of DPPC vesicles and DSPC vesicles held at 45°C. The sample was cooled after 5 min and data recorded on reheating. (d) Trace obtained after cooling sample in Fig. 2c and reheating. In Figs. 2a–2d, buffer was 50 mM sodium phosphate, pH 8 and heating rate was 2 K/min.

profile for DPH in a mixture of vesicles of the phospholipids DPPC and DSPC (β,γ-distearoyl-L-α-phosphatidylcholine). DSPC has a similar phase behaviour to DPPC, but the characteristic phase transition temperature is raised. A mixture of vesicles of these two lipids shows both phase transitions clearly. In contrast, vesicles formed from a mixture of DPPC and DSPC show a broad featureless transition intermediate between the characteristic temperatures of its constituent lipids. Fig. 2b shows the effect of δ-toxin on a sample of a mixture of DPPC and DSPC vesicles. When the toxin is added above the phase transition temperatures of both lipids and allowed to equilibrate, the individual lipid transitions are abolished on a subsequent heating after cooling to room temperature. The broad transition resulting is characteristic of vesicles of mixed lipids. The experiment was repeated, but adding the toxin to a mixture of DPPC vesicles and DSPC vesicles held at 45°C, where DPPC is in its liquid-crystalline phase while the DSPC is below its phase transition, and in a relatively rigid gel phase. The sample was cooled to 20°C after 5 min incubation. A temperature/polarisation profile was recorded while heating this sample to 60°C (above T_c for DSPC). This is shown in Fig. 2c. Two sharp transitions are clearly evident. Since DPPC was in a fluid phase during the first incubation at 45°C, it is evident that DPPC vesicles did not fuse with vesicles of DSPC during this incubation. Had they done so, an intermediate transition would have been seen on subsequent heating. The fact that the DPPC transition is sharpened suggests strongly that SUV of DPPC in fact fused with one another during the first incubation. During the subsequent temperature/polarisation scan to 60°C, the DSPC vesicles pass through their phase transition, and the profile shows that this too is sharpened, suggesting that again small DSPC vesicles preferentially interact (initially) with each other. On cooling this sample to 20°C (after several minutes at 60°C) and reheating, a broad featureless transition is seen (Fig. 2d), showing that total lipid mixing occurs on standing above the phase transition of both lipids. On adding δ-toxin to a mixture of DSPC vesicles and DPPC vesicles at 20°C, two sharp characteristic transitions are observed on first heating to 60°C. After cooling to 20°C, a broad transition is again seen on subsequent reheating.

The results show clearly that δ-toxin induces lipid mixing, and suggests that in order to do so, lipids involved must be in the 'fluid' phase.

The high curvature of small vesicles tends to destabilise them, and might itself favour fusion between vesicles, or enhance lipid exchange through aggregates. However, when experiments were repeated using large unilamellar vesicles (LUV) prepared as in Ref. 8, similar results were obtained to those given above. Initial vesicle size is not therefore a major factor in the lipid mixing process. The fluorescent probe used in the above experiments, DPH, is freely exchangeable through solution, and cannot be localised within a subset of lipid vesicles. To further investigate the nature of the δ-toxin effect on lipid vesicles, experiments were performed using a non-exchangeable fluorescent probe. The probe chosen was the phospholipid DPH-PC, which has the fluorophore DPH covalently bound within one of its acyl chains [9]. Vesicles of DSPC were prepared containing the probe DPH-PC, and these were mixed with an excess of unlabelled vesicles of egg-yolk phosphatidylcholine (egg PC). The egg PC vesicles are heterogeneous in lipid composition, and are in a liquid-crystalline state at room temperature and above. The fluorescent probe DPH-PC is highly constrained motionally when bound to gel phase DSPC vesicles, as evidenced by the high value of fluorescence polarisation. If vesicle fusion or other lipid mixing could occur between vesicles of DSPC and egg PC, then the polarisation of DPH-PC fluorescence would be considerably reduced. Fig. 3a shows the effect of adding δ-haemolysin to this lipid mixture held at 37°C. Clearly, no mixing of egg PC with DSPC occurs at this temperature. On increasing the temperature of the mixture to 47°C (close to, but below, the phase transition of DSPC), a slow decrease in polarisation is seen (Fig. 3b). However, even after standing at this temperature for hours, lipid mixing was incomplete. This is shown by the temperature scan in Fig. 3c, which clearly reveals the DSPC transition. The transition is less sharp than that seen using DPH as probe, because DPH-PC is anchored, and its fluorescence is more polarised in fluid phase lipid than that of DPH [9]. After heating for 5 min to 60°C the phase transition of DSPC was lost, indicating

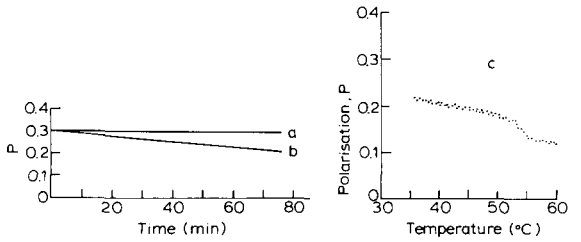


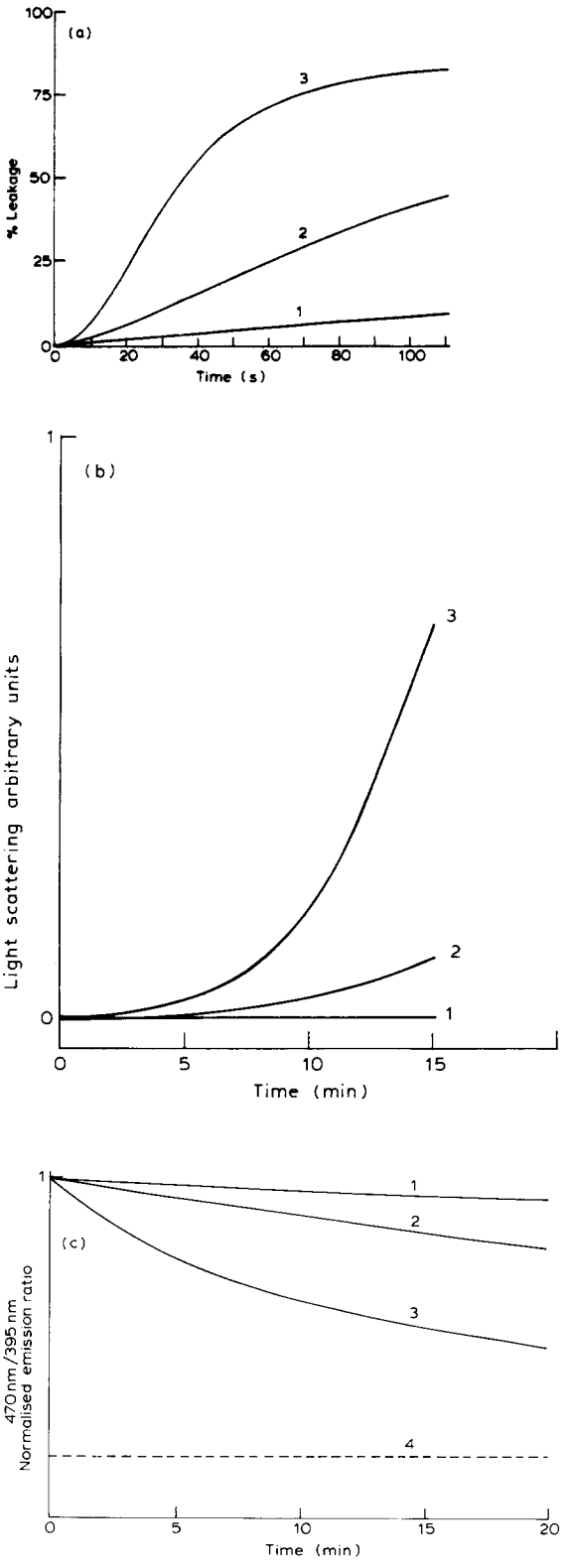
Fig. 3. The effect of δ-haemolysin (2.8 μM) on a mixture of DSPC vesicles labelled with DPH-PC (DSPC $6.3 \cdot 10^{-5}$ M, DPH-PC/DSPC 1:200 mol) with unlabelled vesicles of egg-yolk PC ($3 \cdot 10^{-4}$ M). (a) Fluorescence polarisation vs. time for a sample held at 37°C. (b) Data for a sample held at 47°C. (c) The polarisation/temperature profile of the sample from (b) after standing at 47°C for approx. 3 h. Heating rate was 2 K/min, and buffer was 50 mM sodium phosphate, pH 8 in all cases.

complete lipid mixing. After standing overnight at room temperature, the sample contained lipid structures clearly visible to the unaided eye, while a control containing no δ-toxin remained clear.

In a control experiment, DPPC labelled with DPH-PC was incubated at 47°C with unlabelled vesicles of egg PC in the presence of δ-toxin. After cooling to 20°C, a subsequent heating scan showed that the DPPC transition had been lost. The incomplete DSPC/egg PC mixing under conditions where this DPPC control experiment has shown mixing of vesicles of fluid phase lipids is in accord with the suggestion made earlier that δ-toxin primarily affects interactions between vesicles in the fluid phase. The results also show that the lipid mixing process is progressive, and that lipid structures become larger with time.

Several possible explanations suggest themselves for the simultaneous increase in turbidity and lipid mixing. Vesicles might be induced to aggregate, and lipids rapidly exchanged between apposed bilayers, or vesicle-vesicle fusion might be occurring. Alternatively, aggregation might be occurring at the same time as, but independently of, lipid exchange through solution. These alternatives are very difficult to distinguish in general. The first mentioned alternatives are but extremes of a single process, resulting in large lipid structures with continuous bilayers (as distinct from aggregates of independent bilayer vesicles). The distinguishing feature between these mechanisms might be the mixing of aqueous vesicle contents expected for vesicle fusion. However, this is not an inevitable consequence of vesicle fusion, since destabilisation of bilayer structure necessary to reduce fusion activation energy might also allow rapid leakage of vesicle contents. Thus, a positive evidence of contents mixing is a reliable indicator of vesicle fusion, but a negative result cannot be interpreted to exclude this process. Experiments were attempted using LUV of DPPC containing entrapped calcein, to monitor leakage rate for the marker in the presence of δ-toxin. Unfortunately, at the phase transition temperature vesicle contents were rapidly and totally lost. Moreover, LUV of (fluid) egg PC at 20°C rapidly lost entrapped calcein at δ-toxin concentrations less than those needed to cause detectable changes in light scattering. This is clearly shown by Figs. 4a and 4b, where light scattering and leakage data are presented at constant lipid concentration. Trace (3) of Fig. (4a) can be directly compared with trace (1) of Fig. 4b, since both have the same lipid and δ-toxin concentrations.

In the previous experiments, it might be argued that vesicle lipid mixing, though requiring 'fluid' phase lipid, was a consequence of elevated temperature rather than 'fluidity'. For egg PC, an assay for lipid mixing was therefore tested at room temperature. The method chosen uses the variation in excimer-to-monomer fluorescence emission ratio of a phospholipid probe molecule to measure local probe concentration within a vesicle population. The probe, PyPC, shows strong excimer fluorescence at 470 nm when locally concentrated, but mainly monomer fluorescence at 395 nm when present at low concentration [15]. Vesicles of egg PC containing 4 mol% PyPC were prepared by the deoxycholate dilution method. These vesicles were mixed with a large excess of unlabelled egg PC vesicles, and the excimer-to-monomer fluorescence emission ratio was recorded as a function of time before and after addition of δ-toxin. The results are shown in Fig. 4c. In the absence of δ-toxin, a very slow change in emission ratio is seen. Addition of δ-toxin markedly accelerates this. The emission ratio corresponding to complete mixing of lipids was established by preparation of a sample of a mixture of PyPC and egg PC in the ratio expected for total randomisation, with the addition of δ-toxin to control for possible peptide



effects on fluorescence emission. The lipid concentration in Fig. 4c is the same as that in Figs. 4a and 4b. The lipid mixing shown using PyPC is accompanied by an increase in light scattering, indicating either that vesicles are aggregating with lipid exchange, or are fusing together. Electron micrographs of LUV of egg PC show very large amorphous structures with multilamellar regions for samples incubated at 20°C with δ-toxin (Fig. 5a). A similar experiment using DPPC vesicles showed no evidence of aggregation or fusion for LUV or SUV incubated with δ-toxin at 20°C. However, incubation of DPPC vesicles with δ-toxin at 55°C (above T_c) caused larger structures to be formed. After short incubation (about 20 min), products formed from LUV are larger and apparently mainly unilamellar, with developing multilamellar regions. After longer incubation at 55°C (> 2 h), multilamellar structures are clearly evident. Fig. 5b shows an electron micrograph of a sample of DPPC LUV after prolonged incubation with δ-toxin at 55°C; the result of a similar experiment with SUV is shown in Fig. 5c. These observations suggest that the process we are observing is vesicle-vesicle fusion or exchange through aggregates, rather than lipid exchange

Fig. 4. Leakage, light scattering and lipid mixing data are shown for egg PC vesicles in the presence of δ-toxin. In all cases buffer was 50 mM sodium phosphate, 35 mM EDTA, 0.02% sodium azide (pH 8). All experiments were performed at 20°C. The lipid dispersion was $2.6 \cdot 10^{-4}$ M and vesicles were LUV prepared as described in Ref. 12. (a) The effect of δ-toxin on leakage of entrapped calcein from vesicles. The concentrations of δ-toxin were: trace 1, 0.65 μM (1:400 mol relative to lipid); trace 2, 0.98 μM (1:267 mol); trace 3, 1.3 μM (1:200 mol). (b) Light scattering data at 470 nm for egg PC vesicles incubated with δ-toxin. The concentrations of toxin were: trace 1, 1.3 μM (1:200 mol relative to lipid); trace 2, 1.95 μM (1:133 mol); trace 3, 2.6 μM (1:100 mol). (c) The effect of δ-toxin on lipid mixing monitored by the fluorescent probe PyPC. Labelled vesicles were egg PC LUV containing PyPC (25:1 mol egg PC/PyPC). Vesicles of egg PC containing no probe ($2.43 \cdot 10^{-4}$ M) were mixed with labelled vesicles ($1.73 \cdot 10^{-5}$ M). Fluorescence was excited at 335 nm and emission monitored at 470 nm and at 395 nm. Trace 1 is a control sample containing no δ-toxin. Trace 2 contains 2.6 μM δ-toxin (1:100 mol relative to lipid). Trace 3 contains 5.2 μM δ-toxin (1:50 mol relative to lipid). Trace 4 shows the emission ratio (470 nm/395 nm) measured with a sample containing 0.27 mol% PyPC in egg PC. This concentration is that expected for total lipid randomisation in the mixed vesicle systems.

through solution. This is consistent with the markedly nonlinear change of light scattering with time, which we reported in an earlier paper [8] for DPPC vesicles incubated with δ-haemolysin above the phase transition temperature. Such behaviour would be expected for a progressive fusogen, since fusion between ever-growing vesicles leads naturally to an exponential growth in size with time. This is to be contrasted with vesicles which grow by accretion, with small vesicles unstable and able to fuse with larger vesicles, but large vesicles unable to fuse with each other.

The packing imperfections and dynamic fluctuations in density which occur when lipid vesicles are close to phase transition temperatures do not appear to be directly involved in δ-toxin mediated fusion. Vesicles do not need to pass through a phase transition in order to fuse together, but merely need to be in a 'fluid' liquid-crystalline phase. This is shown by results quoted for egg PC, which forms large structures on δ-toxin addition at 20°C. If small DPPC vesicles are incubated at 55°C and δ-toxin added, the phase transition recorded on cooling has an onset at a higher temperature than that of the DPPC SUV (i.e. fusion has occurred at a temperature above T_c for DPPC). These data very closely resemble the heating curve shown in Fig. 1b, and hence are not shown. These data do not, of course preclude changes in fusion at or near lipid phase transitions.

In the Introduction, it was mentioned that the lytic activity of δ-haemolysin towards DPPC vesicles is lost in buffer of high phosphate concentration. High phosphate concentration did not, however, inhibit the fusogenic effect of the peptide towards DPPC in its liquid-crystalline phase since the fluorescence polarisation data measured in 250 mM phosphate buffer are very similar to those seen at much lower phosphate concentration. It is not possible to directly compare these observations, since the polarisation measurements are made at higher temperature than the measurements of bilayer permeability. Increase in temperature might, for example, affect the conformation or aggregation state of the δ-toxin in high phosphate media. However, using vesicles of egg PC, which is liquid crystalline at 20°C, we have observed that high phosphate concentration (500 mM) does not prevent δ-toxin mediated lysis of vesicles leading to contents loss (results not shown). Using the PyPC assay mentioned earlier, lipid exchange is also clearly seen and light scattering changes indicate the formation of large structures in the presence of δ-toxin. Effects seen from 500 mM phosphate qualitatively parallel those at low ionic strength reported above, but are considerably slower. We conclude that the lack of effect of δ-toxin for gel phase phospholipid is related to the physical state of the lipid rather than to inherent loss of toxin activity. These data will be published separately.

The fusogenic effects of δ-haemolysin can be contrasted to those of melittin. Unlike δ-toxin, melittin is not a progressive fusogen under normal conditions [9,10], and vesicles of DPPC fuse only when they pass through a phase transition for neutral lipids. Vesicles, once fused by heating with melittin will not fuse further with small unilamellar vesicles subsequently added. For vesicles containing acidic lipids, melittin induces fusion much more readily than calcium ion [16]. Unlike melittin, δ-haemolysin does not appear to aggregate gel phase lipid, since no change in light scattering is seen with time after toxin addition [8]. The products of melittin fusion are not multilamellar aggregates [10], whereas structures formed by δ-haemolysin addition are multilamellar, at least when samples have been incubated for some time.

In view of the rapid loss of vesicle contents induced by δ-toxin, under conditions where light scattering and lipid mixing indicate slow aggregation and fusion, it seems reasonable to suggest that toxin binding initially disorders bilayer lipids. Leakage is not likely to be primarily a consequence of bilayer-bilayer interactions during vesicle fusion. The disorder induced by δ-toxin binding would be expected to reduce activation energy for bilayer mixing through initial encounter complexes or vesicle aggregates.

The similarities and differences in activity of the lytic peptides are worthy of further study and might lead to a better understanding of structure-activity relationships in membrane-lytic toxins generally. Synchrotron-radiation studies of the aggregation equilibria for δ-haemolysin are underway, and should shed light on the influence of high phosphate concentrations on the peptide.

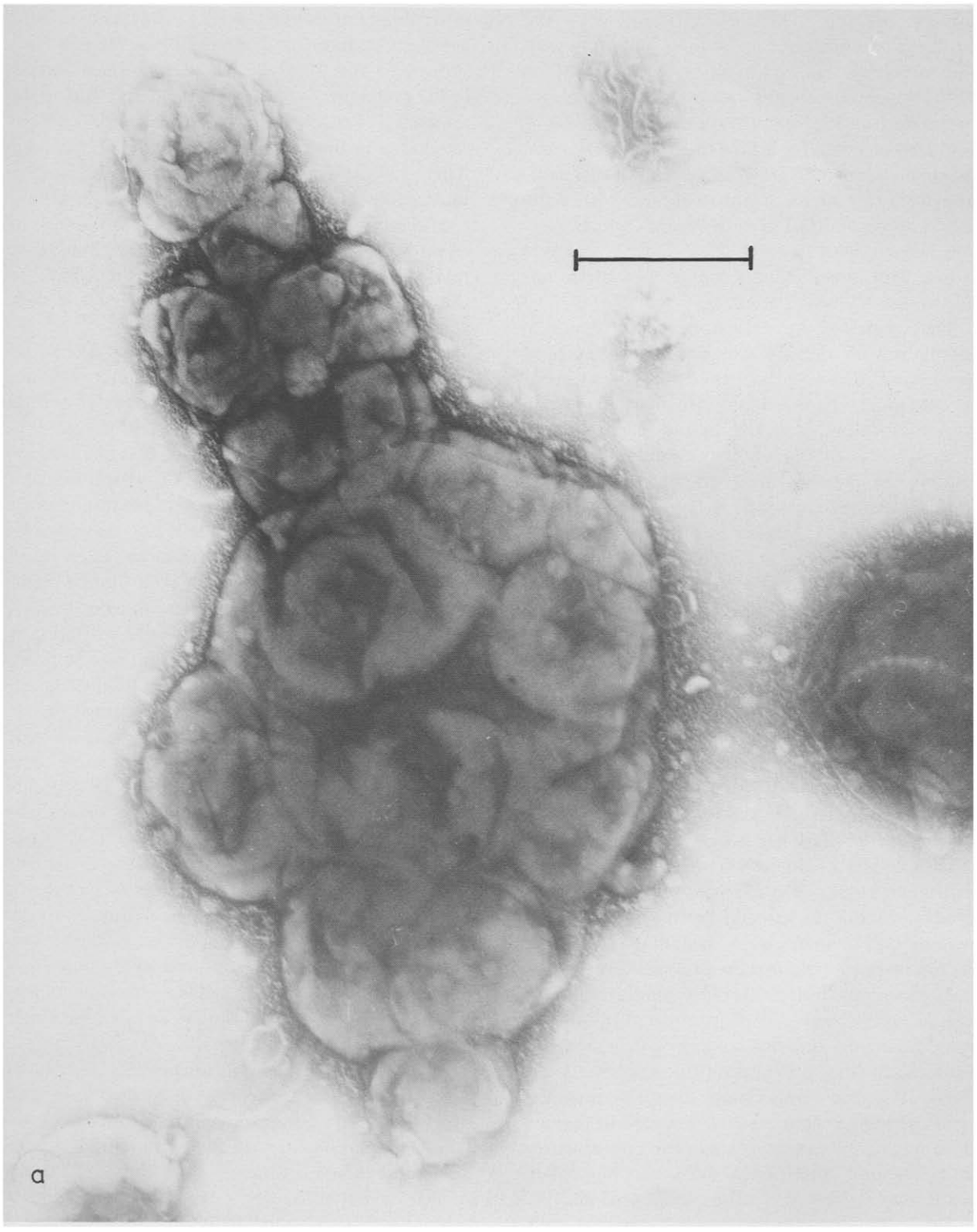


Fig. 5a.

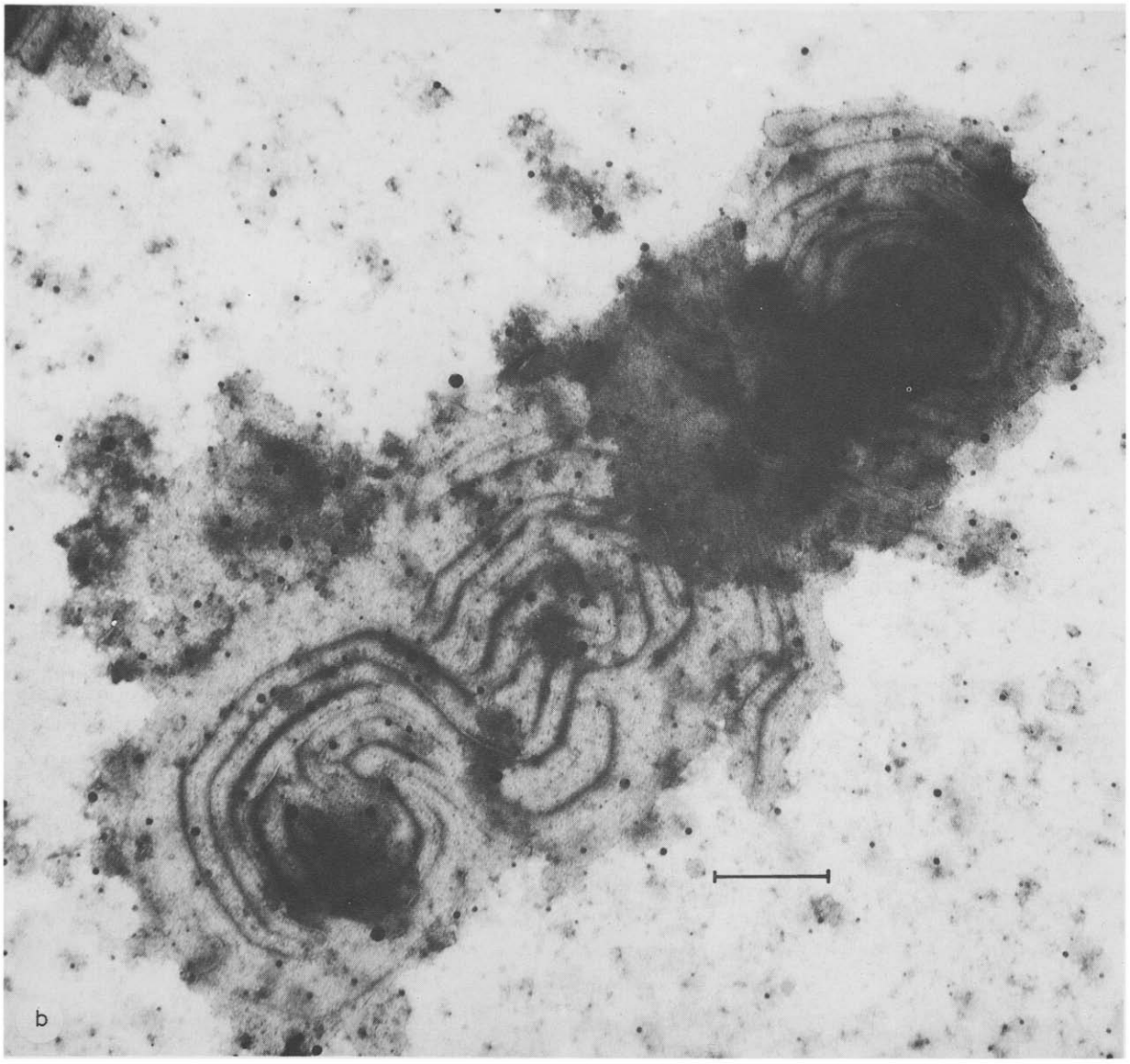


Fig. 5. Electron micrographs are shown for vesicles of egg PC and of DPPC after incubation with δ-toxin. Vesicle concentration was $2.6 \cdot 10^{-4}$ M and δ-toxin was 5.2 μM in all cases. (a) The effect of δ-toxin on LUV of egg PC after incubation at 20°C for 2 h. Magnification is 20000× and the bar equals 400 nm. (b) DPPC LUV which have been incubated for 2 h at 55°C with δ-toxin. Magnification is 24000× and the bar equals 200 nm. (c, see p. 138) DPPC SUV incubated with δ-toxin at 55°C for 2 h. Magnification is 80000× and the bar equals 100 nm.

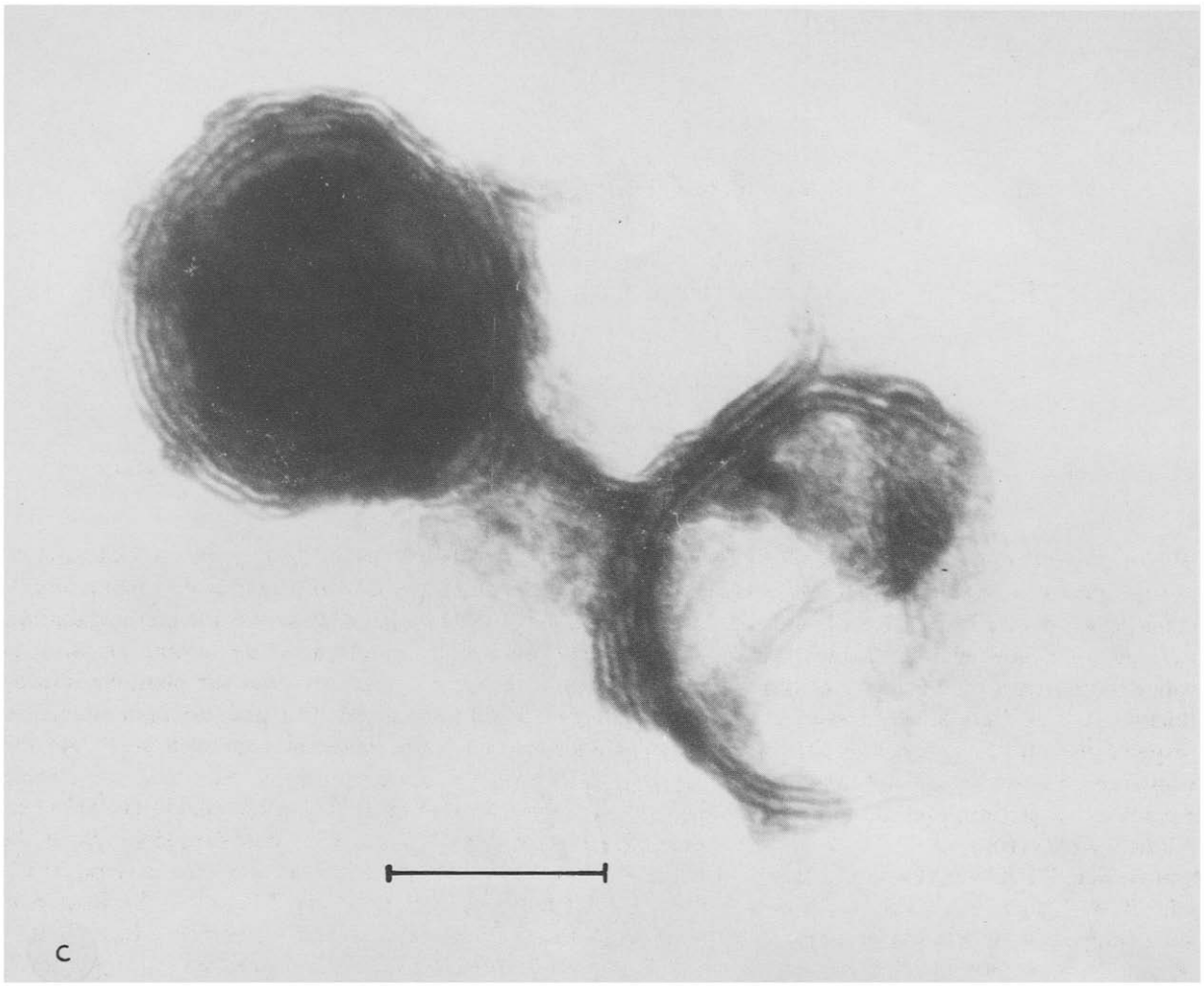


Fig. 5c. For legend see p. 137.

Time-resolved fluorescence and low-angle X-ray scattering data are presently being processed, and will be reported separately.

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