Biochimica et Biophysica Acta, 471 (1977) 412—420

BBA 77882

PREFERENTIAL INTERACTION OF PENTAGASTRIN WITH THE GEL STATE OF DIMYRISTOYL GLYCEROPHOSPHOCHOLINE

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(Received July 12th, 1977)

Summary

Fluorescence and circular dichroism spectra indicate that pentagastrin interacts with dimyristoyl glycerophosphocholine more strongly below the phase transition temperature of the lipid than above it. Studies on the interaction of several peptides with dimyristoyl glycerophosphocholine suggest that this property may be related to the ability of these peptides to form amphipathic structures containing two hydrophobic amino acids separated by two other amino acids. Pentagastrin has a marked effect on the proton magnetic resonance spectra of dipalmitoyl glycerophosphocholine below the phase transition temperature indicating that the peptide decreases the motional freedom of the lipid.

Introduction

Synthetic phosphatidylcholine preparations with uniform fatty acid chains undergo a cooperative thermal transition from gel to liquid crystalline state with increasing temperature. For dimyristoyl glycerophosphocholine this transition occurs at 23°C. Substances can, in general, interact more readily with the liquid crystalline state where the phospholipid has greater fluidity and lower density. For example, the proteins of human erythrocyte membranes are more exposed to the aqueous environment when the microviscosity of the membrane is increased [1]. However, it has recently been noted that glucagon interacts more strongly with the gel state of dimyristoyl glycerophosphocholine [2]. Glucagon has an amino acid sequence which would allow it to form amphipathic helices. Other small, physiologically active peptides, including pentagastrin and Met-enkephalin, have two hydrophobic amino acids separated by two other amino acids and thus have the potential of forming amphipathic structures. We wish to determine if other peptides, in addition to glucagon, can interact more readily with the gel state of dimyristoyl glycerophosphocholine and if this property is related to their ability to form amphipathic structures.

Materials and Methods

Dimyristoyl glycerophosphocholine (Sigma Chemical Co.) showed a single spot when visualized with iodine vapors after thin-layer chromatography. The following peptides were obtained from commercial sources: Glucagon (Elanco Corp.), Met-enkephalin (H-Tyr-Gly-Gly-Phe-Met NH_2, Peninsula Labs., lot 791009), pentagastrin (*N-t*-butoxycarbonyl-β-Ala-Trp-Met-Asp-Phe NH_2, Bachem, lot 1898), tetragastrin (H-Trp-Met-Asp-Phe NH_2, Bachem, lot 8970), gastrin-related peptide (H-Trp-Met-Arg-Phe NH_2, Peninsula Labs., lot 72161), H-Phe-Gly-Gly-PheOH (Bachem, lot 9249), H-Phe-Gly-Phe-GlyOH. H_2O (Bachem, lot 8409), EAE peptide, synthetic, residues 114—122 of human basic protein of myelin (H-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-ArgOH, Beckman, lot 02705).

Fluorescence emission spectra were measured with a Perkin-Elmer, MPF-44 in the ratio mode with 295 nm excitation and using 2 nm slit widths. The circular dichroism (CD) spectra were measured with a Cary, model 61. No corrections were made for light scattering. CD results are expressed in terms of the mean residue ellipticity, $[\theta]$. For fluoresence measurements the peptides at concentrations of approximately 2 μg/ml were dissolved in 0.02 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) and the pH adjusted to the desired value with small volumes of 10 M or 0.1 M NaOH. For CD measurements the peptides at concentrations of approximately 50 μg/ml were dissolved in 0.001 M HEPES, pH 7.4. Spectral properties of identical solutions of peptide in the presence and absence of dimyristoyl glycerophosphocholine were compared. To 5-ml aliquots of peptide solution were added 50 μl of dimyristoyl glycerophosphocholine solution in ethanol by rapid injection above the phase transition temperature to give unilamellar vesicles [3] at a concentration of 220 μg/ml. An equal amount of ethanol was added to peptide solutions not containing lipid. The concentrations of the peptides used for CD were determined from their absorption spectra in the near ultraviolet, measured on a Cary 118 spectrometer. The extinction coefficient for each peptide was calculated from its constituent amino acids using the spectral data given by Wetlaufer [4].

Proton magnetic resonance (PMR) spectra were obtained from a Bruker WH 90 fourier transform spectrometer operating at 90 MHz. Normally, 100 (90°) pulses were required to provide adequate signal to noise enhancement although up to 1000 pulses were used in some cases to adequately resolved weaker peptide signals. A Bruker B-ST 100/700 Temperature Controller was used to maintain sample temperature within ±1°C. Samples were prepared by dissolving pentagastrin in ^2H_2O at a pH meter reading of 8. Dipalmitoyl glycerophosphocholine was dissolved in chloroform and the solvent was evaporated under a stream of nitrogen while vortexing so as to deposit a film of lipid on the walls of a glass test tube. Final traces of solvent were removed by drying for at least 1 h under high vacuum. The pentagastrin solution or ^2H_2O at pH 8 was added to the lipid film and the lipid was suspended by vortexing above the phase transition temperature. The sample was then sonicated at 42°C for 1 h under nitrogen in a Bransonic 12 bath-type sonicator.

Results

The fluorescence properties of several tryptophan-containing peptides are altered upon addition of dimyristoyl glycerophosphocholine. The intrinsic fluorescence of the tryptophan in these peptides was measured at 6° and 40°C (Table I). The ratio of intensities are precise to ±2% and except for tetragastrin the wavelength of maximum emission is independent of pH within a precision of ±1 nm. The fluorescence intensity in the absence of dimyristoyl glycerophosphocholine varies only about 15% over the pH range studied for all of the peptides listed in Table I except for tetragastrin which exhibits a 4-fold variation in fluorescence intensity over this pH range. The fluorescence enhancement observed for glucagon is less than that previously reported using other conditions [2]. The difference may be due, in part, to the presence of HEPES since the fluorescence enhancement induced by dimyristoyl glycerophosphocholine increases to 1.45 when the HEPES concentration is reduced to 0.001 M HEPES, pH 7.4, 0.15 M NaCl. In all cases, however, the lipid only affects the fluorescent properties of glucagon at temperatures below the phase transition temperature of the lipid. The effect of the molar ratio of lipid to pentagastrin on the extent of fluorescence enhancement is given in Fig. 1. At the highest ratios of dimyristoyl glycerophosphocholine to pentagastrin the fluorescence emission spectra of the peptide is shifted to 335 nm with no change in the bandwidth.

The effect of dimyristoyl glycerophosphocholine on the magnitude of the major CD band in the spectral region 250—210 nm are given in Table II. The wavelength of this band is independent of both temperature and the presence of dimyristoyl glycerophosphocholine. The effect of dimyristoyl glycerophosphocholine on the CD spectra does not arise because of scattering artifacts since the lipid is always found to increase the magnitude of the CD band while scattering artifacts would decrease it.

Dipalmitoyl instead of dimyristoyl glycerophosphocholine was used for the PMR studies since increased turbidity in the pentagastrin-dimyristoyl glycero-

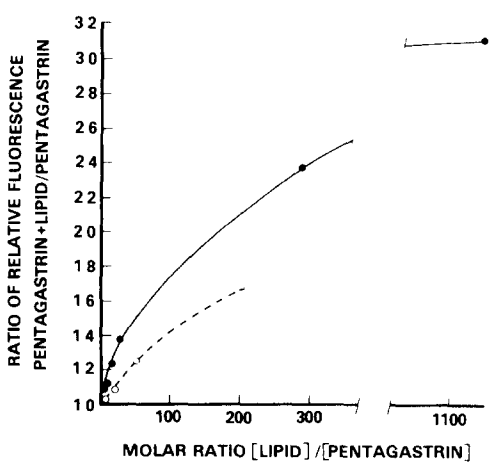

Fig. 1. Dependence of enhancement of pentagastrin fluorescence at 6°C in 0.1 M ammonium acetate, pH 7.4 on the molar ratio of lipid to pentagastrin ●———●, Dimyristoyl glycerophosphocholine, ○- - - - -○, dipalmitoyl glycerophosphocholine

TABLE I

EFFECT OF DIMYRISTOYL GLYCEROPHOSPHOCHOLINE ON THE FLUORESCENT PROPERTIES OF TRYPTOPHAN-CONTAINING PEPTIDES

Ratio of relative fluorescence of peptides (2 μg/ml) in the presence to that in the absence of dimyristoyl glycerophosphocholine (220 μg/ml) 0.02 M HEPES.

pH	Temperature (°C)									
	Glucagon		Pentagastrin		Gastrin-related		EAE peptide		Tetragastrin	
	6°	40°	6°	40°	6°	40°	6°	40°	6°	40°
6	1 30	1 0	1 61	1.26	1.57	1.25	1 17	1.31	0 611	0.571
7	1.23	0.98	1.58	1.32	1.66	1 30	1 14	1 20	1 12	1 0
8	1 18	0.98	1 60	1.38	1.58	1 25	0 89	1.24	1.24	1 15
9	1.16	0.99	1.38	1.19	1 74	1.39	0 62	0.89	1 13	1.12
Wavelength of Maximum Fluorescence Emission										
With dimyristoyl glycerophosphocholine	345	352	345	352	343	349	352	353	348 *	353 *
Without dimyristoyl glycerophosphocholine	350	352	354	356	353	354	352	353	350 *	353 *

* Values for pH 7, varies somewhat with pH.

TABLE II

EFFECT OF DIMYRISTOYL GLYCEROPHOSPHOCHOLINE ON THE CD SPECTRA OF SEVERAL PEPTIDES

Peptide (50 μg/ml) in the presence and absence of dimyristoyl glycerophosphocholine (220 μg/ml) 0.001 M HEPES, pH 7.4

Peptide	Mean residue weight	CD band λ (nm)	$[\theta] \times 10^{-3}$ *		% Increase of magnitude of CD band in the presence of dimyristoyl glycerophosphocholine	
			6 °C	40 °C	6 °C	40 °C
Glucagon	120 17	220 **	−2 9	2 8	167	0
Met-enkephalin	114 55	220	+3.1	+2 1	22	13
Pentagastrin	153 59	226	+2 1	+2 5	35	0
Tetragastrin	149 18	226	+5 4	+4 0	0	0
Gastrin-related peptide	159 45	226	+2 4	+1 0	64	22
L-Phe-Gly-Gly-L-Phe	106 63	217	+8 1	+7 0	7	2
L-Phe-Gly-L-Phe-Gly	106 63	218	+6 2	+5 0	5	10
EAE peptide	115 25	222	+3 8	+2 6	8	13

* Units of deg/cm^2 per decimole^{-1}.

** Shoulder in the absence of dimyristoyl glycerophosphocholine and at 40°C in the absence or presence of dimyristoyl glycerophosphocholine

phosphocholine was noted at temperatures below the phase transition temperature of the lipid at the high vesicle concentrations needed for the PMR measurements. Pentagastrin had no effect on the right-angle light scattering properties of a sonicated preparation of dipalmitoyl glycerophosphocholine as observed with the fluorimeter. A decrease in light scattering intensity was observed at 41°C for vesicle preparations of dipalmitoyl glycerophosphocholine both with and without peptide, indicating that the peptide had little effect on the phase transition temperature of the lipid.

The major effect of pentagastrin on the PMR spectra of dipalmitoyl glycerophosphocholine is to broaden the lipid methylene and terminal methyl protons beyond detection by fourier transform PMR spectroscopy at 35°C (Fig. 2). The

TABLE III

EFFECT OF PENTAGASTRIN ON THE FRACTION OF LIPID PROTONS DETECTED BY PMR

Dipalmitoyl glycerophosphocholine (7 mM) in the presence and absence of pentagastrin to give the indicated molar ratio, ^2H_2O, p^{2}H 8

Temperature (°C)	Molar ratio of dipalmitoyl glycerophosphocholine to pentagastrin	Fraction of protons observed	
		$-N^+(CH_3)_3$	$-CH_2-$ and terminal CH_3
35°	(Lipid alone) *	1	0 14
35°	45 1	0.7	0
35°	20 1	0 5	0
35°	10 1	0 15	0
55°	(Lipid alone) *	1	0.75
55°	20 . 1	0 8	0 45
55°	10 1	0 7	0 3

* Data from ref. 5

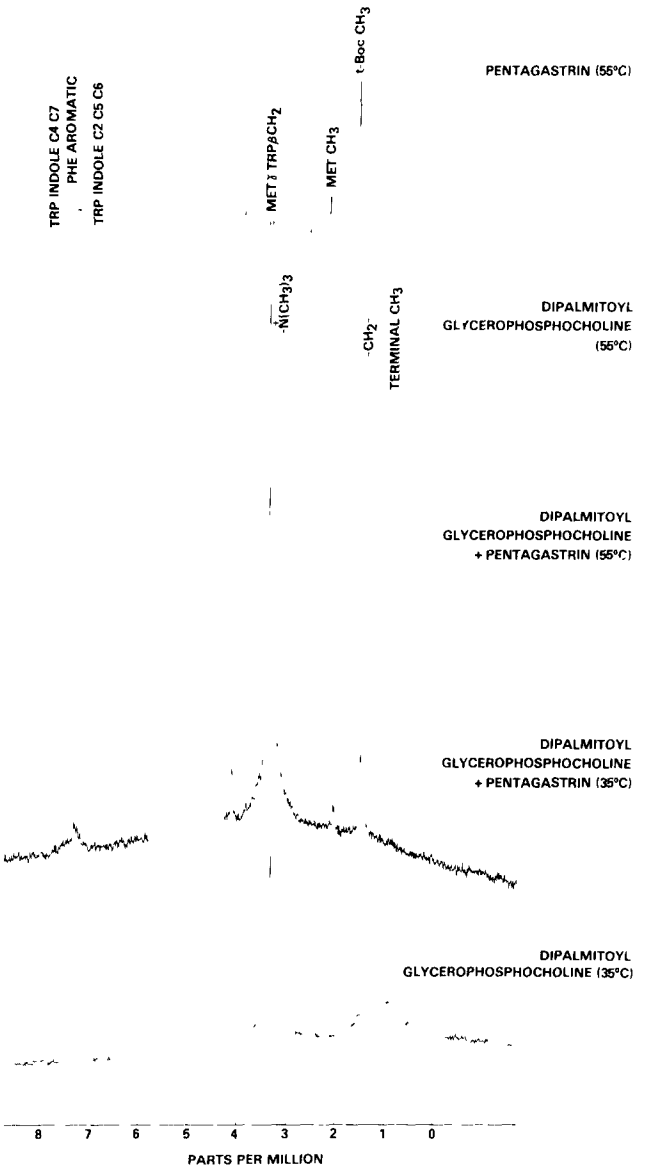

Fig. 2. Effect of pentagastrin (0.35 mM) on the PMR of a sonicated dispersion of dipalmitoyl glycerophosphocholine (7 mM). ^2H_2O, p^{2}H 8.

fraction of lipid protons detected at 35° and 55°C, above and below the phase transition temperature are summarized in Table III. Their values are calculated from the peak areas, measured by planimetry, knowing the molar ratio of lipid to peptide and assuming that all of the peptide protons are detected. This is a reasonable assumption since a large ratio of lipid to peptide is required to obtain maximum fluorescence enhancement (Fig. 1). If not all of the peptide protons are being observed then the effect of the peptide on the lipid would be even larger. Generally, areas of the *t*-butoxy and aromatic signals of the peptide

TABLE IV

AMINO ACID SEQUENCE OF PEPTIDES USED IN THIS STUDY

Peptide	Sequence
Glucagon	H-His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Thr-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr-OH
Met-enkephalin	H-Tyr-Gly-Gly-Phe-Met-NH_2
Pentagastrin	*N*-t-Butoxycarbonyl-β-Ala-Trp-Met-Asp-Phe-NH_2
Tetragastrin	H-Trp-Met-Asp-Phe-NH_2
Gastrin-related peptide	H-Trp-Met-Arg-Phe-NH_2
H-Phe-Gly-Gly-Phe-OH	H-Phe-Gly-Gly-Phe-OH
H-Phe-Gly-Phe-Gly-OH	H-Phe-Gly-Phe-Gly-OH
EAE peptide	H-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg-OH

corresponding to 9 and 10 protons respectively were used to calculate the area per proton observed. The number of quaternary ammonium methyl protons of the lipid were corrected for the overlapping signals of the methionine-γ-CH_2 and tryptophan-β-CH_2. Because of the uncertainty of the baseline, the fraction of protons observed are precise to ±20%.

Discussion

The fluorescence emission spectrum of tryptophan is shifted to shorter wavelengths in a more hydrophobic environment. This is usually accompanied by an increase in quantum yield. Dimyristoyl glycerophosphocholine causes a blue-shift in the emission spectrum of glucagon, pentagastrin and the gastrin-related peptide at 6°C but at 40°C the shift is smaller for the latter two peptides and absent with glucagon (Table I). This shift is also absent with the EAE peptide at both 6° and 40°C (Table I) as well as with L-tryptophan, L-tryptophan amide and *N*-acetyl-L-tryptophan amide at pH 7.4 (unpublished observations) and with H-Gly-Trp-Gly-OH [6]. CD results (Table II) also indicate that the interaction of glucagon, pentagastrin and gastrin-related peptide as well as met-enkephalin with dimyristoyl glycerophosphocholine is greater at 6°C than at 40°C. All of these peptides have in common a structure containing two hydrophobic amino acids, separated by two other amino acids (see Table IV for the structures of these peptides). The two $(L\text{-Phe})_2$ $(Gly)_2$ peptides show only small changes in CD spectra with dimyristoyl glycerophosphocholine as does the EAE peptide, but the trend is suggestive indicating that the peptide having its hydrophobic amino acids separated by two other amino acids, i.e., H-Phe-Gly-Gly-Phe-OH, interacts more strongly with dimyristoyl glycerophosphocholine at low temperatures, while the other two interact more strongly at higher temperatures. Tetragastrin is an exception to this generalization and appears to interact weakly with dimyristoyl glycerophosphocholine, although at pH 7 it does show a small change in position and intensity of fluorescence suggesting a greater interaction at low temperatures. These results are difficult to interpret because of the marked pH dependence of the fluorescence emission of tetragastrin and may result from changes in the p*K* of the ionizable groups of the peptide. This could be among the effects which result in dimyristoyl glycerophosphocholine decreasing the fluorescence of tetragastrin and the EAE pep-

tide. In the latter case this occurs without any shift in the emission maximum. It has been shown that non-specific interactions occur between dimyristoyl glycerophosphocholine and small peptides [7] but in the examples where there is a blue shift of the tryptophan fluorescence, the interaction must at least in part be hydrophobic. This is also confirmed by the insensitivity of the changes in fluorescence with dimyristoyl glycerophosphocholine to pH. At pH 9, where the dimyristoyl glycerophosphocholine may have a partial negative charge, the fluorescence enhancement of the positively-charged gastrin-related peptide increases while that of the negatively-charged pentagastrin decreases.

It is highly probable that in spite of any effect of these peptides on the phase transition temperature of 23°C for dimyristoyl glycerophosphocholine, 6° and 40°C will still be below and above the phase transition temperatures, respectively. Glucagon has little effect on the phase transition temperature of dimyristoyl glycerophosphocholine [2,8] and pentagastrin has no effect on the phase transition temperature of dipalmitoyl glycerophosphocholine as measured by changes in turbidity. In both systems the peptide has a pronounced effect on broadening the PMR signals from the methylene and terminal methyl protons of the gel state lipid suggesting a decrease in the rate of lateral diffusion of the lipid [8]. Glucagon which is capable of forming longer amphipathic helices has a much stronger interaction with dimyristoyl glycerophosphocholine, resulting in a solubilization of the lipid [9] and a large increase in the helix content of the peptide [2]. The perferential interaction of glucagon for gel state lipid is much greater than for the small peptides studied here and the conformation of the peptide can be more accurately described as an amphipathic helix in the case of glucagon. Nevertheless, other peptides do show preferential interaction with the gel state of dimyristoyl glycerophosphocholine and this property may be correlated with the ability of the peptide to form amphipathic structures. However, other proteins such as the serum lipoproteins [10,11] and amyloid A [12] which are capable of forming amphipathic helices do not interact preferentially with gel-state lipid. In these cases, only segments of the molecule can form amphipathic helices, while in the case of glucagon it can involve the entire molecule [2]. In addition to the peptides studied here, other peptides and proteins which cannot form amphipathic helices interact preferentially with the liquid crystal state and include mellitin [6] and cytochrome b_5 [13]. Thus, although there are many examples of peptides and proteins which interact more readily with the liquid crystal state of phospholipid bilayers there are exceptions to this rule and in the cases of glucagon and pentagastrin there is preferential interaction with the gel state.

Acknowledgements

This work was supported by grant A-9849 from the National Research Council of Canada and from the Multiple Sclerosis Society of Canada. We are greatful to Dr. M.R. Dufresne of the Ayerst Laboratories, Montreal for a sample of Peptavalon (pentagastrin), with which preliminary experiments were carried out, and to Mr. Brian Sykes of the Chemistry Department, McMaster University, for running the PMR spectra.

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