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Opioid peptide interactions with lipid bilayer membranes

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The interaction of the δ-opioid receptor selective peptides, cyclic [D-Pen^2, D-Pen^5]-enkephalin [DPDPE] and its acyclic analog, DPDPE(SH)$_2$, with neutral phospholipid bilayer membranes was examined by permeability and calorimetry measurements. The permeabilities were accomplished by entrapping either peptide inside of unilamellar liposomes (composed of a mixture of a molar ratio 65:25:10 phosphatidylcholine/phosphatidylethanolamine/cholesterol) then monitoring the peptide efflux through the bilayer. The initial permeability of DPDPE (first 12 h) averaged over four experiments was $(0.91 \pm 0.47) \cdot 10^{-12}$ cm s^{-1}. In contrast the average permeability of the acylic DPDPE(SH)$_2$ was $(4.26 \pm 0.23) \cdot 10^{-12}$ cm s^{-1}. The effect of these peptides on the phase transition, T_m, of 1,2-dipalmitoylphosphatidylcholine (DPPC) bilayers was examined by high sensitivity differential scanning calorimetry. The T_m, the calorimetric enthalpy, and the van 't Hoff enthalpy of DPPC were not significantly altered by the presence of DPDPE, whereas the calorimetric data for DPPC with DPDPE(SH)$_2$ showed a small, yet significant, increase (0.2°C) in the T_m with a 30% decrease in the cooperative unit. Both the permeability and calorimetry data reveal a stronger peptide–membrane interaction in the case of the more flexible acyclic peptide.

Introduction

The discovery of endogenous opioid peptides in the 1970s has accelerated research in opioid chemistry and biology, which led to the concept of multiple opioid receptors with different structural requirements [1–3]. The design and synthesis of ligands for these receptors led to the development of peptides with high receptor selectivity for δ and μ receptors [4–8]. The cyclic [D-Pen^2, D-Pen^5]-enkephalins are noted for their high potency and selectivity for δ receptors.

Water-soluble peptides are known to have limited permeability from the bloodstream into the brain. It was postulated that transport, from blood to the brain, of opiates like methionine enkephalin and the opiate antagonist, Tyr-MIF-1 [Tyr-Pro-Leu-Gly amide], share a saturable carrier-mediated transport system [9]. Banks and Kastin [10] suggested that lipophilicity was an important predictor of permeability. The recent study by Davis and coworkers [11] on the body distribution of ^{3}H-labeled peptides, DPDPE and [*p*-X Phe^4]DPDPE where X = F, Cl, Br and I, indicated that the amount of [*p*-ClPhe^4]DPDPE that reached the brain was approximately twice that found for DPDPE and [*p*-IPhe^4]DPDPE. This suggested that lipophilicity did not play an important role in their ability to cross the blood-brain barrier. Since there has not been a study of the passive diffusion of these water soluble peptides through model phospholipid membranes we have undertaken a series of studies to examine the effect of peptide structure, conformational constraints, and lipophilicity on the membrane permeability of [D-Pen^2, D-Pen^5]-enkephalins. Initial results from these studies of peptide-membrane interactions are reported here with particular attention to the role of conformational freedom in the passive permeability of the conformationally constrained cyclic DPDPE, and the more flexible acyclic DPDPE(SH)$_2$.

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Abbreviations: DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPE, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine; Hepes, *N*-2-hydroxyethyl-*N'*-2-ethanesulfonic acid; D-Pen, D-penicillamine; DPDPE, cyclic [D-Pen^2, D-Pen^5]-enkephalin; DPDPE(SH)$_2$, acyclic [D-Pen^2, D-Pen^5]-enkephalin; D-Pen(*S*-pMeBzl), *S-para*-methylbenzyl-D-pencillamine; DSC, differential scanning calorimetry; RP-HPLC, reverse phase high pressure liquid chromatography.

Materials and Methods

1-Palmitoyl-2-oleoylphosphatidylcholine (POPC), 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE)

and 1,2-dipalmitoylphosphatidylcholine (DPPC) were purchased from Avanti Polar Lipids. Cholesterol, sodium aside and Triton X-100 were purchased from Sigma. Hepes was obtained from Calbiochem. Sodium chloride AR grade was from Mallinckrodt and acetone was AR grade from EM Science. Fluorescamine was from Aldrich. Spectrapor-2 dialysis membranes and Nuclepore filter membranes were purchased from VWR Scientific. Sephacryl S-300 was obtained from Pharmacia-LKB Biotechnology. The buffer solutions (10 mM Hepes, 150 mM sodium chloride, 1 mM sodium azide at pH 7.4) were filtered through 0.22 μm filters prior to use.

Synthesis of enkephalin analogs

Cyclic [D-Pen^2, D-Pen^5]-enkephalin was synthesized and purified in a fashion similar to that described earlier [6,12]. Sequential couplings of Boc-amino acids to D-Pen (*S*-pMeBzl)-resin were followed by simultaneous removal of the peptide from the resin and deprotection by treatment of 10.0 g of the peptide-resin with 90 ml of anhydrous HF in the presence of 5.0 g cresol and 5.0 g thiocresol. After removal of the solvent in vacuo, the peptide was extracted from the resin and purified by reverse-phase HPLC. A reverse-addition method was employed to overcome the huge volume of solvent that would be encountered in large scale intramolecular cyclization when adding $K_3Fe(CN)_6$ to a dilute aqueous solution of the peptide. The purified DPDPE(SH)$_2$ (1.0 g) was dissolved in 100 ml of 25% CH_3OH/25% CH_3CN/50% H_2O and the pH of the solution was adjusted to 8.0. Argon was continually bubbled through this solution while it was added, dropwise over a period of 4 h, to a stirred solution of 600 ml of 50% CH_3CN/50% H_2O and 155 ml of 0.1 M $K_3Fe(CN)_6$ while maintaining the pH at ~ 8.5 with 8.0 M NH_4OH. After the addition of the DPDPE(SH)$_2$ solution was complete, the mixture was stirred for an additional 4 h, the pH adjusted to 4.0 with glacial acetic acid, then Amberlite IRA-68 (chloride form) was added. The solution was stirred for 20 min, filtered, stripped of the CH_3CN on a rotary evaporator, and lyophilized. Final purification of the cyclized peptide (DPDPE) was achieved on a preparative HPLC system. Purity of the DPDPE was greater than 99% as measured on analytical HPLC monitored at 230 nm, 254 nm and 280 nm.

Preparation of lipid-peptide liposomes

The lipids were mixed from their individual chloroform solutions to give a molar mixture of POPC/POPE/cholesterol 65 : 25 : 10. This solution was evaporated to dryness under high vacuum (at least 6 h), then hydrated at room temperature with the buffer and sonicated in a bath type sonicator for 1 min to yield a 10 mg/ml lipid suspension. The desired peptide (2 mg) was added to the lipid suspension and bath sonicated to ensure complete dissolution of peptide in the suspension. The lipid/peptide sample was taken through ten freeze-thaw cycles to form extended bilayers which were then extruded ten times through 0.2 μm Nuclepore membranes at a constant pressure of 200 psi at 40°C. Liposomes with entrapped peptide were separated from untrapped peptide by gel-permeation chromatography on a Sephacryl S-300 column.

Permeability measurements

Three 1 ml aliquots of the chromatographed liposome fractions were each placed in a dialysis bag (10 mm × approx. 2.5 cm, M_r CO: 12000–14000) and dialyzed against 100 ml of Hepes buffer at 25°C. Aliquots (1 ml) were taken from the dialyzate at regular intervals and replaced with fresh buffer solution. After 40 h the contents of the dialysis bags were carefully transferred into borosilicate tubes, and 100 ml of 5% aqueous solution of Triton X-100 was added. These suspensions were bath sonicated until clear and transferred back to their respective dialysis bags. Final aliquots for the infinity values were taken 6 to 8 h after Triton X-100 treatment. DPDPE in Hepes buffer (without liposomes) was placed in dialysis bags and dialyzed against 100 ml of buffer to determine the rate of peptide efflux from the bag.

Fluoresamine peptide assay

All fluorescence measurements were made with the Fluorolog 2 Series spectrophotometers (SPEX Industries) controlled by the SPEX DM3000F data analysis software using a PC computer. Fluorescamine (25 μl of $7.2 \cdot 10^{-5}$ M solution in acetone) was added to samples taken during the duration of the experiment. The emission at 475 nm upon excitation at 380 nm of peptide-fluorescamine conjugate was used to determine the peptide concentration per aliquot.

Permeability data analysis

The permeability data were analyzed by the method of Johnson and Bangham [13,14] which yields the following equations,

$$\ln\left[\frac{N_t V_0}{V_0 + V_1} - N_0\right] = \left[N_0 + \frac{V_0}{V_0 + V_1}\right] - k_1\left[\frac{V_0 + V_1}{V_0}\right]t$$

where,

$$k_1 = \left[\frac{A_1}{V_1}\right]P_1$$

A_1 = surface area of the membranes, V_1 = internal volume of the bag, V_0 = volume of the dialyzate, N_t = counts in the liposomes at $t = 0$ and N_0 = counts in the

dialyzate at time $t = t'$. N_1 was determined from control samples of peptide containing liposomes, which were dissolved with Triton X-100 to release all the peptide. A plot of the left side of the equation as function of time gave a straight line whose slope, $k_1 = (A_1/V_1)P_1$, is related to the size and the permeability of the liposomes. The liposome size was determined by quasi-elastic light scattering.

Quasi-elastic light scattering (QELS)

All measurements were made with a Brookhaven Instruments multiangle laser light scattering system consisting of a 5 mW vertically polarized helium-neon laser as a light source, a BI-200SM multiangle goniometer and detector, which was interfaced to a BI-8000AT digital autocorrelator. Typically, a 25 μl aliquot of the liposome fraction was diluted with buffer (prefiltered through 0.22 μm filter) to adjust the sampling rate to 50 kHz at 90°. The liposome suspension was filtered through 0.8 μm Nuclepore filter to eliminate scattering from dust. Measurements were made at 60°, 90° and 120° until the total counts were between 10^6 and 10^7 with non-linearly spaced (1:2) channels. At least three measurements were made at each angle and the data were deconvoluted using the methods of Cumulants [15], Exponential Sampling [16], NNLS [17] and CONTIN [18] provided with the autocorrelator. Liposomes size is reported as the mean diameter of measurements at three angles.

Differential scanning calorimetry (DSC)

Calorimetric experiments were performed with a Microcal MC-2 scanning calorimeter (Microcal) interfaced to a PC computer. Extended multilamellar lipid suspensions were prepared as follows: DPPC (1 mg) was hydrated with outgassed Hepes buffer and mixed with peptide (0.2–0.4 mg) dissolved in the buffer. This suspension was briefly bath sonicated to ensure complete mixing then repeatedly freezed and thawed (10 cycles). Both reference (buffer) and sample (lipid) cells were pressurized (40 psi with N_2 tank) and heated at a constant scan rate of 10 deg/h up to 60°C. Data points were collected at 4 s intervals, and the instrument was programmed to run four scans. A base line was obtained by filling both the cells with Hepes buffer and scanning at the same rate.

All the scans were normalized by dividing each data point by the scan rate to obtain millicalories/degree, and the normalized buffer-buffer baseline was subtracted. Each thermogram was then divided by $1000 \cdot N$, where N is the number of mol of lipid in the cell, to calculate cal/deg per mol. Finally, the excess heat capacity curve was obtained by connecting data points before and after the transition followed by point by point subtraction of data points outside the transition in the curve. Deconvolution of the data yields the temperature at the maximum of excess heat capacity (T_m), calorimetric enthalpy (ΔH_{cal}) and the van 't Hoff heat (ΔH_{vh}). The cooperativity unit was calculated as the ratio of van 't Hoff heat to the calorimetric enthalpy.

Results

Liposome-peptide formation

The lipids used in this study to prepare liposomes are a mixture of POPC, POPE and cholesterol in the molar ratio of 65:25:10. This composition and ratio were selected as a reasonable first approximation to neutral lipid membranes. Variations of this composi-

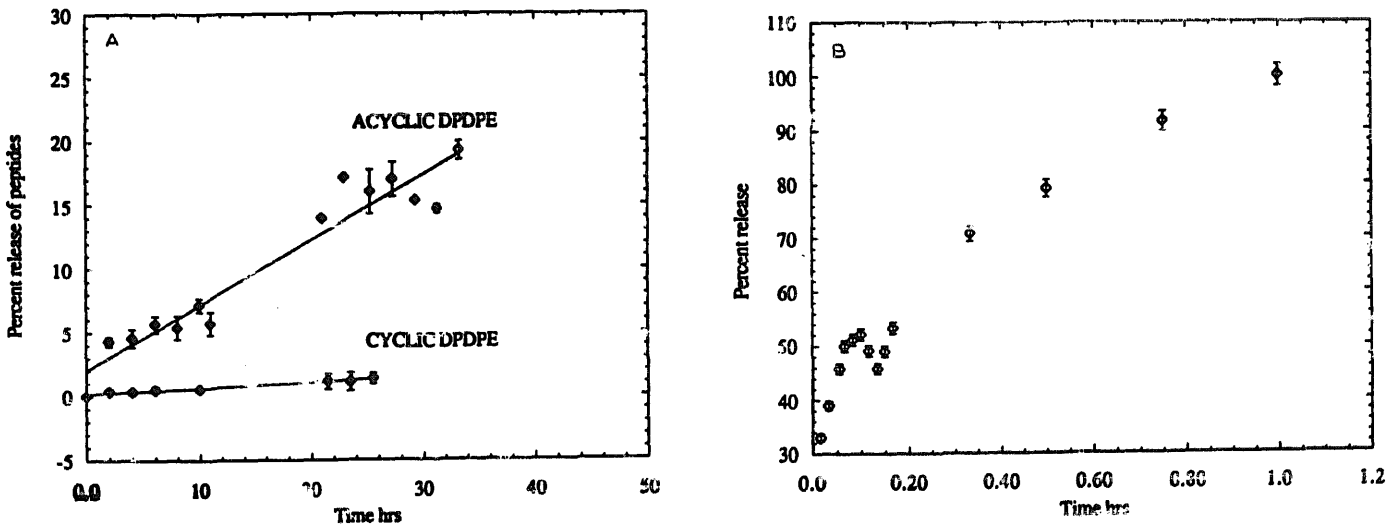

Fig. 1. Percent release of peptides vs. time. (A) The percent release of DPDPE and DPDPE(S^{t}1$_2$) from POPC/POPE/cholesterol liposomes. (B) The percent release of DPDPE from the dialysis membrane in the absence of liposomes. The data are averages of three identical samples for each experiment.

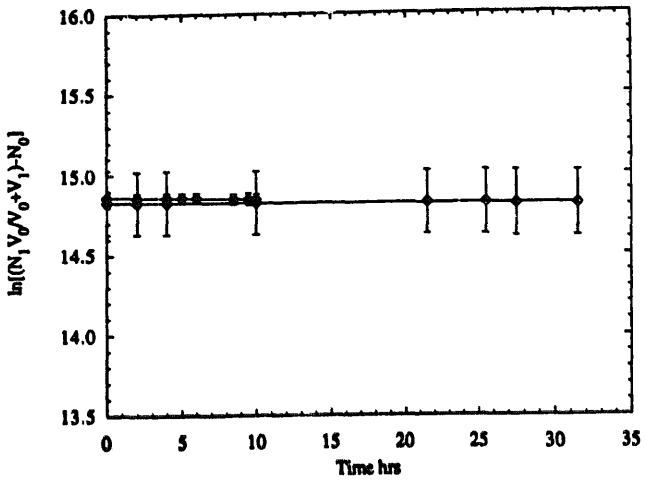

Fig. 2. Permeability profiles for the release of DPDPE from POPC/POPE/cholesterol liposomes. Data for two separate experiments are shown.

tion, e.g., a higher cholesterol content, and/or incorporation of negatively charged lipids, will be examined later. The palmitoyl and oleoyl chains were chosen to reflect the mix of *sn*-1 saturated and *sn*-2 unsaturated acyl chains. At 25°C these bilayers are in the liquid crystalline phase.

Liposomes were prepared by freeze-thaw, followed by extrusion through 0.2 μm Nuclepore filter membranes according to the method of Hope and coworkers [19]. This procedure yielded predominantly unilamellar liposomes of uniform size and narrow polydispersity, which was confirmed by quasi-elastic light scattering measurements at three angles: 60° (forward); 120° (backward); and 90°. The apparent diameter for liposomes with entrapped DPDPE varied from 151 nm at 120° to 171 nm at 60°. The average diameter based on the three angle measurements for liposome with DPDPE was 162 ± 10 nm. The average diameter for liposomes with entrapped DPDPE(SH)$_2$ was 177 ± 14 nm. The light scattering data was supported by negative stain transmission electron microscopy (not shown).

Permeability measurements

The permeability of the bilayer membranes to various solutes may be measured by dialysis [20] of the liposome-entrapped solute provided the rate of permeation through the dialysis bag is fast relative to the rate through the bilayer. Detection of the solute can be accomplished by radiochemical techniques, absorption and emission spectroscopy, as well as enzymatic or redox reactions. A convenient peptide assay is the rapid (milliseconds) reaction of the free amino group with fluorescamine [21] to yield highly fluorescent derivatives (Ex Max, 380 nm and Em Max, 475 nm). This assay is very sensitive with a reported detection limit of 50 pmol for amino acids and has been used to detect peptide hormones, e.g., vasopressin and oxytocin in individual rats, and opioids, e.g., β-endorphins [22]. Our calibration experiments with fluorescamine gave a detection limit of 5–10 nmol/ml for DPDPE and 2–5 nmol/ml for DPDPE(SH)$_2$.

The percent leakage of DPDPE from liposomes and from the dialysis bag are shown in Figs. 1a and b, respectively. Only a few percent of the peptide leaks out of the liposomes in 8 h, whereas virtually all the peptide leaks out of the bag in less than an hour. The GPC separation of the liposome entrapped peptide from the free peptide requires 45 min, during which time only a few tenths of a percent of the encapsulated peptide will escape the liposomes. Therefore, the fraction of peptides outside the liposomes but inside the dialysis bag is negligible at the start of the permeability experiment.

Permeability profiles for two different extruded lipid-DPDPE samples are shown in Fig. 2. This figure is a plot of natural log of the rate of appearance $[\ln\{(N_1V_0/V_0 + V_1) - N_0\}]$ as a function of time. The variation in intercept between experiments occurs because the intercept is related to the fraction of peptide entrapped. The constant K_1, which is a function of the size and permeability of the liposome, was determined from the slope of the plot. Permeabilities (P) were calculated from K_1, based on the measured liposome diameter (see Materials and Methods).

Both the peptide rate constant (K_1) and permeability (P) show some variations between liposome-peptide experiments. The permeabilities of DPDPE based on data collected over the first 12 h in four separate experiments (each performed in triplicate) averaged $(0.91 \pm 0.46) \cdot 10^{-12}$ cm s^{-1}. The measured permeabilities of the individual experiments were 0.32, 0.85, 0.98 and $1.47 \cdot 10^{-12}$ cm s^{-1}. The standard deviations within each set of samples was no more than 20%.

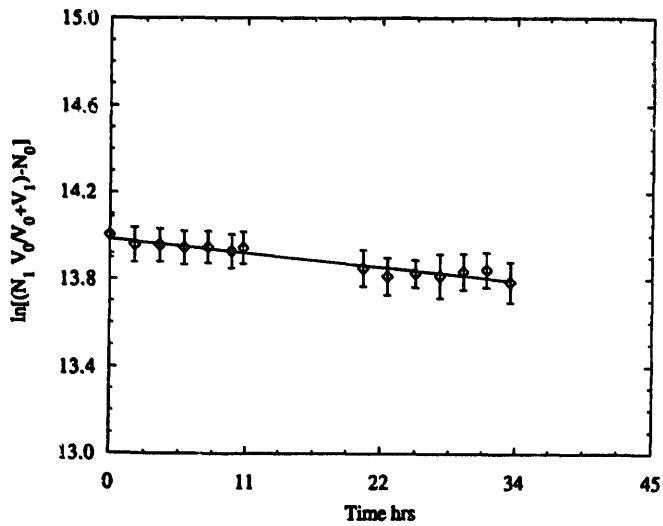

Fig. 3. Permeability profile for DPDPE(SH$_2$) from POPC/POPE/cholesterol liposomes.

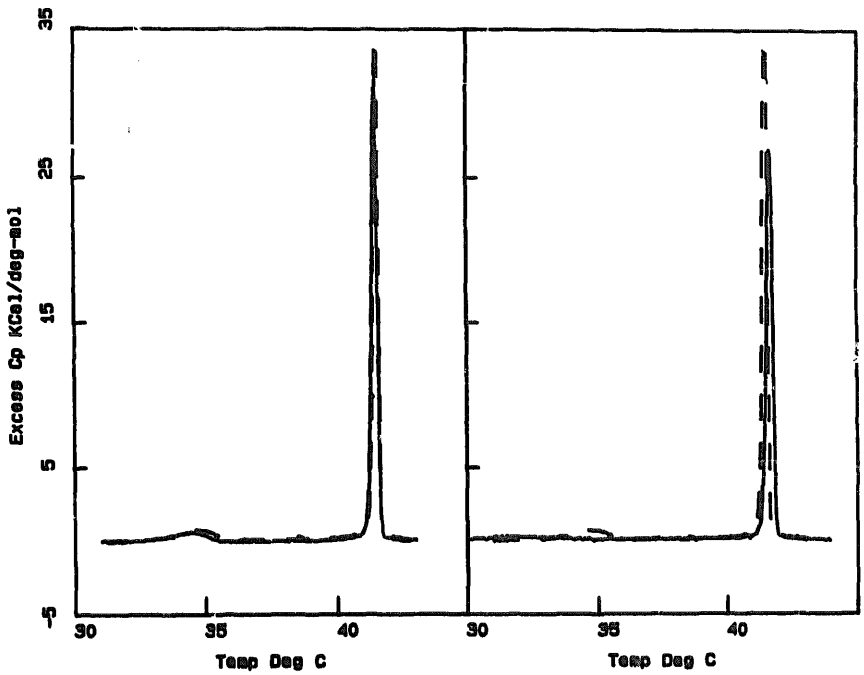

Fig. 4. DSC thermograms for the heating scans of DPPC (left and right, dashed line) and DPPC/DPDPE mixture (left, solid line) and DPPC/DPDPE(SH_2) mixture (right, solid line).

A somewhat greater permeability for DPDPE was observed if the data was collected at longer times (20 to 40 h). These data suggest that the peptide may modify the liposomal barrier over time, thus only the initial permeability data were used for comparison between peptides. The average liposome size as determined by light scattering did not change over the course of the experiment.

The permeablity of acyclic DPDPE($SH)_2$ was determined for comparison to DPDPE. Fig. 3 shows the permeability profile of DPDPE($SH)_2$. The initial rate constant (K_1) based on two individual preparations (each in triplicate) was $(1.6 \pm 0.13) \cdot 10^{-16}$ s^{-1}. The calculated permeability (P), based on a 180 nm diameter liposome, was $4.26 \pm 0.23 \cdot 10^{-12}$ cm s^{-1}, which is approx. 4-fold the average value observed for DPDPE. The increased permeability is readily apparent when the percent leakage of DPDPE($SH)_2$ is compared with that of DPDPE (Fig. 1a).

Calorimetric studies

In order to examine the interaction of DPDPE with bilayer membranes, the phase behavior of a pure lipid bilayer in the presence and absence of peptide was determined. A lipid such as DPPC with a very sharp main transition to the L_α phase facilitates such a study. High sensitivity DSC thermograms of lipid with and without peptide are shown in Fig. 4. The heating scan of DPPC bilayers (Fig. 4A) exhibits a sharp transition at 41.49 ± 0.03°C (Table I) with $\Delta H = 8.19 \pm 0.72$

TABLE I

Differential scanning calorimetry of DPDPE and DPDPE($SH)_2$ with DPPC membrane bilayers

Lipid concentrations were 1 mg/ml; lipid/peptide molar ratios were 3.5:1. The data shown are the average of four heating scans each at a scan rate of 10 deg/h. ΔH_{cal} (kcal/mol per deg) = calorimetric enthalpy calculated from the numerical integration of the area under the excess heat capacity curves. ΔH_{vH} = van 't Hoff's heat of transition for a two-state process and is calculated from the excess heat capacity (ΔC_p) at T_m. $\Delta H_{vH} / \Delta H_{cal}$ = cooperative unit of lipid molecules undergoing the main transition.

Peptide	ΔH_{cal} (kcal/mol per deg)	ΔH_{vH} (kcal/mol)	$\Delta H_{vH} / \Delta H_{cal}$	T_m (°C)
DPPC	8.19±0.72	3091± 90	397±21	41.49±0.03
DPDPE	8.17±0.87	2714± 31	335±40	41.52±0.03
DPDPE	8.17±0.74	2850±171	351±37	41.47±0.03
DPDPE	7.74±0.72	2876±113	371±36	41.40±0.03
DPDPE($SH)_2$	8.15±0.40	2429±115	290± 7	41.68±0.02

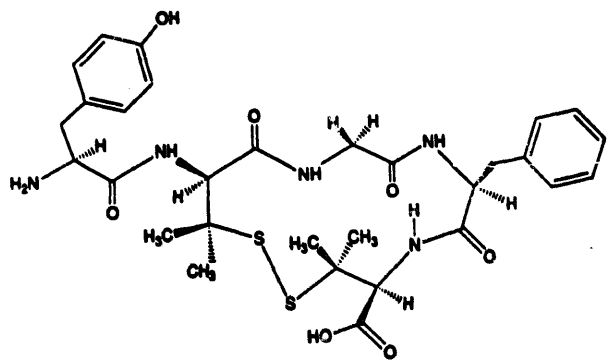

Fig. 5. Structure of [D-Pen^2, D-Pen^5]-enkephalin.

kcal/mol per deg, consistent with the reported values [23]. The thermogram of DPPC, when mixed with DPDPE in molar ratios as high as 3.5:1 (lipid/peptide), did not exhibit significant changes (Fig. 4A). Both the main transition at 41.5°C and the pretransition at 34°C were undisturbed. The T_m and the calorimetric enthalpy for the two state transition were within the standard deviations of DPPC alone, whereas the van 't Hoff enthalpy was reduced by only 10%. Thus, the cooperative unit (which is ratio of the van 't Hoff enthalpy to the calorimetric enthalpy) was somewhat smaller in the presence of the peptide than for pure DPPC. These data indicate that DPDPE does not perturb the acyl chain organization and cooperativity of DPPC bilayers, and therefore very little of the DPDPE inserts into the hydrophobic region of the bilayer.

The calorimetric data for DPDPE(SH)$_2$ showed a stronger bilayer interaction (Fig. 4B and Table I). There was a small, yet significant, increase (0.2°C) in T_m and notably the pre-transition at 34°C is abolished. Although the calorimetric enthalpy of 8.15 ± 0.72 kcal/mol per deg is within the standard deviation for bilayers of DPPC, the van 't Hoff enthalpy was significantly decreased, resulting in an estimated 30% reduction in the lipid cooperative unit of DPPC.

Discussion

DPDPE, a cyclic pentapeptide with a conformationally constrained 14-member ring (Fig. 5), was found to have limited permeability across zwitterionic bilayers prepared from a mixture of POPC/POPE/Cholesterol. The low passive permeability of DPDPE is consistent with the amount of ^{3}H-labeled DPDPE found associated with the opioid receptors in rat brain 40 min after intravenous administration [11]. Davis and coworkers [11] observed that 0.12–0.19% of the total peptide activity was bound to the opioid receptors. In our experiments approx. 1% or so of the entrapped DPDPE escaped from bilayer liposomes over an 8 h period. When the cyclic peptide was reduced to form the conformationally flexible acyclic DPDPE(SH)$_2$ the bilayer permeability increased about 4-fold.

Model building and conformational considerations reveal that the number of possible conformations for the cyclic peptide, DPDPE, is greatly reduced compared to the acyclic peptide, DPDPE(SH)$_2$. A tentative model for the 'biologically active conformation' has been proposed by Hruby et al. [24] which suggest that the lipophilic side chain groups of Tyr^1, Phe^4, and D-Pen^2 are on same face of the fourteen member ring, with all the carbonyl groups pointing to the other face of the three-dimensional structure giving an amphiphilic-like conformation. Relative lipophilicity of these peptides can be estimated from the RP-HPLC capacity factors, $k' = t - t_0/t_0$ where t = retention time for solute and t_0 = retention time for solvent. In a C_{18} bonded reversed phase HPLC column lipophilic peptides are retained longer than hydrophilic ones. The k' of DPDPE has been reported [25] to be between 1.85 and 1.95 and the k' for DPDPE(SH)$_2$ is 3.5 ± 0.1, which indicates DPDPE is less lipophilic. This could explain the somewhat higher permeability of the acyclic DPDPE(SH)$_2$ relative to that of DPDPE. In principle the low membrane permeability of any solute could be due to either high water solubility and limited interaction with membranes or strong association with hydrophobic region of the bilayer. The high water solubility of these peptides indicates that the first explanation is correct. This is substantiated by the DSC studies which show limited peptide-bilayer interaction.

The total enthalpic change associated with the main lipid chain melting transition (T_m) is related to molecular packing and length of acyl chains. Any perturbation of the bilayer packing or the head groups is manifested as a change in the calorimetric enthalpy and the degree of cooperativity at phase transition. These properties have been effectively used to study the effect on the bilayer of small molecules (alkanes, alcohols, fatty acids, tertiary alkyl ammonium salts etc.) [26], as well as peptides and proteins [27]. The highly cooperative T_m of DPPC is especially sensitive to the partitioning of small molecules into the bilayer. Short chain (≤ 10 carbons) alkanes, alcohols, fatty acids and tertiary alkyl ammonium salts decrease the T_m, whereas longer chain analogs increase the T_m. Short chain alkanes (≤ 10 carbons) have been shown [26] by X-ray and electron diffraction to intercalate into the interior of the hydrophobic core, which increases in the disorder in the bilayer and results in a decrease in the T_m. Longer chain alkanes (≥ 10 carbons) are also found in the hydrophobic core, but they extend outward to intercalate between the acyl chains, which increases the van der Waals hydrophobic interaction and increases the T_m. Acyclic polar molecules, e.g., alcohols, fatty acids, and tertiary alkyl ammonium salts, also decrease the T_m though these are shown to anchor with the polar

groups near the head group and the methylenes of the chains intercalating between the acyl chains. In the case of short chain solutes this causes a void near the acyl hydrocarbon core, which is compensated by the increase in the ratio of gauche to trans conformers that destabilizes the lamellar gel phase and lowers the T_m. On the other hand, longer polar chain compounds ($\geq$ 12 carbons) intercalate between the lipid acyl chains and are long enough that no voids are formed. This increases the van der Waals interaction which produces a higher T_m.

The nature of interaction of hydrophobic peptides with lipid membranes is dependent on the length [28], amino acid sequence [29] and net charge of the polypeptides [29]. McLean et al. [28] have reported that the optimal amphipathic peptide length for effective lipid binding to be eight amino acid residues for α-helical peptides. Epand et al. [29] examined the interaction of pentagastrin-related peptides with DMPC membranes. The substitution of Gly or Phe for Asp^4 in pentagastrin (*N-t*-Boc-D-Ala-Trp-Met-Asp-Phe-NH_2) had very little effect on temperature of the phase transition, but broadened the phase transition. Replacement of the Asp^4 with Arg^4 resolved the calorimetric curves into two components at high ratios of lipid to peptide. Jacobs and White [30] studied the effect of amino acid substitution on the nature of interaction of tripeptides (Ala-X-Ala-*O-tert*-butyl) with DMPC membranes where X = Gly, Ala, Phe and Trp. Gly and Ala substituted peptides did not alter the T_m but lowered the enthalpy of the main transition with increasing peptide content. The tripeptide with aromatic residues Phe or Trp considerably lowered the T_m and broadened the main transition.

Calorimetric studies on the interaction of DPDPE with DPPC membranes showed that main transition endotherm is not altered by the presence of the peptide (Table I). The negligible effect on T_m indicates, that unlike nonpolar alkanes, polar alcohols and fatty acids, amphipathetic and hydrophobic peptides, the water soluble DPDPE does not intercalate into a neutral bilayer to a detectable extent. Both calorimetric and van 't Hoff enthalpies are not very different from that of the pure DPPC membranes, thus the low bilayer permeability of these water soluble conformationally constrained peptides is due to weak interaction of the peptides with the neutral lipid bilayer. The acyclic DPDPE$(SH)_2$, on the other hand, increases the T_m by 0.2°C and decreases the lipid cooperativity by approx. 30%. These small but real changes are probably due to the insertion into the membrane of a small fraction of this water soluble peptide. Although the preferred conformation of DPDPE$(SH)_2$ in nonpolar media, e.g., the bilayer interior, is unknown, the peptide chain is long enough to penetrate into the bilayer and nearly span the region of the lipid head groups to the bilayer midline in a manner that would stabilize the gel phase and increase the T_m.

Conclusions

The cyclic analogs of enkephalin have low permeabilities through neutral model membranes. Calorimetric studies indicate that these peptides interact to a negligible extent with the lipid hydrocarbon chains. These data demonstrate that these important water soluble peptides only weakly interact with neutral lipid bilayer membranes. The conversion of the cyclic peptide to the acyclic DPDPE$(SH)_2$ increases the number of low energy conformations that are available for peptide–bilayer interactions with a corresponding increase in bilayer interaction. The permeability data correlates well with the biodistribution of these δ selective peptides in various organs in mice [11]. Extensions of these studies to other cyclic and acyclic pentapeptides and their homologs are in progress in order to provide a clearer understanding of the effect of peptide conformation and sequence on peptide–bilayer interactions.

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