Role of Peptide Structure in Lipid–Peptide Interactions: A Fluorescence Study of the Binding of Pentagastrin-Related Pentapeptides to Phospholipid Vesicles†

Witold K. Surewicz‡ and Richard M. Epand*

ABSTRACT: The binding of pentagastrin and three other structurally related pentapeptides to phospholipid vesicles has been studied by fluorescence spectroscopy. The fluorescence of the tryptophan residues of these peptides exhibits an increased quantum yield upon binding to phospholipid vesicles. This is accompanied by a blue shift of the maximum emission, indicative of the incorporation of the tryptophan residue into a more hydrophobic environment. The affinity of the peptides for a zwitterionic phospholipid, dimyristoylphosphatidylcholine (DMPC), increases in the following order: *N-t*-Boc-β-Ala-Trp-Met-Gly-Phe-NH_2 > *N-t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 > *N-t*-Boc-β-Ala-Trp-Met-Asp-Phe-NH_2 > *N-t*-Boc-β-Ala-Trp-Met-Phe-Asp-NH_2. Comparison of the interaction of these various peptides with this phospholipid indicates that although the interaction is largely of hydrophobic nature, the structure of the polar amino acids and their electrostatic charge have significant influence on the nature of the bindings. In addition, the sequence of polar and apolar amino acids appears to be of importance. The higher affinity for DMPC of *N-t*-Boc-β-Ala-Trp-Met-Asp-Phe-NH_2 as compared to its "reversed" analogue *N-t*-Boc-β-Ala-Trp-Met-Phe-Asp-NH_2 suggests that the ability of the peptides to fold into amphiphatic structures can enhance their lipid binding affinity. For all peptides the interaction with DMPC is greater at 8 °C, i.e., below the lipid phase transition temperature, than at 40 °C, i.e., above the lipid phase transition temperature. The interaction of the peptides with phosphatidylcholine bilayers has also been studied by following the peptide-induced release of intravesicular contents. The relative abilities of the peptides to induce leakage of vesicle-entrapped carboxyfluorescein fully coincide with the order of increased affinities derived from fluorescence titration curves.

The interaction of proteins with lipids determines many of the structural and functional properties of biological membranes. This interaction is, however, not yet well understood at the molecular level. One approach to gain further insight into the nature of lipid–protein interactions is to use simpler, well-defined lipid–peptide systems (Viletto et al., 1979; Dufourcq et al., 1981; Prendergast et al., 1982; Sixl & Galla, 1982; Davis et al., 1983; Epand et al., 1983; Gierasch et al., 1983). This approach may be of particular significance if a series of structurally related peptides is available, allowing the systematic investigation of the role of specific chemical structures involved in lipid–peptide interaction.

A system suitable for such studies is a group of peptides related to the pentapeptide, pentagastrin. Although water soluble, pentagastrin associates with zwitterionic phosphatidylcholines (Epand, 1977). This is in contrast to many other small water-soluble peptides that interact only with acidic phospholipids by electrostatic forces (Deber & Young, 1979; Dufourcq et al., 1981). The previous study with pentagastrin (Epand, 1977) is now extended to the following series of structurally related peptides: *N-t*-Boc-β-Ala-Trp-Met-Asp-Phe-NH_2 (pentagastrin) (I); *N-t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 (II); *N-t*-Boc-β-Ala-Trp-Met-Gly-Phe-NH_2 (III); *N-t*-Boc-β-Ala-Trp-Met-Phe-Asp-NH_2 (IV). This series allows the systematic study of the involvement of both electrostatic and hydrophobic effects on lipid–peptide interaction. The role of the electrostatic charge in the interactions of peptides with phosphatidylcholine vesicles is studied by replacing negatively charged aspartic acid, present at the fourth position in pentagastrin, with positively charged arginine or with neutral glycine. Further, the importance of the amphiphatic structure of pentagastrin to its binding to phospholipids is explored by reversing the order of the last two amino acids (structure IV). This latter peptide would have the same overall hydrophobicity as pentagastrin but would not be able to form an amphiphatic loop.

The present paper compares the extent of interaction of each member of the series of pentagastrin-related peptides with phospholipid vesicles. The effects of the phospholipid phase transition and phospholipid structure on the interactions are explored. In this work the nature and extent of peptide–lipid interaction are studied by fluorescence spectroscopy, utilizing the intrinsic fluorescnece of tryptophan, as well as by measurement of the ability of each of the peptides to lyse phospholipid vesicles.

Materials and Methods

Materials. Dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylglycerol (DMPG) were obtained from Calbiochem Co. and used without further purification. The phase transition temperatures of aqueous suspensions of these preparations were 23.6 and 22.5 °C, respectively, as measured high-sensitivity scanning calorimetry (Microcal, Model MC-2). Egg yolk phosphatidylcholine (type VE) was from Sigma. Pentagastrin (*N-t*-Boc-β-Ala-Trp-Met-Asp-Phe-NH_2), *N-t*-Boc-β-Ala-Trp-Met-Phe-Asp-NH_2, and *N-t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 were from Peninsula Labs, and *N-t*-Boc-β-Ala-Trp-Met-Gly-Phe-NH_2 was from Bachem, Inc. Each of the peptides was shown to be at least 95% pure by high-performance liquid chromatography (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% 2-propanol to pure isopropanol. The only exception was pentagastrin, which had a closely related contaminant amounting

† From the Department of Biochemistry, McMaster University Health Sciences Centre, Hamilton, Ontario L8N 3Z5, Canada. *Received May 10, 1984.* This work was supported by the Medical Research Council of Canada (Grant MA7654).

‡ Permanent address: Department of Biophysics, University of Lodz, Poland.

0006-2960/84/0423-6072$01.50/0

to about 10% of the product. Carboxyfluorescein was from Eastman Kodak.

Fluorescence Titration Study. Phospholipid vesicles used in fluorescence titration studies were prepared by ultrasonic irradiation of a suspension of phospholipid in PIPES buffer (20 mM PIPES, 1 mM EDTA, 150 mM NaCl, 0.02 mg/mL NaN_3, pH 7.4). Sonication was performed for 30 min under a nitrogen atmosphere in a Bransonic 12 bath-type sonicator at a temperature above the lipid phase transition.

Fluorescence measurements were performed with a Perkin-Elmer MPF-44 fluorometer operating in the ratio mode and equipped with a variable temperature accessory. The binding of phospholipid to peptides was studied by following changes in the fluorescence spectra of the tryptophan residue of the peptides upon the addition of lipid vesicles. For this purpose small aliquots of a vesicle suspension were successively added to a solution of 10^{-5} M peptide in PIPES buffer. The suspension was continuously stirred, and after each addition of lipid it was left to equilibrate for 20 min before fluorescence measurements were made. It is assumed that after such an incubation the system was at equilibrium, as all changes in fluorescence spectra proceeded in a much shorter time scale. Fluorescence intensities were corrected for blank measurements from suspensions of lipid in buffer and for dilution effects.

Fluorescence titration curves were analyzed according to (Bashford et al., 1979)

$$\epsilon - 1 = (\epsilon_b - 1) - \frac{K_d(\epsilon - 1)}{nm} \quad (1)$$

where K_d is the dissociation constant of the lipid–peptide complex, m denotes the lipid concentration, and n is the number of binding sites per lipid. The quantity ϵ characterizes the relative change of the spectral parameter accompanying binding of the peptide to the membrane at a particular lipid concentration, m. This quantity is defined either as the relative change of fluorescence intensity I

$$\epsilon = I/I_0 \quad (2)$$

or as the relative change of the position of maximum emission λ

$$\epsilon = \lambda_0/\lambda \quad (3)$$

Unlike I/I_0, the ratio λ_0/λ is not necessarily linearly related to the amount of peptide bound even for a simple two-state system. The ratio λ_0/λ was used only to analyze the data from the titrations at 40° of pentagastrin and its "reversed" isomer where I/I_0 is close to 1. Therefore, in these cases there is little increased weighting of the more intense peak and the analysis based on the wavelength shift is more precise and reasonably accurate. The parameter ϵ_b characterizes the spectral properties of membrane-bound peptide. It is equal to the limiting value of ϵ when all peptide present in the suspension is bound to the membrane. This parameter will have a specific value for each peptide and lipid system. As predicted by eq 1 the slope of a plot of $\epsilon - 1$ vs. $(\epsilon - 1)/m$ yields K_d/n. The value of K_d/n represents the reciprocal of the classic first association constant for the peptide–lipid interaction (Scatchard, 1949) and provides a basis for comparing the various peptides studied (Bashford et al., 1979). Although in principle further manipulation of the data could give estimates of both K_d and n, no attempt has been made to resolve these quantities. As shown by Bashford et al. (1979), when this type of titration is used for comparative purposes, K_d/n provides a more reliable indication of the affinity of the ligand to the membrane than the more conventional parameter K_d. In contrast to the K_d/n ratio, the estimates of K_d and n are strongly dependent on the analytical procedure chosen to fit the experimental data (Bashford et al., 1979). This makes the separation of K_d and n especially difficult for comparative purposes since for the peptide–lipid systems studied they are not very sensitive to changes in peptide structure or to temperature. The ordinate intercept of the $\epsilon - 1$ vs. $(\epsilon - 1)/m$ plot yields the value of $\epsilon_b - 1$, from which fluorescence properties of membrane-bound peptide may be derived according to eq 2 or 3.

Release of Carboxyfluorescein from Vesicles. Leakage from vesicles was examined by using the technique of Weinstein et al. (1977). With this technique vesicles are prepared with a highly self-quenched fluorescent compound, carboxyfluorescein, entrapped. As the dye leaks from vesicles it becomes diluted, and as a result an increase in fluorescence is seen. To prepare vesicles containing entrapped carboxyfluorescein, a chloroform solution containing 30 mg of egg yolk phosphatidylcholine was dried under nitrogen and left under vacuum for several hours to remove residual solvent. The sample was resuspended in 2 mL of PIPES buffer (20 mM PIPES, 1 mM EDTA, 0.2 mg/mL NaN_3, pH 7.4) containing 0.1 M carboxyfluorescein. This was followed by vortexing and then sonicating under nitrogen for 30 min at 4 °C. Extravesicular carboxyfluorescein was removed by passing the suspension through a column (20 × 1 cm) of Sephadex G-50 with elution at room temperature with a PIPES buffer containing 150 mM NACl. A 50-μL aliquot of the gel-filtered vesicle suspension was added to 2 mL of the elution buffer containing peptide at the desired concentration. The time course of fluorescence change, corresponding to carboxyfluorescein efflux, was recorded at room temerature with excitation and emission wavelengths of 490 and 520 nm, respectively. To calculate the percentage of carboxyfluorescein released, vesicles were disrupted by addition of 50 μL of 1% Triton X-100, and fluorescence corresponding to maximum dye release was measured.

Results

The wavelengths of maximum fluorescence emission of pentagastrin and related peptides in aqueous solution are in the range of 352–354 nm, indicating a highly polar environment of the tryptophan residues. The fluorescence properties of these peptides are markedly altered upon addition of DMPC. Representative fluorescence spectra of pentagastrin (Figure 1) demonstrate that in the presence of lipid the maximum emission is shifted to shorter wavelength, and there is an enhancement of the relative fluorescence intensity. The observed blue shift reflects the decreased polarity of the solvent environment of the tryptophan residue (Cowgill, 1967), indicating the incorporation of the peptide into the lipid bilayer. Qualitatively similar changes in the fluorescence emission spectra have been observed for the other peptides studied. The degree of the shift and intensity enhancement markedly depend, however, both on the peptide structure and on the physical state of the interacting lipid. The titration curves of different peptides with gel (8 °C) and with liquid-crystalline-state (40 °C) DMPC are shown in Figure 2. The most striking feature of these curves is that the lipid-induced blue shift and fluorescence intensity enhancement are more pronounced at 8 °C than at 40 °C, indicating preferential interaction of the peptides with gel-state DMPC. Quantitative analysis of these titration curves (see Materials and Methods) yields the affinity parameter K_d/n and the value of shift in the emission maximum as a result of membrane incorporation (Table I). The relative affinity of the peptides toward both gel-state and liquid-crystalline-state DMPC decreases in the following order: *N-t*-Boc-β-Ala-Trp-Met-Gly-Phe-NH_2 >

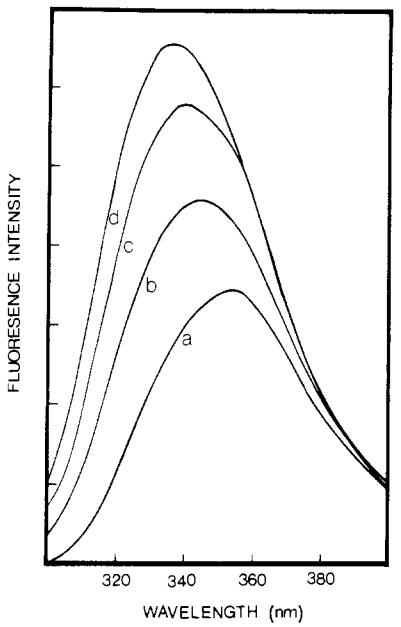

FIGURE 1: Representative fluorescence spectra (uncorrected) of *N-t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 (1×10^{-5} M) in the absence of (a) and in the presence of 0.32 (b), 0.73 (c), or 1.22 mM (d) DMPC. Excitation wavelength 290 nm; temperature 8 °C.

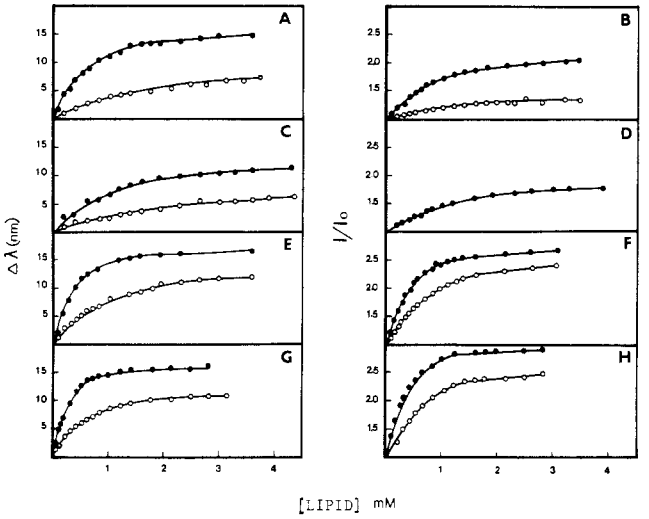

FIGURE 2: Fluorescence properties of the tryptophan residues of pentapeptides in the presence of DMPC vesicles as a function of lipid concentration. Peptide concentration 1×10^{-5} M. I/I_0 is the ratio of fluorescence intensities at 340 nm, Δλ is the blue shift in the wavelength of maximum emission. (●) Titrations performed at 8 °C; (○) titrations performed at 40 °C. (A and B) *N-t*-Boc-β-Ala-Trp-Met-Asp-Phe-NH_2; (C and D) *N-t*-Boc-β-Ala-Trp-Met-Phe-Asp-NH_2; (E and F) *N-t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2; (G and H) *N-t*-Boc-β-Ala-Trp-Met-Gly-Phe-NH_2.

N-t-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 > *N-t*-Boc-β-Ala-Trp-Met-Asp-Phe-NH_2 > *N-t*-Boc-β-Ala-Trp-Met-Phe-Asp-NH_2. Substitution of the negatively charged aspartic acid present at the fourth position in pentagastrin by the positively charged arginine markedly increases the affinity of the peptide to the DMPC bilayer. The K_d/n parameter is further decreased (i.e., affinity is increased) when neutral glycine is present at the fourth position in the peptide chain. Comparison of the data obtained for pentagastrin (*N-t*-Boc-β-Ala-Trp-Met-Asp-Phe-NH_2) and *N-t*-Boc-β-Ala-Trp-Met-Phe-Asp-NH_2 indicates that the ability of the peptides to interact with DMPC depends not only on their amino acid composition but also on the sequence of amino acids. Reversing of the order of last two amino acids in pentagastrin results in about a 2-fold decrease in the lipid affinity of the peptide.

Table I: Binding Parameters of Peptide–Lipid Complexes[a]

peptide	lipid	K_d/n (mM) 8 °C	K_d/n (mM) 40 °C	Δλ (nm) 8 °C	Δλ (nm) 40 °C
N-t-Boc-β-Ala-Trp-Met-Asp-Phe-NH_2	DMPC	0.81	2.08	17.0	10.9
N-t-Boc-β-Ala-Trp-Met-Phe-Asp-NH_2	DMPC	1.40	4.15	14.3	11.5
N-t-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2	DMPC	0.50	1.18	18.5	15.0
N-t-Boc-β-Ala-Trp-Met-Gly-Phe-NH_2	DMPC	0.39	0.60	17.5	13.2
N-t-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2	DMPG	0.29	0.35	21.0	16.5

[a] Equilibrium data were calculated from fluorescence emission spectra. 20 mM PIPES, 1 mM EDTA, 150 mM NaCl, 0.02 mg/mL NaN_3, pH 7.4.

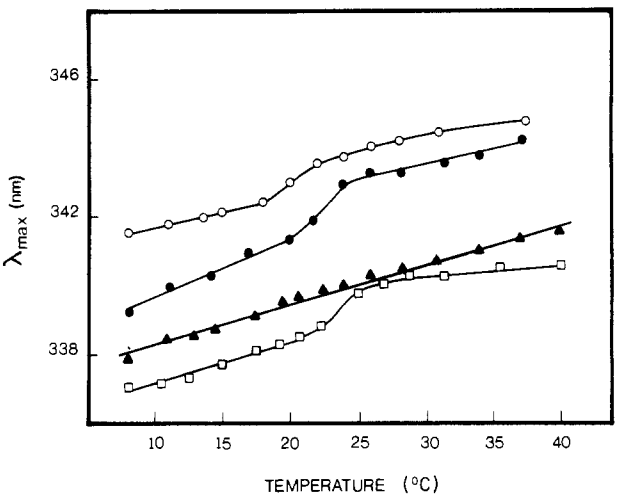

FIGURE 3: Effect of temperature on the wavelength of maximum fluorescence emission of pentapeptides in the presence of DMPC vesicles. Peptide concentration 1×10^{-5} M; DMPC concentration 5 mM. (●) *N-t*-Boc-β-Ala-Trp-Met-Asp-Phe-NH_2; (○) *N-t*-Boc-β-Ala-Trp-Met-Phe-Asp-NH_2; (□) *N-t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2; (▲) *N-t*-Boc-β-Ala-Trp-Met-Gly-Phe-NH_2.

Although all peptides studied interact with DMPC both below and above the phase transition temperature, they exhibit marked preference for gel-state lipid. For pentagastrin, its reversed analogue, and the arginine-containing peptide the affinity toward DMPC vesicles is 2–3 times higher at 8 °C than at 40 °C. This preference for gel-state lipids is significantly less marked in the case of the more hydrophobic peptide containing glycine at the fourth position. The differences in peptide interaction with DMPC at 8 and 40 °C manifest themself also in the degree of blue shift of the maximum fluorescence (Table I). The emission maximum of membrane-incorporated peptides is more blue-shifted at 8 °C than at 40 °C. This indicates a less polar environment of the tryptophan residue at lower temperature, presumably due to the deeper penetration of the peptide into the bilayer. The temperature dependence of the maximum emission wavelength of different peptides in the presence of 5 mM DMPC is shown in Figure 3. For all peptides there is a gradual increase in λ_{max} with increasing temperature, with a significantly more rapid change between approximately 20 and 25 °C, i.e., around the phase transition temperature of DMPC. No such rapid change is observed with *N-t*-Boc-β-Ala-Trp-Met-Gly-Phe-NH_2 where λ_{max} increases gradually over the whole temperature range studied.

Analysis of the data shown in Table I suggests that two independent factors may contribute to the observed increase

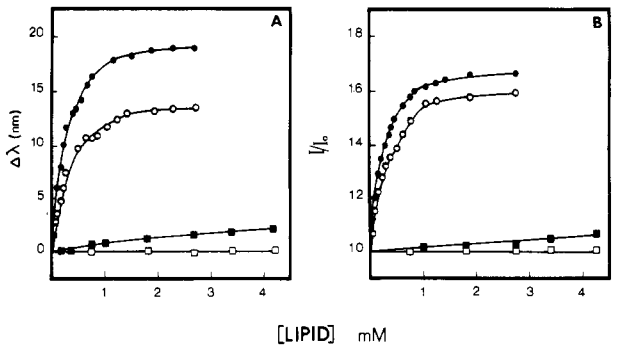

FIGURE 4: Fluorescence properties of tryptophan residues of pentapeptides in the presence of DMPG vesicles as a function of lipid concentration. I/I_0 is the ratio of fluorescence intensities, $\Delta\lambda$ is the blue shift in the wavelength of maximum emission. Closed symbols (● and ■) represent titrations performed at 8 °C for *N-t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 and *N-t*-Boc-β-Ala-Trp-Met-Asp-Phe-NH_2, respectively. Open symbols (○ and □) represent titrations performed at 40 °C for *N-t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 and *N-t*-Boc-β-Ala-Trp-Met-Asp-Phe-NH_2, respectively.

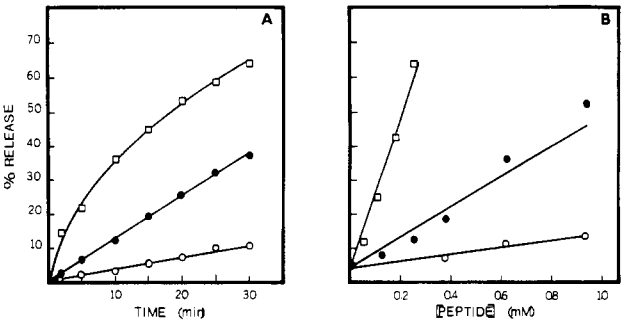

FIGURE 5: Peptide-induced carboxyfluorescein leakage from egg yolk phosphatidylcholine vesicles. (A) Time course of carboxyfluorescein efflux in the presence of 0.63 mM *N-t*-Boc-β-Ala-Trp-Met-Asp-Phe-NH_2 (●), 0.63 mM *N-t*-Boc-β-Ala-Trp-Met-Phe-Asp-NH_2 (○), and 0.25 mM *N-t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 (□). (B) Carboxyfluorescein leakage as a function of peptide concentration. (●) *N-t*-Boc-β-Ala-Trp-Met-Asp-Phe-NH_2; (○) *N-t*-Boc-β-Ala-Trp-Met-Phe-Asp-NH_2; (□) *N-t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2.

in λ_{max} with increasing temperature: the displacement of membrane-incorporated peptide into a less hydrophobic environment and the dissociation of the lipid–peptide complex. The latter phenomenon should occur to a much lesser extent with *N-t*-Boc-β-Ala-Trp-Met-Gly-Phe-NH_2 than with the other peptides. The glycine-containing peptide exhibits the highest lipid affinity, which is only slightly temperature dependent. The rapid increase in λ_{max} around the lipid phase transition temperature for the other peptides is thus most likely caused by the partial dissociation of the lipid–peptide complex around the transition temperature. Titration studies at temperatures close to the melting point of the phospholipid could not be performed since incubation of the DMPC vesicles in the presence of peptides for longer periods of time around the phase transition temperature results in massive aggregation. The resulting light scattering effects severely impede any precise fluorescence studies.

In order to gain deeper insight into the involvement of hydrophobic and electrostatic effects in the formation of lipid–peptide complexes, the interaction of *N-t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 and *N-t*-Boc-β-Ala-Trp-Met-Asp-Phe-NH_2, the peptides bearing net positive and negative charge, respectively, with acidic DMPG has been studied. Dimyristoylphosphatidylglycerol has been chosen here as a model acidic phospholipid due to its very similar thermotropic behavior to DMPC (Van Dijck et al., 1975). Titration curves are shown in Figure 4 and the binding parameters obtained for *N-t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 in Table I. As expected, *N-t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 interacts more readily with DMPG than with DMPC. Interestingly, however, electrostatic effects seem to play a much more significant role above than below the lipid phase transition temperature. Whereas at 40 °C the affinity of the arginine-containing peptide is about 3.5 times higher toward DMPG than DMPC, at 8 °C it is only about 1.5 times higher. Consequently, the affinity of *N-t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 toward DMPG is almost the same at 8 and 40 °C. As in the case of DMPC, the blue shift in the fluorescence emission spectrum of *N-t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 incorporated into DMPG is greater at 8 °C than at 40 °C. However, both below and above the phase transition temperature the tryptophan environment of this peptide is slightly more hydrophobic in DMPG than in DMPC. Figure 4 also shows that there is practically no interaction between DMPG and acidic pentagastrin. Apparently, in this case the repulsive electrostatic forces provide too strong a barrier for any association to take place.

In addition to fluorescence titration studies, the relative abilities of the peptides to interact with phosphatidylcholine bilayers have been assayed by following peptide-induced release of intravesicular contents. Figure 5A illustrates the typical time course for carboxyfluorescein efflux from egg yolk phosphatidylcholine vesicles. In the absence of peptides the carboxyfluorescein permeability of these vesicles is very low (less than 5% of entrapped marker is released in 30 min). Addition of peptides causes a significant increase in the rate of carboxyfluorescein release. This leakage is, however, not characterized by an initial burst but develops more gradually. It indicates that the release of intravesicular content is not due to a rapid solubilization or lysis of the membranes by peptides. As shown in Figure 5B, the relative ability of the pepties to induce carboxyfluorescein release is in the order *N-t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 > *N-t*-Boc-β-Ala-Trp-Met-Asp-Phe-N_2 > *N-t*-Boc-β-Ala-Trp-Met-Phe-Asp-NH_2; i.e., it fully coincides with the order of increased affinities derived from fluorescence titration curves. No measurement could be done with *N-t*-Boc-β-Ala-Trp-Met-Gly-Phe-NH_2 due to the limited water solubility of this peptide.

Discussion

The present fluorescence study indicates that pentagastrin-related pentapeptides provide a particularly suitable model for investigating the molecular mechanisms of peptide–lipid interaction. Although several other small peptides have been previously used for this purpose, most of them, unless very hydrophobic and sparsely soluble in water (Gierasch et al., 1983), interact specifically with acidic phospholipids largely by electrostatic forces (Deber & Young, 1979; Dufourcq et al., 1981). The pentapeptides used in this study associate readily with the bilayers prepared from phosphatidylcholines, the most abundant phospholipid class in plasma membranes (Rouser et al., 1968). This association is accompanied by the penetration of the indole ring of the tryptophan residue into a less polar environment, probably in the vicinity of the aliphatic chains. Such a penetration suggests the involvement of hydrophobic effects in the formation of these lipid–peptide complexes. The largely hydrophobic nature of the interaction is further supported by the observation that both acidic pentagastrin and its basic and neutral analogues associate with phosphatidylcholine vesicles, and in all cases the localization of tryptophan rings in the bilayer is very similar

(Table I). Nonetheless, the nature of polar amino acids and their electrostatic charge are not without significance. The higher affinity for DMPC of *t*-Boc-β-Ala-Trp-Met-Gly-Phe-NH_2 as compared with the other peptides is most likely related to the fact that this peptide has no charged groups to be accommodated in the hydrophobic environment of the lipid bilayer. As a consequence, the water solubility of this peptide is markedly reduced, promoting its partitioning into the lipid phase. There is also an almost 2-fold increase in the apparent affinity for DMPC arising from substitution of aspartic acid by arginine. It is possible that the formation of hydrogen bonds between phosphate groups of phospholipids and guanidinium groups of arginine residues (Cotton et al., 1973) may be involved in the interaction between *t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2 and phosphatidylcholine molecules. Although further work is required to verify this possibility, the results of structural studies indicate some specific interaction between the arginine-containing peptide and polar head groups of phosphatidylcholine molecules (W. Surewicz and R. Epand, unpublished observation).

Purely electrostatic effects seem to play an unexpectedly small role even in the interaction of the basic peptide, *t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2, with the acidic phospholipid, DMPG. This is particularly striking below the phase transition temperature, where the affinity of this peptide for DMPG is only slightly higher than for DMPC. However, even in the liquid-crystalline state the difference in the affinity of the arginine-containing peptide for acidic vs. zwitterionic phospholipids (Table I) is much smaller than is usually observed for other cationic amphiphatic molecules. For example, with some cationic anesthetics a 20–70-fold increase in affinity has been reported upon substitution of neutral phospholipid vesicles by acidic ones (Papahadjopoulos et al., 1975; Surewicz & Leyko, 1981) compared with only 3.5-fold for the arginine-containing peptide studied in this work.

Another factor controlling the association of the peptides with lipid bilayers appears to be the relative sequence of polar and apolar amino acids. Pentagastrin, having its hydrophobic amino acids separated by two other amino acids, i.e., the Trp and Phe residues in -Trp-Met-Asp-Phe-, has markedly higher lipid affinity than its reversed analogue containing the sequence -Trp-Met-Phe-Asp-, although both peptides have the same overall hydrophobicity. Hydrophobic amino acid side chains occurring at every 3.6 residues in the peptide chain can result in the formation of amphipathic helices in longer peptides. Several small biologically active peptides such as pentagastrin, the enkephalins, and molluscan cardioexcitatory neuropeptide (Phe-Met-Arg-Phe-NH_2) have sequences in which two hydrophobic amino acids are separated by two other amino acids. It is possible that such a structure is sufficient to allow hydrophobic and hydrophilic amino acid residues to become segregated as a result of peptide folding. There is some evidence from NMR studies that this can occur with metenkephalin (Stimson et al., 1979; Deber & Behnam, 1984). In addition, theoretical calculations suggest a bend conformation for the sequence of amino acids in pentagastrin (Abillon et al., 1981). Thus, the conformational properties of the peptides, which will be governed in part by the distribution of hydrophobic and hydrophilic amino acid residues, will also influence their ability to interact with phospholipids. Because of the small size of these peptides, it would be expected that their conformational properties would be highly dependent on their environment. This is demonstrated by the fact that the same pentapeptide sequences in different globular proteins have different conformations, depending on their environment (Kabsch & Sander, 1984). Our results suggest that peptides that are able to fold into amphiphatic structures in the presence of lipids have increased affinity for lipids.

The striking feature of the interactions studied here is that the peptides have a higher affinity for DMPC at 8 °C than at 40 °C. Although the peptides affect the thermotropic properties of phospholipid bilayers (Epand & Sturtevant, 1984), under the conditions used in the titration studies, 8 and 40 °C are still far below and above the phase transition temperture, respectively, of the DMPC–peptide complexes (unpublished observation). The observed preferential interaction of the peptides with the gel-state DMPC is rather unusual. The much more common situation is that proteins and peptides associate more readily with lipids in a liquid-crystalline state (Faucon et al., 1976; Mateu et al., 1978) or that they partition into the membrane preferentially only over a narrow temperature range around the phase transition (Pownall et al., 1978; Epand, 1980; Hanssens et al., 1980; Jonas & Mason, 1981; Epand et al., 1982; Prendergast et al., 1983). Although rare, the situation observed here with pentapeptides is, however, not a unique one. The greater interaction with the gel-state lipids compared with liquid-crystalline ones has been reported also for a few other small peptides (Epand, 1977) as well as for the pulmonary surfactant apolipoprotein A (King et al., 1983).

The molecular basis for the preferential interaction of the peptides with gel-state DMPC is at present not fully understood. Experiments performed with the reversed analogue of pentagastrin demonstrate that the presence of two hydrophobic amino acids separated by two other residues, a structural feature previously thought to be important for such a preferential interaction (Epand, 1977), is not a requirement. Several independent and mutually not exclusive factors could contribute to the observed behavior. A positive enthalpy of association between lipid and peptide would lead to a stronger association at low temperatures, i.e., when the lipid is in the gel state. We do not believe that this is a major factor since the lipid penetration of the peptide decreases most rapidly in a narrow temperature range around the phase transition temperature (Figure 3) rather than gradually decreasing with temperature. It is possible that peptides interact preferentially with defect sites in the gel phase. Such defect sites, containing an enhanced proportion of lipids in gauche conformation, are likely to be present in the gel-state lipids even at temperatures as much as 20 °C below their main transition (Sackmann et al., 1980; Lee, 1977), and as discussed by Jain (1983), interaction with them would be energetically very favorable. The number of defect sites should be particularly high at temperatures slightly below the phase transition. Some kind of special interaction in this temperature range is indicated by the massive aggregation of DMPC vesicles in the presence of peptides, the effect being particularly marked between approximately 18 and 23 °C (unpublished observation). Unfortunately, the resulting light scattering effects prevent precise definition of binding parameters by fluorescence techniques. The other factor that could potentially contribute to the preferential peptide interaction with gel-state lipids is the strong lipid–lipid interaction below the phase transition tempertaure, causing, under certain conditions, lipids to self-associate rather than to associate with proteins (Uemura et al., 1983). This may be especially important in the case of small peptides that have a relatively small hydrophobic surface to be buried. The resulting peptide–peptide interactions would lower the free energy of the lipid–peptide complex in the gel state relative to what it would be in the lipid-crystalline state

where the peptide would be more dispersed in the plane of the membrane.

Registry No. I, 5534-95-2; II, 92762-75-9; III, 92762-76-0; IV, 92762-74-8; DMPC, 13699-48-4; DMPG, 61361-72-6.

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