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# EFFECTS OF LIPID STRUCTURE ON PEPTIDE-LIPID INTERACTIONS

## COMPLEXES OF SALMON CALCITONIN WITH PHOSPHATIDYLGLYCEROL AND WITH PHOSPHATIDIC ACID

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**The interactions of salmon calcitonin with a number of phospholipids are studied by electron microscopy, circular dichroism and the leakage of carboxyfluorescein. At room temperature, calcitonin reacts strongly with dimyristoylphosphatidylglycerol and egg phosphatidic acid, while only moderate or no interaction is observed with several other phospholipids. The interaction is judged by the dissolution of the phospholipid dispersion and by electron microscopic observation and is in general concomitant with an increase in the helical content of the peptide. The electrostatic charge and the transition temperature of each of the phospholipids are important factors in determining the extent of reaction with salmon calcitonin. An exception is the sulphatide from bovine brain. The resulting morphology of the complex formed between salmon calcitonin and phosphatidic acid is quite different from that formed with phosphatidylglycerol. In the case of phosphatidylglycerol and most other negatively charged phospholipids, disc-shaped complexes are observed under the electron microscope by negative staining. The calcitonin-DMPG complexes are about 7 nm thick and their diameter increases with an increasing lipid-to-peptide ratio. In contrast, phosphatidic acids form spherical complexes with salmon calcitonin causing large multilamellar structures to spontaneously break-up into smaller particles of about 10 to 20 nm in diameter independent of the lipid-to-peptide ratio. The contrasting effects of salmon calcitonin on the morphology of these two phospholipids is explicable by consideration of the size of the lipid headgroup. Phosphatidic acid can accommodate the peptide without rupture of the bilayer, while the larger headgroup of phosphatidylglycerol requires the bilayer to rupture. This model is supported by studies of calcitonin-induced leakage of carboxyfluorescein from sonicated vesicles of 75% egg phosphatidylcholine and 25% either egg phosphatidic acid, egg phosphatidylglycerol or dimyristoylphosphatidylglycerol. There was a much greater increase in carboxyfluorescein leakage from phosphatidylglycerol-containing vesicles induced by salmon calcitonin demonstrating the greater ability of the peptide to rupture bilayers containing this phospholipid.**

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Abbreviations: DMPG, dimyristoylphosphatidylglycerol; DMPA, dimyristoylphosphatidic acid; PC, egg phosphatidylcholine; PG, phosphatidylglycerol from PC; PA, phosphatidic acid from PC; PS, phosphatidylserine from bovine brain; PI, phosphatidylinositol from yeast. Pipes, 1,4-piperazinediethanesulfonic acid.



## Introduction

Calcitonin is one of a number of peptide hormones which contain regularly spaced hydrophobic amino acid residues at every third or fourth position along the chain [1]. Such sequences can

fold into amphipathic helices, structures which are also thought to be important in enabling certain serum apolipoproteins to solubilize phospholipids [2]. Calcitonin can solubilize certain acidic phospholipids [3]. Like the case of many other polypeptides with amphipathic helices [2,3], the interaction between lipids and the polypeptide is highly dependent on the structure of the lipid. This 'lipophobic effect' has been recently discussed by Jähnig [4]. In this work, we study the effects of lipid headgroups and the lipid/polypeptide ratio on the nature of the peptide-lipid complex formed.

## Experimental

*Materials*

Salmon calcitonin was synthesized by Armour Pharmaceutical Co., Kankakee, IL.

Dimyristoylphosphatidylglycerol (DMPG) was purchased from Avanti Polar Lipids, Inc. and dimyristoylphosphatidic acid (DMPA) from Calbiochem. Egg phosphatidylcholine (PC) was purchased from Sigma Chem. Co. as were phosphatidylglycerol (PG) and phosphatidic acid (PA), both products of phospholipase D treatment of PC.

*Methods*

*Buffer.* Unless otherwise indicated the buffer used was 20 mM Pipes, 1 mM EDTA, 150 mM NaCl containing 0.02 mg/ml $NaN_3$ (pH 7.40).

*Formation of salmon calcitonin-PA complexes.* PA was first deposited as a film from a 2:1 (v/v) chloroform/methanol solution by solvent evaporation under nitrogen followed by removal of traces of solvent for at least 1 h in a vacuum oven at 40°C with a liquid nitrogen trap. To the dry lipid, a solution of salmon calcitonin in Pipes buffer was added and the mixture vortexed above the phase transition temperature of the lipid. The mixture was then cooled and heated between 10 and 40°C to aid in equilibration. For the circular dichroism experiments the solution, which was stored frozen, was thawed and centrifuged for 3 min at room temperature in an Eppendorf centrifuge.

*Peptide concentration.* The concentration of salmon calcitonin was determined by weight or by its absorbance at 275 nm using 1515 $cm^{-1} \cdot M^{-1}$ as the extinction coefficient [3].

*Lipid concentration.* Lipid concentrations were calculated from the phosphate content of the solutions after digestion with perchloric acid [5].

*Carboxyfluorescein leakage experiment.* Carboxyfluorescein is impermeable to phospholipid bilayers and is a highly fluorescent substance which undergoes concentration dependent self-quenching. An assay for the leakage of carboxyfluorescein which had been entrapped at high concentration within sonicated liposomes was employed [6]. Dried lipid films were suspended in Pipes buffer containing 250 mM carboxyfluorescein by vortexing. The resulting suspension was sonicated for about 20 min in a Bransonic 12 bath type sonicator. The vesicles containing entrapped carboxyfluorescein were then separated from extra-vesicular carboxyfluorescein by passage through a 1.5 × 30 cm column of Sephadex G-50 at room temperature.

Leakage of carboxyfluorescein from these vesicles was measured by monitoring the increase in fluorescence emission at 520 nm with time using a Perkin-Elmer MPF-44 spectrofluorimeter in the ratio mode. The excitation wavelength was set at 490 nm and the slit widths at 4 nm. The sample was continuously stirred and maintained at 25°C. 2 ml of buffer were added to the cuvette and the fluorimeter output signal set at zero. 15 μl of the vesicle-containing void volume eluent from the gel-filtration and containing approx. 50 nmol of phosphate were added to the buffer and the zero-time fluorescence recorded. 5 to 20 μl of a solution of salmon calcitonin were then added to the stirred suspension of liposomes and the rate of increase of fluorescence was measured. Finally 100 μl of 1.0% Triton X-100 was added to determine the total concentration of carboxyfluorescein present.

In the case of PG, the vesicles were found to be very leaky to carboxyfluorescein even before the addition of salmon calcitonin, while with PA little carboxyfluorescein was found entrapped in the vesicles after gel filtration. The lipid composition was therefore changed to stabilize the bilayer by adding 75% PC and mixed with 25% of one of the acidic phospholipids. Leakage from these vesicles was also compared with that from vesicles composed of PC as the sole lipid component. Before addition of peptide, rates of carboxyfluorescein

leakage from any of these vesicles was always less than 2%/min. Peptide-induced release of carboxyfluorescein was essentially instantaneous at high peptide concentrations or in the presence of Triton X-100. At lower peptide concentrations there was generally a biphasic release with a very rapid release of a significant portion of the carboxyfluorescein followed by a continued slower rate of release which was somewhat more rapid than the rate of release in the absence of peptide. For comparative purposes we have used the fraction of carboxyfluorescein released during the first two minutes after addition of the peptide. During this time period all of the release of carboxyfluorescein which occurs during the rapid phase is essentially complete and there is an additional contribution from the slower release phase of carboxyfluorescein. The data are expressed as % carboxyfluorescein release = $100(F - F_0)/(F_T - F_0)$, where $F$ is the fluorescence intensity measured after 2 min, $F_0$ is the fluorescence intensity measured at zero-time, just prior to the addition of the peptide solution and $F_T$ is the total fluorescence of the carboxyfluorescein measured after vesicle rupture with Triton X-100.

*Circular dichroism.* Circular dichroism spectra were recorded on a Cary 61 spectropolarimeter with the temperature of the sample maintained at 25°C. The data are expressed as the mean residue ellipticity using 107.4 as the mean residue weight of salmon calcitonin.

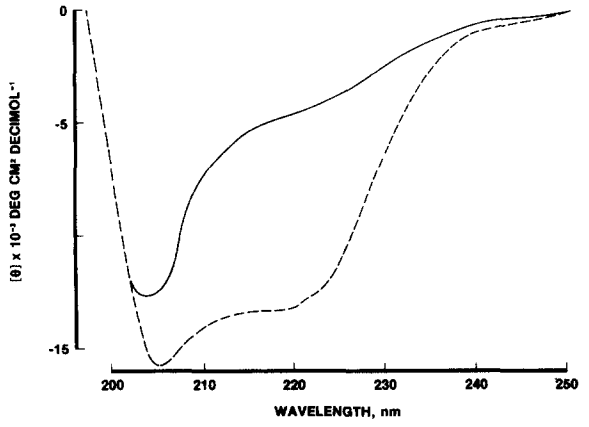


Fig. 1. Circular dichroism of salmon calcitonin (100 $\mu$M) alone (solid line) or in the presence of 3.5 mM PA (dashed line). Pipes buffer, 25°C.

*Electron microscopy.* Negative-stained samples were made at room temperature (25°C). The grids were pretreated with 0.01% bacitracin to render the Formvar-carbon film hydrophilic. Untreated films were used for comparison and the results were identical. The samples were allowed to settle on the Formvar-carbon film for 1 min and then stained with 2% ammonium molybdate. The air-dried specimens were examined in a Siemens 101 microscope at 40000 to 80000 ×. For morphological measurements, at least 50 similar features (vesicles, discoidal complexes) photographed from different areas of the grid, and from different grids, were measured. Standard deviations were calculated.

## Results

*Phosphatidic acid*

The circular dichroism spectrum of salmon calcitonin is markedly altered by the presence of PA (Fig. 1). The magnitude of the ellipticity at 222 nm of 100 $\mu$M salmon calcitonin in the presence of 1 mM egg PA decreases with increasing temperature, having values of $-14000$, $-13300$ and $-7800$ deg · cm$^2$ · dmol$^{-1}$ at 5, 25 and 50°C, respectively. The ellipticity at 222 nm of a solution of 90 $\mu$M salmon calcitonin with 1.6 mM DMPA at 25°C was only $-8000$ deg · cm$^2$ · dmol$^{-1}$, indicating a weaker interaction of this synthetic phospholipid with salmon calcitonin. The affinity of salmon calcitonin for egg PA was further evaluated from measurements of the dependence of the circular dichroism spectra on the ratio of salmon calcitonin to PA. Freshly prepared solutions of salmon calcitonin with PA occasionally contained some particulate material and generally exhibited circular dichroism spectra of lower magnitude than was observed for the same solutions after centrifugation. To obtain more accurate data for the dependence of the circular dichroism on lipid concentration, solutions were centrifuged to removed unreacted lipid and the circular dichroism as well as the lipid and peptide concentrations of the supernate were determined. The resulting binding curve still did not have a high degree of precision and the final lipid/peptide ratio could not be predetermined resulting in an uneven distribution of data points (Fig. 2). The resulting curve could

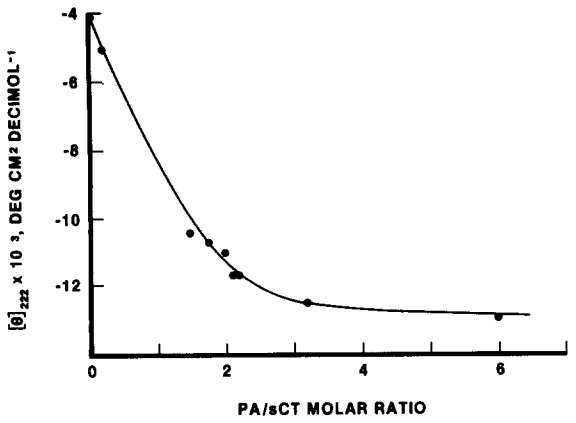


Fig. 2. Dependence of the mean residue ellipticity at 222 nm of salmon calcitonin (115 $\mu$M) on the molar ratio of PA to salmon calcitonin (sCT). Pipes buffer, 25°C. Experimental points fitted to a single association constant of $2 \cdot 10^5$ $M^{-1}$ for the binding of salmon calcitonin to two molecules of PA.

be well described by a single set of independent binding sites with two lipid molecules bound per salmon calcitonin and an affinity constant of approximately $2 \cdot 10^5$ $M^{-1}$.

The morphology of PA is greatly affected by the presence of salmon calcitonin. In the absence of salmon calcitonin, the PA suspension is 'milky'. Many vesicles are observable by light microscope. Electron microscope observation reveals that these vesicles are multilamellar, with diameters varying from 0.5 $\mu$m to 2 $\mu$m. In the presence of salmon calcitonin, the egg PA suspensions become clear, especially upon cooling slightly below the room temperature. Electron micrographs show that large vesicles of egg PA are broken up into very small particles of 10–20 nm size (Fig. 3a). Unlike sonicated vesicles which appear round and usually have a stain-filled center and definite walls when observed by negative staining technique, these particles appear solid and irregular in shape. Unlike the salmon calcitonin-DMPG complexes (see later), the size of these particles do not seem to vary with the initial lipid/peptide ratio. In samples with high lipid/peptide ratio, excess PA multilamellar vesicles are observed (Fig. 3b). At extremely high lipid/peptide ratio (e.g., 68), the suspension appears translucent by visual observation. Many small vesicles (0.1 $\mu$m) are seen by negative staining electron microscopy; intermixed with the 10–20 nm particles and larger vesicles (Fig. 3c). If DMPA is used instead of egg PA, many vesicles of about 0.3 $\mu$m in diameter are observed together with small (20 nm) particles. The morphology and the extent of interaction involving the two phosphatidic acids examined are qualitatively different. This observation is in accord with the different extent of helix formation induced in the peptide by the two forms of PA as observed by CD. Small

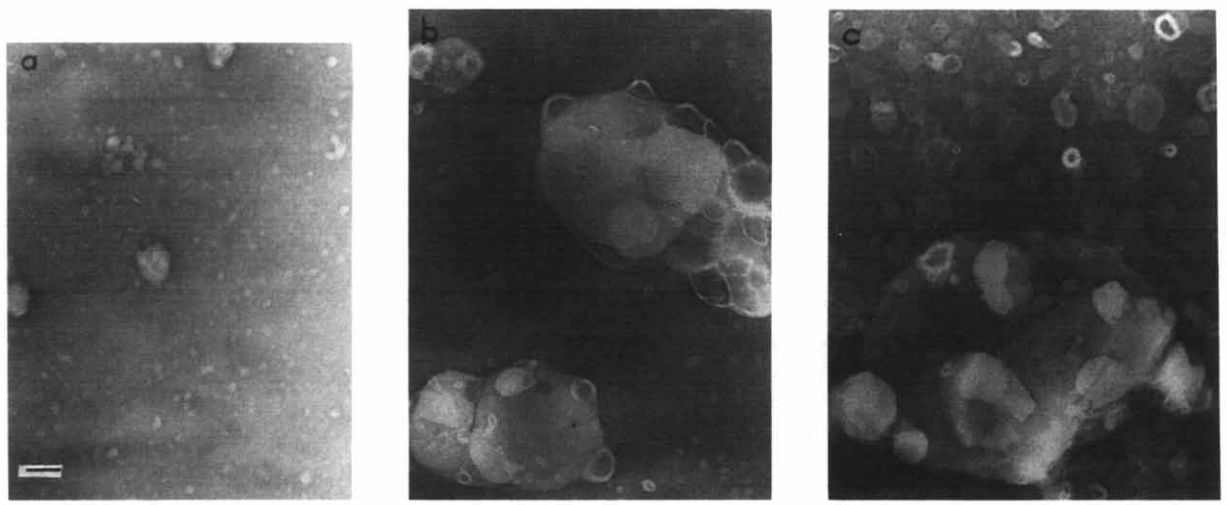


Fig. 3. Negative stain electron micrographs of egg PA/salmon calcitonin mixture of various lipid/peptide (L/P) ratios. (a) L/P = 4.0, (b) L/P = 30 and (c) L/P = 68. Some small vesicles appear to be 'blebbing' from large vesicles in (b) and (c). Bar = 0.1 $\mu$m.

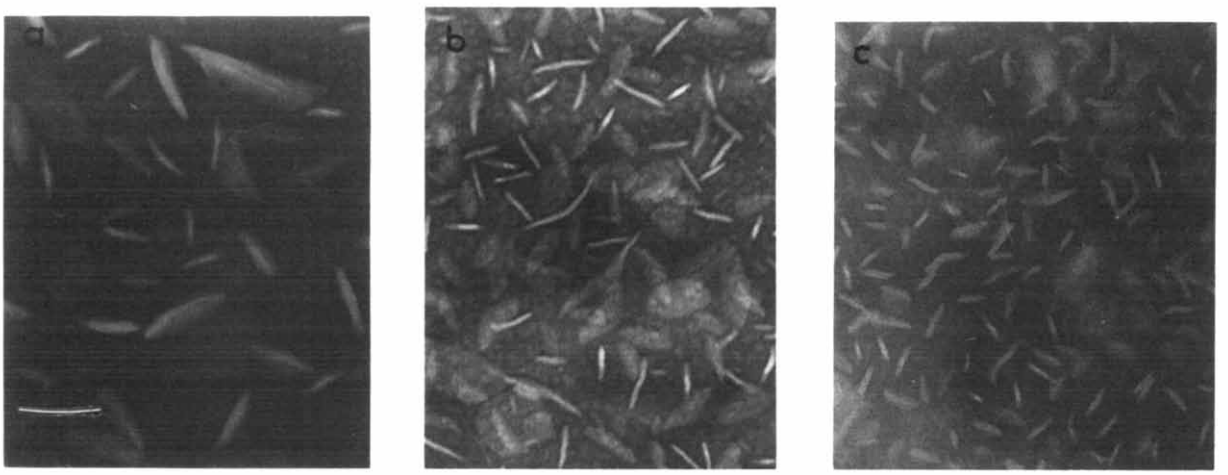


Fig. 4. Negative stain electron micrographs of samples from various fractions of DMPG/salmon calcitonin mixtures eluted from a Sepharose 4B column. The samples have a L/P ratio of (a) 43.5, (b) 23.5 and (c) 13.7. Bar = 0.1 $\mu$m.

unilamellar vesicles of PA can also be produced by transiently raising the pH to 10–11 [7].

*Phosphatidylglycerol*

The resulting morphology of the salmon calcitonin-PG complex is again qualitatively different from those of salmon calcitonin-PA. The salmon calcitonin-DMPG complex is disk or flake [3] in shape, the size of which depends critically on the lipid/peptide ratio. The 'disks' are typically 7 nm thick, suggesting that they are formed at the most by a single bilayer. Some samples from different fractions of a column eluent are shown in Fig. 4. Complexes of varying size are separated by a Sepharose 4B column [3], with higher lipid/peptide ratio (larger size) complex eluting earlier. The dependence of the long dimension on the lipid/peptide ratio of the complexes is shown in Fig. 5. The data include measurements both from column eluents and from direct mixing of predetermined quantities of lipid and peptide. The resultant lipid/peptide ratio of complexes formed by both methods were determined after the complexes were isolated. The size of the complexes increases with increasing lipid/peptide ratio.

Discoidal complexes are also formed between salmon calcitonin and egg PG, but the reaction is incomplete. Many large multilamellar vesicles were still observable at an initial lipid/peptide ratio of 10. The comparison between DMPG and egg PG is opposite to that between DMPA and egg PA; egg PA is more readily disrupted than DMPA by salmon calcitonin.

The presence of salmon calcitonin can accelerate the release of carboxyfluorescein which had been entrapped in phospholipid vesicles (Fig. 6). The sensitivity of these vesicles to rupture by carboxyfluorescein is dependent on their phospholipid composition with the addition of 25% PA making PC vesicles slightly more sensitive to lysis by salmon calcitonin but with the addition of 25% PG (or DMPG) making the vesicles much more sensitive to salmon calcitonin-induced leakage.

*Other phospholipids*

Apart from PA and PG, salmon calcitonin also

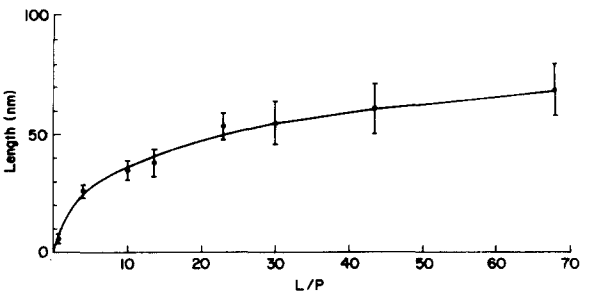


Fig. 5. The long dimension of DMPG-salmon calcitonin complexes as a function of their lipid/peptide ratio. The error bars represent standard deviations of the data points.

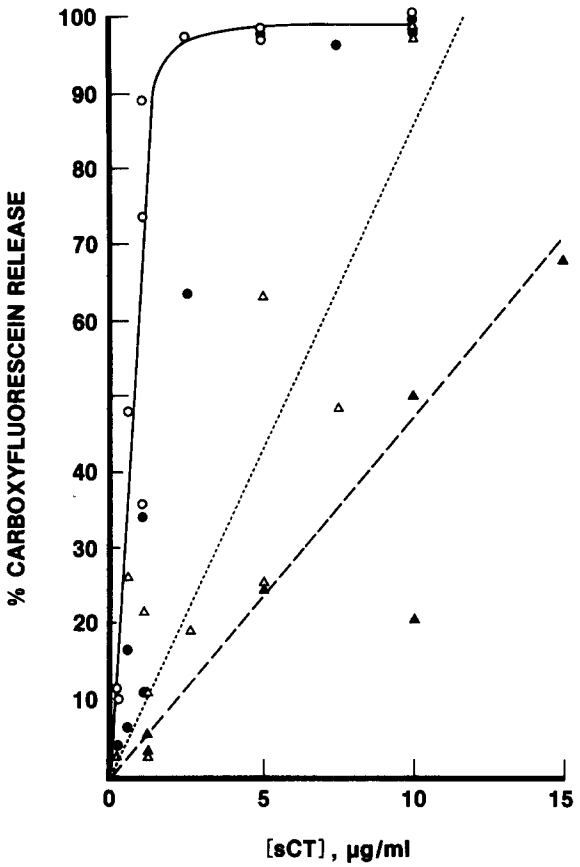


Fig. 6. Extent of carboxyfluorescein release from sonicated vesicles induced by salmon calcitonin (sCT). Pipes buffer, 25°C; PC (▲- - - - -▲); 75% PC, 25% PA (△- - - - -△); 75% PC, 25% PG (○———○); 75% PC, 25% DMPG (●———●).

reacts with other phospholipids including PS, PI and sulfatide. At room temperature, the reactions of salmon calcitonin with PS and PI are less complete than with egg PA or DMPG. Figs. 7a and 7b show the resulting mixtures of lipid/peptide complexes and the remaining lipid vesicles when salmon calcitonin is added to PS and PI, respectively. The PS vesicles are mostly unilamellar, their surfaces appear to be contorted by domains of contingent salmon calcitonin-PS complexes. This morphology is also apparent in the rough freeze-fracture faces (results not shown) of these vesicles. The PI vesicles, on the other hand, remain multilamellar. The complexes appear to be 'peeling off' the outer layers of the vesicles to form the discoid or flaky units about a single bilayer thick. The intermediate stage of forming these complexes is more apparent in higher magnification (Fig. 7c). The reaction between salmon calcitonin and sulfatide is complete at room temperature, resulting in the complete solubilization of the lipid into discoidal complexes (Fig. 7d). In general, lipids whose structures are disrupted by salmon calcitonin induce more secondary structure in the peptide. For example DMPG and egg PA which form low molecular weight complexes with salmon calcitonin exhibit large negative ellipticities at 222 nm ($-12350$ and $-14600$ deg · cm$^2$ · dmol$^{-1}$, respectively). However, the low molecular weight complex which forms between sulfatide and salmon calcitonin exhibits a much weaker CD band at 222 nm than does the less soluble salmon calcitonin-PI complex ($-4900$ vs. $-13100$ deg · cm$^2$ · dmol$^{-1}$).

## Discussion

Among the phospholipids we have studied so far, DMPG and egg PA are most reactive with salmon calcitonin at room temperature. Both PA

TABLE I

COMPARISON OF THE PHASE TRANSITION TEMPERATURE OF PHOSPHOLIPIDS AND THEIR INTERACTION WITH SALMON CALCITONIN

| | $T_c$ [a] | $\Delta T_{25}$ [b] | $[\theta]_{222}$ [c] | Reaction [d] |
|---|---|---|---|---|
| DMPC | 24 | −1 | −4.2 | − |
| Egg PC | −15 | −40 | −4.2 | − |
| DMPG | 23 | −2 | −12.4 | + + + |
| Egg PG | −15 | −40 | −6.7 | + |
| DMPA | 50 | 25 | −8.0 | + + |
| Egg PA | 17 | −8 | −14.6 | + + + |
| Bovine Brain PS | 5 | −20 | −5.7 | + + |
| Yeast PI | 50 | 25 | −13.1 | + + |
| Bovine brain sulfatide | 50 | 25 | −4.9 | + + + |

[a] Phase transition temperature in °C.

[b] $\Delta T_{25} = T_c - 25$ in °C.

[c] In units of deg · cm$^2$ · dmol$^{-1}$ ($\times 10^{-3}$). For salmon calcitonin in the absence of phospholipid $[\theta]_{222} = -4200$ deg · cm$^2$ · dmol$^{-1}$.

[d] Reactions are rated by morphology. At a lipid/peptide ratio of 10, those lipids completely 'dissolved' by salmon calcitonin into complex particles are rated + + +; partially 'dissolved' are rated + +; sparsely 'dissolved' are rated +; and those remaining vesicular dispersion thus unaffected by the presence of salmon calcitonin are rated −.

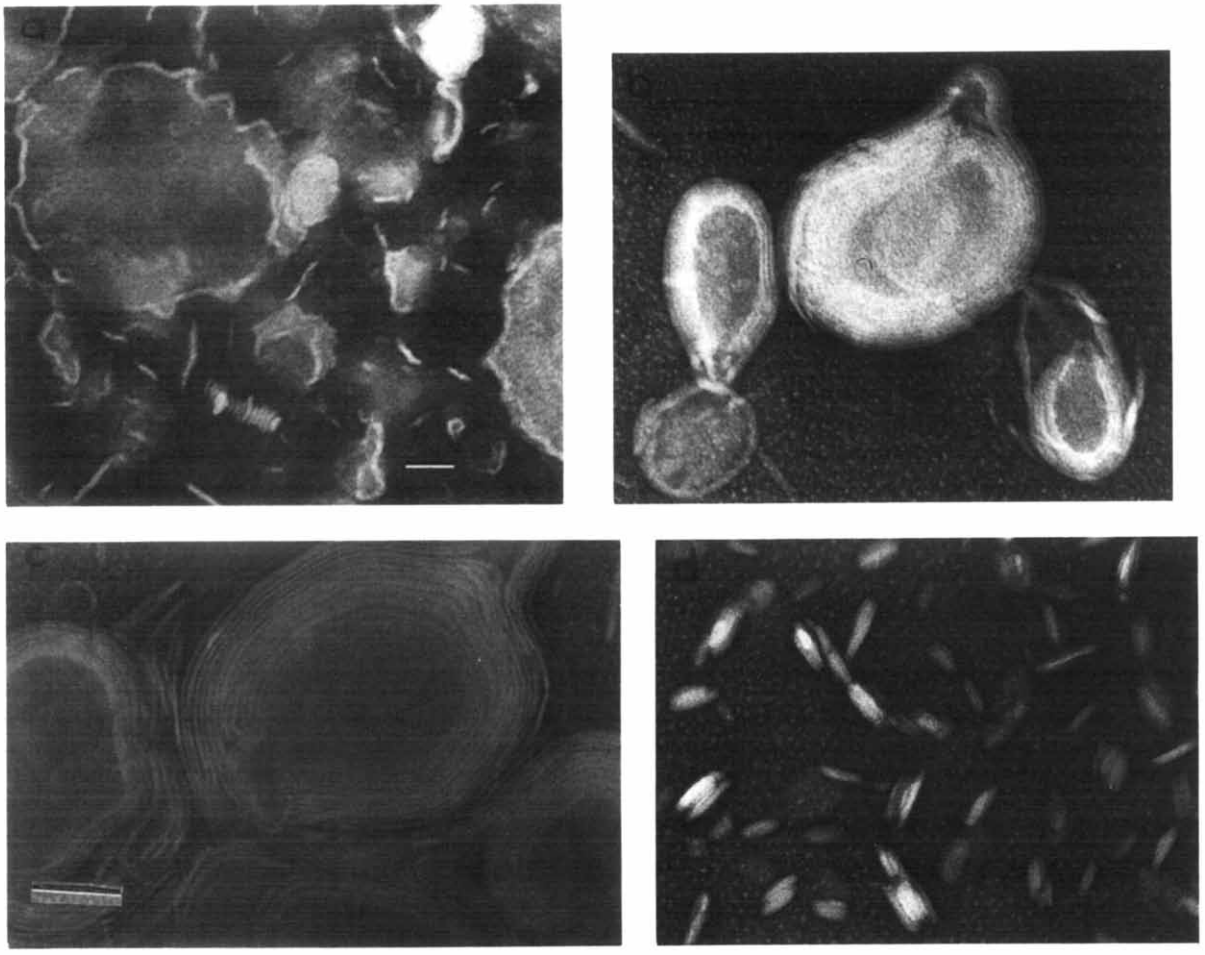

Fig. 7. Negative stain elution micrographs of salmon calcitonin/phospholipid mixtures at L/P = 10. (a) salmon calcitonin/PS, (b and c) salmon calcitonin/PI, (d) salmon calcitonin/sulfatide. Bar = 0.1 $\mu$m.

(Fig. 1) and DMPG [3] induce a similar change in the CD of the peptide. Both of these lipids bind to salmon calcitonin with similar affinity constants, approx. $10^5$ $M^{-1}$, with 2 PA or 5 DMPG binding per salmon calcitonin. The smaller number of PA molecules suggests less penetration of salmon calcitonin into the bilayer of this phospholipid compared with DMPG. Egg PG [3] or DMPA induce considerably less helical content in salmon calcitonin than do DMPG or egg PA, and the reactions are less complete as judging by morphology, possibly because the former two phospholipids have phase transitions at $-15$°C [8] or about 50°C [9], respectively, which is further removed from 25°C, the temperature at which we have

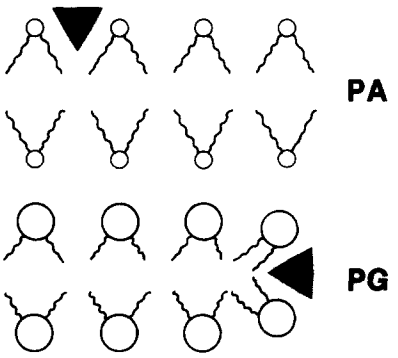


Fig. 8. Diagrammatic model indicating the different nature of salmon calcitonin penetration into phospholipid bilayers dependent on the relative size of the lipid headgroup. salmon calcitonin is represented by a triangle.

studied interactions with salmon calcitonin. In contrast, egg PA and DMPG have transition temperatures at 17°C [10] and 23°C [11], respectively. The results are summarized in Table I.

From the studies of the interaction between salmon calcitonin and a number of phospholipids with different headgroups, and acyl chains, we found that there are some general rules governing the degree of the lipid-peptide interaction. (1) salmon calcitonin does not react with zwitterionic lipids such as PC. It reacts favorably with negatively charged lipids such as PA, PS and PG. (2) When other conditions are equal, reaction with salmon calcitonin proceeds most readily at temperatures close to the phase transition temperatures of the lipid (Table I). The rate of reaction is generally very rapid. At a concentration of 400 $\mu$M of polypeptide and 4 mM of DMPG, the reaction is completed within 30 s as monitored by rapid freezing electron microscopy (results not shown). This observation supports the proposition that maximal structural defects at the phase transition facilitates lipid-peptide interaction [12,13] and that peptide-promoted changes in the phase of the lipid may contribute to a stabilization of the transition state [13] and thereby increase the rate. (3) In general, circular dichroism increases with an increasing degree of reaction as judged by morphology (Table I). An exception to these rules is the bovine brain sulfatide which has a high transition temperature. Although it induces a low CD value for salmon calcitonin ($-4900$ degree $\cdot$ cm$^2$ $\cdot$ dmol$^{-1}$ at 222 nm) it is completely 'dissolved' by this peptide into discoid complexes. In addition, PI which is one of the lipids inducing the greatest amount of helical structure, shows only moderate morphological changes. Thus, lipid charge and phase transition properties are clearly important factors determining the extent of their interaction with peptides. However, other factors, such as the nature and extent of lipid-lipid interactions, must also come into play in individual cases.

Despite the apparent weaker interaction of salmon calcitonin with egg PG, compared with egg PA, vesicles containing the former phospholipid are lysed more readily by salmon calcitonin than those containing an equivalent amount of PA. This is not caused by a difference in binding affinity to salmon calcitonin or to the phase transition temperature of this phospholipid component since increased lysis of vesicles is observed when salmon calcitonin is added to vesicles containing either egg PG or DMPG. Egg PG induces less helical structure in salmon calcitonin compared with egg PA and it has a lower transition temperature while DMPG binds salmon calcitonin with similar affinity to egg PA and has a higher transition temperature. Yet both egg PG and DMPG render vesicles more sensitive to lysis by salmon calcitonin.

These effects on vesicle lysis can be explained by the different morphology of the salmon calcitonin complex with PG and with PA resulting from the difference in the structure of the lipid. The salmon calcitonin/PG complexes like the salmon calcitonin/PI and salmon calcitonin/PS complexes, are discoidal shape, with a thickness slightly more than that of a bilayer. Their formation are likely to begin by 'peeling' off the vesicle wall as depicted by Figs. 7b and c. The mechanism is similar to the glucagon/DMPC reaction [14]. The salmon calcitonin/PA complexes, on the other hand, are spherical shape. The formation of these spherical complexes is likely to begin by 'blebbing' from the vesicle as depicted by Figs. 3b and 3c. The reason for this difference may be in the different molecular shapes of PG and PA. PA has a smaller head group than PG, hence it may be able to accommodate a peptide incorporation without requiring a complete rupture of the bilayer. PA therefore forms vesicular or micellar complexes with salmon calcitonin and does not greatly promote carboxyfluorescein leakage from vesicles. In contrast, PG has a crowded headgroup region which does not permit salmon calcitonin to enter unless the bilayer is fragmented to form discoidal complexes, resulting in leakage of vesicle contents. This model is described diagrammatically in Fig. 8. Our results demonstrate the importance of lipid structure in determining the characteristics of the complexes which these lipids can form with certain peptides. A number of factors, such as the amphipathic nature of the peptide, the net electric charge of the lipid, the phase transition and the molecular shape of the lipid, all play a role in determining the nature of the lipid-peptide interaction.

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## References

1 Epand, R.M. (1983) Mol. Cell. Biochem. 57, 41–47
2 Segrest, J.P., Jackson, R.L., Morrisett, J.P. and Gotto, A.M. (1974) FEBS Lett. 38, 247–253
3 Epand, R.M., Epand, R.F., Orlowski, R.C., Schlueter, R.J., Boni, L.T. and Hui, S.W. (1983) Biochemistry 22, 5074–5084
4 Jähnig, F. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 3691–3695
5 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468
6 Steer, C.J., Klausner, R.D. and Blumenthal, R. (1982) J. Biol. Chem. 257, 8533–8540
7 Gains, N. and Hauser, H. (1983) Biochim. Biophys. Acta 731, 31–39
8 Ladbrooke, B.D. and Chapman, D. (1969) Chem. Phys. Lipids 3, 304–356
9 Träuble, H. and Eibl, H. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 214–219
10 Boggs, J.M. and Moscarello, M.A. (1978) J. Membrane Biol. 39, 75–96
11 Findlay, E.J. and Barton, P.G. (1978) Biochemistry 17, 2400–2405
12 Pownall, H.J., Massey, J.B. Kusseron, S.K. and Gotto, A.M. (1979) Biochemistry 18, 574–579
13 Epand, R.M. and Epand, R. (1980) Biochim. Biophys. Acta 602, 600–609
14 Epand, R.M., Epand, R.F., Stewart, T.P. and Hui, S.W. (1981) Biochim. Biophys. Acta 649, 608–615