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Phospholipid structure determines the effects of peptides on membranes. Differential scanning calorimetry studies with pentagastrin-related peptides

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The effect of phospholipid structure on the interaction between small peptides and phospholipid membranes has been studied by high-sensitivity differential scanning calorimetry. The peptides used, *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 and *N*-Boc-β-Ala-Trp-Met-Lys-Phe-NH_2, are basic analogs of the hormone pentagastrin. These peptides split the gel-to-liquid crystalline phase transition of synthetic phosphatidylcholines into two components. For dimyristoyl (DMPC), dipalmitoyl (DPPC) and 1-stearoyl-2-oleoyl (SOPC) phosphatidylcholines, one component remains at the temperature corresponding to that of pure lipid and the other one is shifted towards higher temperatures. With increasing peptide concentration there is a gradual increase in the enthalpy of the high-temperature component at the expense of the low-temperature one, and there is also an increase in the total enthalpy of the transition. A mixture of the peptide with distearoylphosphatidylcholine (DSPC) behaves differently, with the transition occurring at a temperature below that of the pure lipid increasing with peptide concentration. The susceptibility of various phosphatidylcholines to perturbation by the peptides increases in the order DMPC > SOPC > DPPC > DSPC. The effect of these peptides on the phase transitions of acidic phosphatidylglycerols is generally greater than with the corresponding phosphatidylcholines, but the dependence on the length of lipid hydrocarbon chains is similar. Perturbation of the thermotropic phase transition is strongest for dimyristoylphosphatidylglycerol, followed by the dipalmitoyl and the distearoyl analogs. The effect of the peptides on the phase transition of dimyristoylphosphatidylserine is significantly smaller compared to that observed with dimyristoylphosphatidylglycerol and it is further reduced for dimyristoylphosphatidic acid. The phase transition of this latter lipid remains virtually unchanged, even in the presence of high concentrations of the peptide. Similar resistance to the perturbation of the phase transitions by the peptides is observed for synthetic phosphatidylethanolamine. The different susceptibility of various phospholipids to perturbation by the peptides is suggested to be related to different degrees of intermolecular interaction between phospholipid molecules, and particularly to different abilities of phospholipids to form intermolecular hydrogen bonding.

Abbreviations: DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; SOPC, 1-stearoyl-2-oleoylphosphatidylcholine; DMPG, dimyristoylphosphatidylglycerol; DPPG, dipalmitoylphosphatidylglycerol; DSPG, distearoylphosphatidylglycerol; DMPS, dimyristoylphosphatidylserine; DMPA, dimyristoylphosphatidic acid; DLPE, dilauroylphosphatidylethanolamine; DSC, differential scanning calorimetry; Pipes, 1,4-piperazinediethanesulfonic acid.

Introduction

Various aspects of the interactions between lipids and peptides are subjects of considerable interest at the present time. Simple, well-defined lipid-peptide systems provide a very convenient model for studying the nature of the molecular interactions which can occur among lipids and

proteins in more complex biological membranes [1–8]. These interactions are important in determining membrane structure and properties. Moreover, the site of action of many biologically active peptide hormones and drugs is at the plasma membrane. The biological potency of some of these peptides is related to their ability to interact with membrane lipids [9,10].

We have recently demonstrated that a group of pentapeptides related to the hormone analog, pentagastrin, may be particularly suitable for detailed studies of factors involved in lipid-peptide interactions [6,7]. From among various structurally related pentapeptides studied, the basic analog of pentagastrin, *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2, has been shown to interact particularly strongly with zwitterionic phosphatidylcholine [6,7]. This interaction was demonstrated in several ways, including the marked changes produced by the peptide in the thermotropic properties of DMPC [7]. Our previous calorimetric study on the interaction between the model peptide, *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2, and DMPC is now extended to other phospholipids to determine how lipid headgroup structure and the nature of hydrocarbon chains affect lipid-peptide interactions.

Materials and Methods

Phospholipids were obtained from Calbiochem (DMPC, DPPC and DSPC), Avanti Polar Lipids (DMPG, DPPG, DSPG, DMPS, SOPC and DSPC) and Sigma (DSPC and DMPA). Saturated phosphatidylcholines were further purified by recrystallization from chloroform/hexane [11]. Other phospholipids were used without further purification. All the phospholipids run as a single spot on TLC. *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 and *N*-Boc-β-Ala-Trp-Met-Lys-Phe-NH_2 were from Peninsula Labs. Each of these peptides was shown to be at last 95% pure by HPLC using a 15 cm Varian MCH-5, C-18 reverse-phase column with a gradient elution from 80% H_2O, 0.1% trifluoroacetic acid, 20% isopropanol to pure isopropanol.

For preparation of the samples used for differential scanning calorimetry, an appropriate amount of lipid was dissolved in chloroform and the solvent was evaporated to dryness first under nitrogen and then in a vacuum dryer for 3–4 h. 2 ml of standard Pipes buffer (20 mM Pipes/1 mM EDTA/150 mM NaCl, pH 7.4) containing peptide at the desired concentration was added to the lipid film. The above mixture was subsequently vigorously vortexed for approx. 10 min, during which time the sample was warmed and cooled repeatedly through the transition temperature. Samples were subsequently incubated for several hours at a temperature around or slightly below the transition temperature of the pure phospholipid. The calorimetric scans were essentially independent of the thermal history of the sample, except for DMPC and DMPG, as previously described [7].

Calorimetric scans were performed with an ultrasensitive differential scanning calorimeter, Microcal-2 (Microcal, Inc., Amhersts, MA). The scanning rate was 24 or 36 deg. C/h. The sample cell volume was 1.29 ml. The temperature of maximal excess heat capacity was defined as the phase-transition temperature.

Phospholipid vesicles used in fluorescence titration experiments were prepared by ultrasonic irradiation of multilamellar liposomes. Sonication was performed for about 30 min under a nitrogen atmosphere in a Bransonie 12 bath-type sonicator at a temperature above the lipid phase transition. Apparent dissociation constants of lipid-peptide complexes were determined from the enhancement of peptide fluorescence as a function of lipid concentration, as described previously [6].

Results

Differential scanning calorimetry

Fig. 1 shows representative DSC scans of various phosphatidylcholines without and in the presence of increasing concentrations of *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2. In agreement with our previous results [7], the sharp phase transition of DMPC centered at 23.6°C is split into two components in the presence of the peptide (Fig. 1A). There is a relatively sharp transition near the transition temperature of pure lipid and a broader component which is gradually shifted towards higher temperatures as the peptide concentration is increased (Fig. 2). With increasing peptide concentration, there is also a gradual increase in the enthalpy of the high-temperature transition at the expense of the low-temperature transition (Fig.

3A) until finally, the lower-temperature component disappears completely. The effects of *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 on the thermotropic behavior of DPPC, although qualitatively similar to those reported for DMPC, are far less dramatic. The peptide also produced splitting of the DPPC transition into two well-defined components (Fig. 1B). Much higher concentrations of the peptide are, however, required to induce the high temperature transition and to suppress the original low-temperature transition of DPPC compared to DMPC. The threshold concentration of *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 necessary for the complete elimination of the low-temperature transition amounts to about 0.5 and 1.2 mM for DMPC and DPPC, respectively. Moreover, the peptide-induced upward shift in the transition temperature of DPPC is only half of that observed with DMPC (Fig. 2).

The effects of the peptide on the transition profile of DSPC contrast with those observed with DMPC and DPPC. With DSPC, *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 also induces the formation of two transition components (Fig. 1C). The temperature of the main transition (54.3°C for pure lipid) is, however, not increased but is rather decreased by the peptide (Fig. 2). This is accompanied by the appearance of a second transition at lower temperature (53.0°C). With increasing peptide concentration, there is a gradual increase in the enthalpy of lower-temperature transition at the expense of the higher-temperature transition

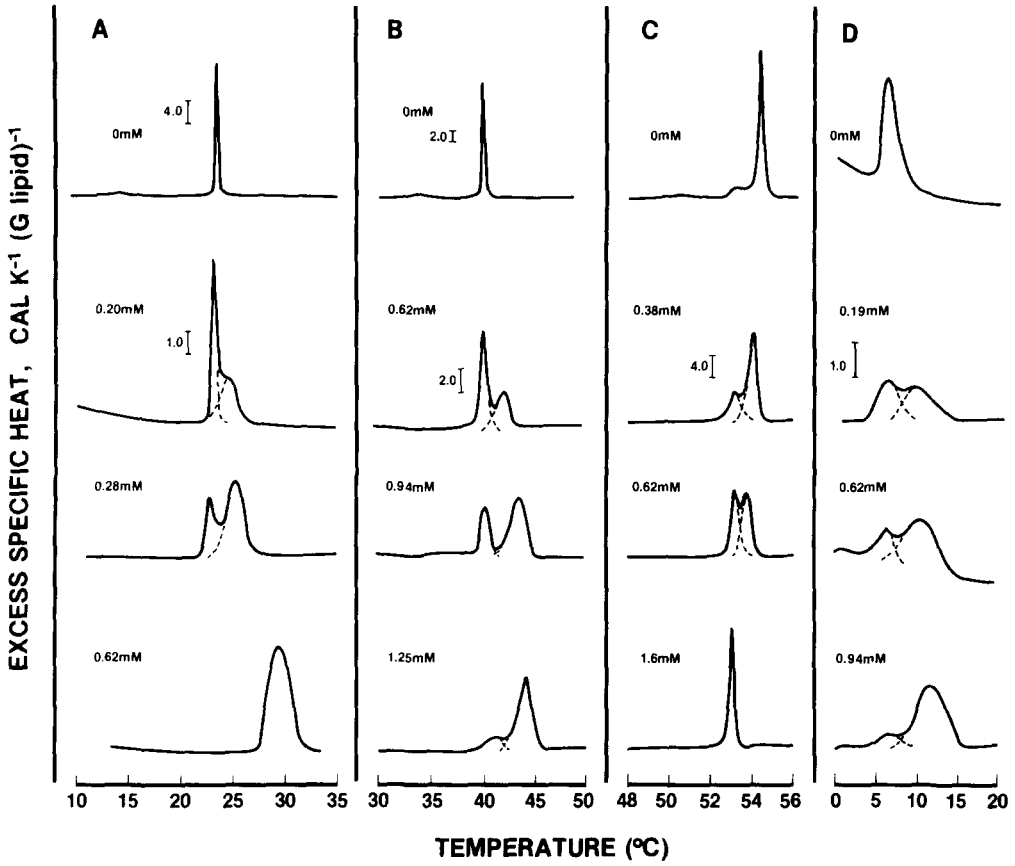

Fig. 1. Representative DSC scans of various phosphatidylcholines without and in the presence of *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2. (A) DMPC, (B) DPPC, (C) DSPC, (D) SOPC. Lipid concentration is 0.5 mg/ml and peptide concentrations are as marked at each curve. The calibration bars give the excess specific heat in cal·K^{-1}·(g lipid)$^{-1}$. The calibration bar for all of the scans in each section is given with the second scan, except for the top scans of sections A and B which have their own calibration bars.

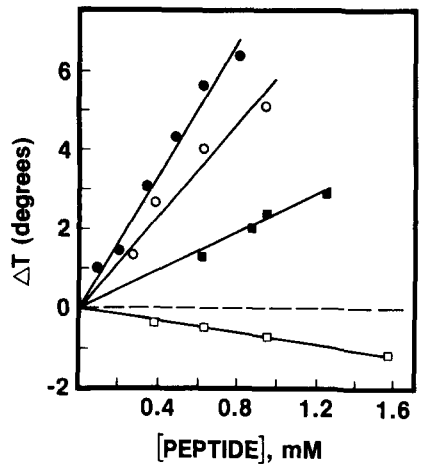

Fig. 2. Shift of the higher-temperature component in the DSC scans of phosphatidylcholine/*N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 dispersions with respect to the transition temperature of pure lipid. ●, DMPC; ■, DPPC; □, DSPC; ○, SOPC. The temperature of the high-temperature transitions was obtained by analysing the observed transition curves in terms of two components (cf. Fig. 1). The transition temperatures of pure phosphatidylcholines are 23.6, 41.1, 54.3 and 6.2°C for DMPC, DPPC, DSPC and SOPC, respectively. Lipid concentration is 0.5 mg/ml.

and, finally, at a peptide concentration above 1.6 mM, only the low-temperature transition can be seen in DSC scans (Fig. 1C). It should be noted here that the interpretation of our results obtained with DSPC is further complicated by the fact that the phase-transition profile of pure DSPC appears to be more complex compared to other phosphatidylcholines. With our ultrasensitive calorimeter, in addition to the main transition of DSPC at 54.3°C and a broader pretransition centered at 51°C, we observed a small, relatively sharp peak at 53.1°C. This additional peak was present in the DSC scans of DSPC obtained from various suppliers, and it could not be eliminated by further purification of the phospholipid. We have not determined the cause for the peak, but it represents only about 14% of the enthalpy of the gel-to-liquid crystalline phase transition. This small peak may overlap with the low-temperature transition induced by the peptide.

The phase transition of SOPC (upper trace in Fig. 1D) is centered at 6.2°C and it is much broader than the transitions of phosphatidylcholines containing only fully saturated fatty acid

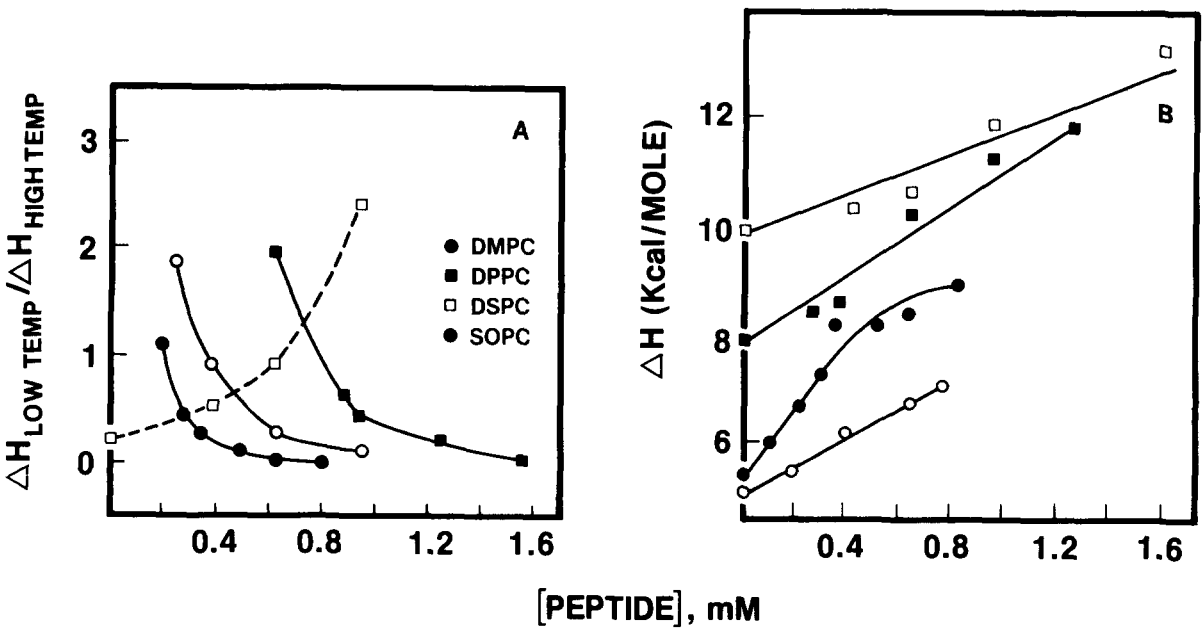

Fig. 3. Effect of *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 on the enthalpy of the phase transition of DMPC (●), DPPC (■), DSPC (□) and SOPC (○). (A) The ratio of the calorimetric enthalpy of the low- to that of the high-temperature transition of various phosphatidylcholines in the presence of the peptide. (B) Total enthalpy of the phase transition of lipid-peptide complexes.

chains. The effects of *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 on the thermotropic behavior of SOPC more closely resemble those observed with DMPC and DPPC than with DSPC. A transition appears which, in the presence of increasing concentration of *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2, is gradually shifted to higher temperatures (Fig. 1D and 2A). The enthalpy of the high-temperature transitions also increases at the expense of the lower transition (Fig. 3A). This latter transition is centered at the same temperature as that of pure SOPC. The susceptibility of the phase transition of SOPC to perturbation by the peptide is greater than that of DPPC but less than that of DMPC.

In addition to the splitting of the transition of phosphatidylcholines into two components and raising the temperature of the transition, *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 also produced an increase in the total enthalpy of the transition. The extent of this effect depends on the length and degree of unsaturation of fatty acid chains and it increases in the order DMPC > SOPC > DPPC > DSPC (Fig. 3B).

Studies similar to those with zwitterionic phos-

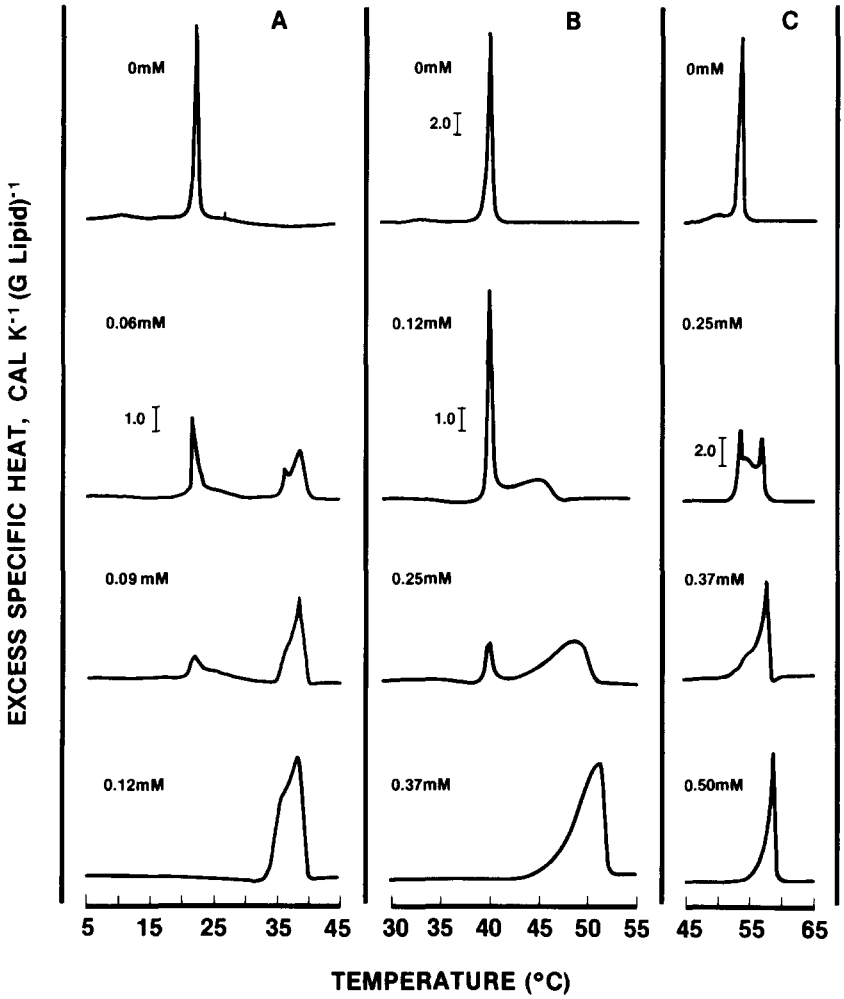

Fig. 4. Representative DSC scans of various synthetic phosphatidylglycerols without and in the presence of *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2. (A) DMPG, (B) DPPG, (C) DSPG. Lipid concentrations is 1 mg/ml and peptide concentrations are as marked at each curve. The calibration bars give the excess specific heat in cal·K^{-1}·(g lipid)$^{-1}$. The calibration bar for all of the scans in each section is given with the second scan, except for the top scan of section B which has its own calibration bar.

phatidylcholines have also been performed with acidic phosphatidylglycerols containing different acyl chains. The thermotropic properties of phosphatidylglycerols at neutral pH are very similar to those of phosphatidylcholines [12,13]. Therefore, these two lipids are particularly suitable for comparative studies of the role of lipid headgroup structure on lipid-peptide interactions.

Addition of *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 to DMPG liposomes results in dramatic changes in the phase-transition profile of this phospholipid. Even at a peptide/lipid molar ratio as low as 0.03, in addition to the original DMPG transition at 22.2°C, a high-temperature peak(s) appears in the DSC scan. With increasing peptide concentration, these high-temperature peaks become more dominant, whereas the transition at 22.2°C is gradually suppressed and finally disappears at a peptide/lipid molar ratio of about 0.1. The high-temperature (34–40°C) portion of the transition curve is relatively complex and most likely represents two or three overlapping transitions. The uppermost peak is centered at about 38°C, independent of the concentration of peptide (Fig. 5). The appearance of the high-temperature transitions is accompanied by a large increase in the enthalpy of the transition (Fig. 6) which at a peptide/lipid molar ratio of 0.1 is about 2.3-times higher than that of pure lipid.

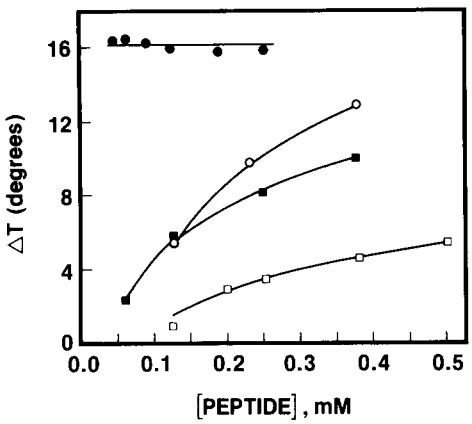

Fig. 5. Shift of the highest-temperature component in DSC scans of phosphatidylglycerol/*N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 and phosphatidylserine/*N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 dispersions with respect to the transition temperature of pure lipid (cf. Fig. 4). ●, DMPG; ■, DPPG; □, DSPG; ○, DMPS. The transition temperatures of pure lipids are 22.2, 40.1, 53.1 and 36.1°C for DMPG, DPPG, DSPG and DMPS, respectively. Lipid concentration is 1 mg/ml.

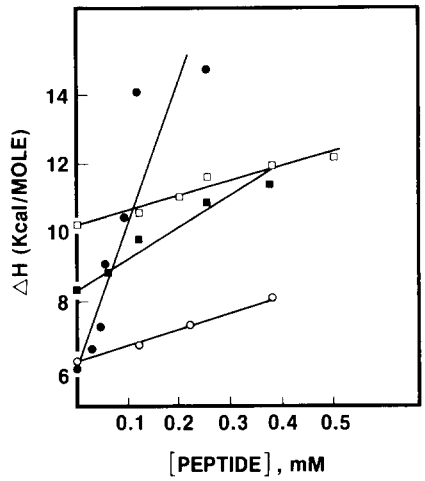

Fig. 6. Effect of *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 on the enthalpy of the phase transition of DMPG (●), DPPG (■), DSPG (□) and DMPS (○).

DSC scans of *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 mixtures with either DPPG or DSPG also indicate a superposition of at least three different components: the relatively sharp peak at a temperature corresponding to that of the transition of pure lipid and a broader peak or peaks at higher temperatures (Fig. 4). In contrast to DMPG, with DPPG or DSPG the temperature of the uppermost peaks shows marked dependence on the peptide concentration (Fig. 5). The upward shift of the highest temperature peaks with respect to the transition temperature of pure lipids is most marked

TABLE I

EFFECT OF *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 ON THE THERMOTROPIC PROPERTIES OF DMPA OR DLPE

Lipid concentration 1 mg/ml.

Lipid	[Peptide] mM	Lipid/peptide molar ratio	Phase transition	
			temperature (T) (°C)	enthalpy (ΔH) (kcal/mol)
DMPA	0	–	49.4	6.0
	0.12	13.1	49.5	5.7
	0.49	3.2	49.0	5.9
DLPE	0	–	29.6	3.6
	1.25	1.4	29.6	3.7

for DMPG, followed by DPPG and then DSPG (Fig. 5). The peptide-induced increase in the enthalpy of the transition is also much more dramatic with DMPG than with DPPG or DSPG (Fig. 6).

In addition to phosphatidylglycerols, two other acidic phospholipids, phosphatidylserine and phosphatidic acid, have been tested with respect to their interaction with *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2. The effects of the peptide on the thermotropic properties of DMPS closely resembles those observed with synthetic phosphatidylglycerols. This is reflected both in the appearance of the high-temperature peak in DSC scans and in the increase in the total enthalpy of the transition. Compared to DMPG, the effects observed with DMPS are, however, significantly smaller (Figs. 5 and 6).

The high susceptibility of synthetic phosphatidylglycerols and DMPS to perturbation by the peptide strongly contrasts with the behavior of DMPA. This phospholipid under present experimental conditions undergoes a thermotropic transition at 49.4°C. The temperature as well as the enthalpy of the transition of this lipid remain virtually unaffected by the presence of *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 (Table I). The only effect of the peptide on the thermotropic properties of DMPA is a small broadening of the transition. Similarly, the phase-transition characteristics of a short-chain phosphatidylethanolamine, DLPE, are not changed by the peptide, even when present at high concentrations (Table I).

Studies similar to those with *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 have also been performed with another basic analog of pentagastrin, *N*-Boc-β-Ala-Trp-Met-Lys-Phe-NH_2. The effects of these two peptides on the thermotropic phase transition of various phospholipids appeared to be virtually indistinguishable (results obtained with *N*-Boc-β-Ala-Trp-Met-Lys-Phe-NH_2 are omitted for brevity).

Fluorescence titrations

The partitioning of the peptide between the aqueous and lipid phases was determined from the effect of lipid concentration on the fluorescence of the peptide [6]. The results are summarized in Table II for a variety of lipids at their phase transition temperature. The partitioning has been analyzed as a ratio of K_d/n where K_d is the equilibrium constant for the dissociation of n peptide molecules from a lipid.

TABLE II

BINDING PARAMETERS OF *n*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2-LIPID COMPLEXES AT THE TEMPERATURE OF LIPID PHASE TRANSITION [a]

Lipid	T (°C)	K_d/n (mM)
SOPC	6	0.58
DMPC	23	0.88 [b]
DPPC	41	1.3
DSPC	54	2.2
DMPG	22	0.32 [b]
DPPG	40	0.42
DSPG	53	0.56
DMPS	36	1.1 [b]

[a] Equilibrium data were calculated from fluorescence emission spectra; 20 mM Pipes/1 mM EDTA/150 mM NaCl (pH 7.4).

[b] Mean value of K_d/n at temperatures 10°C above and below the lipid phase transition. Due to the aggregation of DMPC and DMPG vesicles in the presence of the peptide around the phase-transition temperature, no precise fluorescence measurements could be performed at this temperature [6].

Discussion

A series of pentagastrin-related pentapeptides has proven to be a useful model system for studying various factors controlling the interaction between lipids and peptides [6,7]. Previous studies have focussed mainly on the effect of peptide structure on the mode of its interactions with phospholipids. One of the conclusions of that study was that dimyristoylphosphatidylcholine interacts particularly strongly with the basic analog of pentagastrin, *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2. The present work addresses the problem of the lipid specificity of this interaction. The results show clearly that the interaction between *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 and phospholipids is strongly influenced by the nature of both the hydrocarbon chain and the polar headgroup of the lipid molecule.

The effect of *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 on the thermotropic properties of a series of synthetic phosphatidylcholines and phosphati-

dylglycerols is similar in that the main phase transition of the phospholipids is split into two (phosphatidylcholines) or more (phosphatidylglycerols) components. As discussed in detail previously [7], this most likely reflects the phase separation between a relatively unperturbed bulk lipid domain and a peptide-rich domain(s). The appearance of more than one high-temperature component in DSC scans of some phosphatidylglycerol/peptide mixtures may reflect the coexistence of lipid domains differentially perturbed by the peptide [2]. In addition, this peptide increases the transition enthalpy (Figs. 3 and 6), indicating that it either increases the stability of the gel state or decreases the stability of the liquid-crystalline state. We favor the former explanation, based largely on spin-label studies [7]. In spite of the above general similarity, a detailed analysis of the calorimetric data reveals several important differences among the various peptide/lipid mixtures. These may be summarized as follows. First, the extent of perturbation of the lipid bilayer by the peptide decreases gradually with increasing length of the acyl chains in the series of disaturated phosphatidylcholines and phosphatidylglycerols. Second, the presence of an unsaturated acyl chain enhances the degree of bilayer perturbation by the peptide. Third, the effect of perturbation of the thermotropic behavior of DSPC by the peptide differs from that of other phosphatidylcholines studied. In contrast to DMPC, DPPC and SOPC, the peptide-rich domain of DSPC melts at a temperature lower than that of the pure lipid.

The extent of the effects produced by the peptide on the bilayers prepared from different phospholipids may be due to different partitioning of the peptide between the aqueous and the lipid phase, to different intrinsic resistance of the bilayers to the perturbation by the foreign molecule or to a combination of both factors. The apparent dissociation constants shown in Table II allow for determination of lipid/buffer partition coefficients of the peptide and for calculation of the actual concentration of the peptide in the membrane [14]. Table III shows the shift in the phase-transition temperature of various lipids produced by the same membrane concentration of the peptide corresponding in each case to one peptide molecule per ten lipid molecules (mole fraction of 0.091). Table IV, on the other hand, presents the mole fraction of the peptide in the membrane required to decrease the enthalpy of the low-temperature component of the phase transition (except for DSPC) by a factor of 2. Assuming that the low-temperature transition corresponds to the melting of an unperturbed lipid domain (see above

TABLE III

SHIFT OF THE PEPTIDE-INDUCED COMPONENT IN THE DSC SCANS OF LIPID/*N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 DISPERSIONS

With respect to the transition temperatures of pure lipid at membrane concentration of the peptide corresponding to one peptide molecule per ten lipid molecules (mole fraction of 0.091). ΔT values were determined on the basis of the data of Figs. 2 and 5 extrapolated to a total peptide concentration corresponding to a membrane mole fraction of 0.091. These buffer values were calculated based on K_d/n values of Table I.

Lipid	ΔT
SOPC	+0.7
DMPC	+1.6
DPPC	+0.5
DSPC	−0.2
DMPG	+16.0
DPPG	+6.8
DSPG	+2.2
DMPS	+7.8

TABLE IV

MEMBRANE CONCENTRATION OF *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 REQUIRED TO DECREASE THE ENTHALPY OF THE LOW-TEMPERATURE COMPONENT OF THE PHASE TRANSITION BY A FACTOR OF 2

Lipid	Mole fraction
SOPC	0.25
DMPC	0.14
DPPC	0.33
DMPG	0.05
DPPG	0.12
DSPG	[a]
DMPS	0.08

[a] Not determined due to the poor resolution of the transition components in DSC scans of DSPG-peptide complexes.

and Ref. 7), this situation would correspond to the case when half of the lipid molecules are present in a peptide-depleted domain, whereas the other half is present in a high-temperature-melting, peptide-rich domain. Data of Tables III and IV indicate clearly that among various phospholipids, the differences in the degree of bilayer perturbation by the peptide are not solely due to different partitionings of the solute into the bilayer. Apparently, the same membrane concentrations of the peptide may produce different bilayer perturbation, depending on the nature of both the lipid polar headgroup and acyl chains. This is particularly striking when comparing zwitterionic phosphatidylcholines with their respective acidic phosphatidylglycerols. Thus, the electrostatic interactions between the negatively charged polar headgroups of phosphatidylglycerol and the positively charged arginine residues increase the lipid binding of the peptide (Table II) by greatly enhancing the strength of the interaction between the lipid molecules and membrane-bound peptide. The phospholipid bilayers show increased intrinsic resistance to perturbation by the peptide with increasing length of their hydrocarbon chains. This most likely reflects the enhanced stability of the gel state of these lipids even in the absence of peptide due to increased Van der Waals interaction between the lipid molecules. Thus, the peptide cannot further stabilize the gel state of these phospholipids.

A preliminary study of the morphology of the lipid-peptide complexes indicated that some fragmentation of the multilamellar bilayers occurred at high peptide concentration, but there were no individual lipoprotein particles present as has been observed with larger peptides [15,16]. Because of the large size of the structures and the heterogeneous nature of the morphology, this aspect was not pursued further.

It is notable that the degree of lipid perturbation by the peptide is higher for DMPG than for DMPS, and the thermotropic properties of DMPA remain virtually unaffected even in the presence of high concentrations of the peptide. All of these three phospholipids have the same hydrocarbon chain and all of them, at pH 7.4, possess a single negative charge [17]. The difference in their ability to interact with the basic peptide must be, therefore, due to different intermolecular forces among the polar headgroups of the pure lipids as well as due to the different sizes of the headgroups. There is a great deal of evidence to indicate that phosphatidylserine and phosphatidic acid can interact intermolecularly via hydrogen bonding between adjacent polar headgroups [17–22]. No such intermolecular hydrogen bonding is expected to take place between phosphatidylglycerol molecules. Lipid packing should be particularly tight in the gel phase of phosphatidic acid, as indicated by higher transition temperature of phosphatidic acids compared to their respective phosphatidylserines [17]. This may be due to the strong hydrogen bonding and the particularly small size of the polar headgroup of the phosphatidic acid molecules. This tight packing of phosphatidic acid molecules should be responsible for the inability of the peptide to perturb the phase-transition behavior of DMPA. Similar reasoning may be applied to explain the inability of the peptide to affect the thermotropic properties of DLPE, as this phospholipid is also known to form strong intermolecular hydrogen bonding. The results obtained with phosphatidic acid are, however, particularly notable, as they demonstrate that under certain conditions the interaction of a basic peptide may be much stronger with zwitterionic phosphatidylcholine than with some acidic phospholipids. It should be noted here that the inability of *N*-Boc-Ala-Trp-Met-Arg-Phe-NH_2 to perturb the thermotropic properties of DMPA does not imply that the peptide does not bind to the bilayer surface. Such binding is indicated by peptide-induced aggregation of DMPA vesicles (unpublished observation). The light-scattering effects resulting from the above aggregation prevent a precise determination of the binding parameters by fluorescence methods, although the estimated K_d/n value for the DMPA-peptide complex is in the range of 1–1.5 mM. It should be noted also that the changes in the fluorescence spectrum of the peptide upon binding to DMPA vesicles are generally significantly smaller than those observed with phosphatidylcholines, phosphatidylglycerols or DMPS. This indicates a significantly more polar environment of *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 when bound to DMPA compared to the peptide associated with any of the other phos-

pholipids studied. A purely electrostatic surface absorption of the peptide on the DMPA membrane, without substantial hydrophobic penetration of the bilayer, appears to be insufficient to greatly affect the thermotropic properties of the phospholipid.

Previous studies indicate that intermolecular interactions between phospholipids can affect the mode of binding of larger polypeptides and proteins to lipids [1,23–25]. It is worthwhile noting that myelin basic protein and spectrin can penetrate a phosphatidic acid membrane about as well as a phosphatidylglycerol membrane, whereas the interaction of these proteins with phosphatidylserine is more restricted [23–25]. To explain this unexpected behavior of phosphatidic acid, it has been proposed that although phosphatidic acid is a strong hydrogen bonder in the absence of protein, added protein would bind to the $P\text{-}O^-$ groups of the lipid and prevent hydrogen bonding with P-OH groups of other lipid molecules [24]. The lost lipid-lipid interactions would be compensated for by increased protein penetration into the membrane and resulting lipid-peptide bonding. Apparently, the small pentapeptide carrying only single positive charge is not able to produce a similar disruption of the hydrogen bonding among phosphatidic acid molecules. The difference between myelin basic protein and the small pentapeptide in their relative capacities to penetrate membranes of phosphatidic acid and phosphatidylserine may be also due to the fact that the basic protein is likely to interact more readily with liquid-crystalline lipid whereas the opposite holds for the peptide studied here. Hydrogen bonding in the phosphatidic acid bilayer seems to be stronger than that in the phosphatidylserine membrane when the lipids are in a gel state, but weaker in a liquid-crystalline state. This is indicated by higher transition temperature of phosphatidic acid compared with phosphatidylserine but the lower order parameter of this former lipid in a liquid-crystalline state [24].

The structural requirements for the peptide to induce an increase in the phase-transition temperature of membrane phospholipids are not fully clear at present. The lack of such an effect with uncharged pentagastrin analogs and the much smaller capability to order DMPC bilayer of acidic pentagastrin compared to basic analogs [7] indicate that the electrostatic interaction between the basic residues of the peptides and lipid polar headgroups may be essential to produce the effects observed in this study. Such an electrostatic interaction may facilitate the hydrophobic insertion of the peptide into the bilayer as well as induce a conformational change in the membrane-bound peptide. This electrostatic interaction may occur not only with acidic phospholipids but also with the phosphate group of zwitterionic phosphatidylcholines. The essentially identical results obtained with *N*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 and *N*-Boc-β-Ala-Trp-Met-Lys-Phe-NH_2 indicate that the nature of the basic residue of the peptide is not a determinant of this interaction. A positive charge on the amino-terminal region of the signal peptide has been shown to be important for protein secretion across the membrane [26].

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