Biophysical Chemistry 23 (1985) 39–48
Elsevier

BPC 01023

A COMPARISON OF THE INTERACTION OF GLUCAGON, HUMAN PARATHYROID HORMONE-(1–34)-PEPTIDE AND CALCITONIN WITH DIMYRISTOYLPHOSPHATIDYLGLYCEROL AND WITH DIMYRISTOYLPHOSPHATIDYLCHOLINE

Richard M. EPAND [a], Raquel F. EPAND [a], Ronald C. ORLOWSKI [b], Everett FLANIGAN [b] and Glenn L. STAHL [b,*]

[a] *Department of Biochemistry, McMaster University Health Sciences Centre, Hamilton, Ontario L8N 3Z5, Canada and* [b] *Armour Pharmaceutical Co., Kankakee, IL 60901, U.S.A.*

Received 4th April 1985
Revised manuscript received 11th July 1985
Accepted 28th July 1985

Key words: Glucagon; Parathyroid hormone; Calcitonin; Phase transition; Peptide-lipid interaction; Amphipathic helix

The interaction of glucagon, human parathyroid hormone-(1–34)-peptide and salmon calcitonin with dimyristoylphosphatidylglycerol (DMPG) and with dimyristoylphosphatidylcholine (DMPC) was studied as a function of pH and temperature. The effect of lipid on the secondary structure of the peptide was assessed by circular dichroism and the effect of the peptide on the phase transition properties of the lipid was studied using differential scanning calorimetry. Some peptides interact more strongly with anionic than with zwitterionic phospholipids. This does not require an overall positive charge on the peptide. Increased thermal stability is observed in complexes formed between cationic peptides and anionic lipids. Particularly marked effects of glucagon and human parathyroid hormone-(1–34)-peptide on the phase transition properties of DMPG at pH 5 have been observed. The transition temperature is raised over 10°C at a lipid/peptide molar ratio of less than 30:1 and the transition enthalpy is increased over 2-fold. These effects do not occur with any basic peptide and were not observed with metorphinamide, molluscan cardioexcitatory neuropeptide or myelin basic protein. The results demonstrate that certain peptides can affect the phase transition properties of lipids in a manner similar to divalent cations. The overall hydrophobicities of these peptides can be evaluated by their partitioning between aqueous and organic solvents. None of the above three peptide hormones partition into the organic phase. However, a closely related peptide, human calcitonin, does exhibit substantial partitioning into the organic phase. Nevertheless, human calcitonin has a weaker interaction with both DMPC and DMPG than does salmon calcitonin. The effects of human calcitonin on the phase transition of DMPC are qualitatively different from those of salmon calcitonin in that the human form more readily eliminates the pretransition but causes less change in the main transition. Like overall charge, overall hydrophobicity is not an overwhelming factor in determining the ability of peptides to interact with phospholipids but rather more specific interactions are required for strong complexes to form.

1. Introduction

Several polypeptide hormones bind to phospholipids and it has been suggested that similar interactions can contribute to the binding of these hormones to cell surface receptors [1–3]. One of the properties which allows a peptide hormone to fold into structures which can interact with membranes is the regular distribution of hydrophobic amino acid residues [1,2,4]. Glucagon, calcitonin and the active segment of human parathyroid hormone, hPTH-(1–34), are all of about the same molecular weight and can all form amphipathic helices [1]. However, these three peptides do not

* Present address: Monsanto Co., St. Louis, MO 63167, U.S.A.
Abbreviations: DMPG, dimyristoylphosphatidylglycerol; DMPC, dimyristoylphosphatidylcholine; sCT, salmon calcitonin; hCT, human calcitonin; hPTH, human parathyroid hormone; CD, circular dichroism; DSC, differential scanning calorimetry.

all interact with phospholipids in the same manner. The cationic sCT, for example, interacts with the anionic lipid DMPG more strongly than with the zwitterionic phospholipid DMPC. The phase of the lipid also affects lipid-peptide interactions in some cases. Glucagon, for example, dissociates from DMPC above the phase transition temperature [5] while sCT forms a stable complex with DMPG over a wide range of temperatures [6]. In addition to the amphipathic helix, electrostatic interactions may also have a significant influence on the stability of the interaction between these peptides and phospholipids [6,7]. In order to describe further the characteristics of the binding of these peptide hormones to phospholipids we have compared their interactions with DMPC and DMPG as a function of both temperature and pH.

2. Materials and methods

The sCT, hCT and hPTH-(1–34)-peptide were synthesized using solid-phase methods, by Armour Pharmaceutical Co., Kankakee, IL. Glucagon was isolated from bovine and porcine pancreas by the Elanco Corp., Indianapolis, IN. Synthetic metorphinamide (Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val-NH_2) was purchased from Peninsula Laboratories and molluscan cardioexcitatory neuropeptide (Phe-Met-Arg-Phe-NH_2) from Cambridge Research Biochemicals Ltd. A sample of bovine myelin basic protein was kindly given to us by Dr. M.A. Moscarello of the Hospital for Sick Children, Toronto.

2.1. Buffer solutions

Effects of varying the pH were measured using the following buffered solutions: pH 5.0, 20 mM sodium citrate, 0.15 M NaCl; pH 7.0, 20 mM Pipes, 0.15 M NaCl; pH 9.0, 20 mM sodium borate, 0.15 M NaCl; as well as pH 7.4, 20 mM Pipes, 1 mM EDTA, 0.15 M NaCl containing 0.02 mg/ml NaN_3. Buffers used for the phase partitioning are given in table 3 (see below).

2.2. Peptide solutions

The peptides were dissolved in the appropriate buffer. In the case of glucagon undissolved material was removed by centrifuging at maximum speed in a clinical centrifuge at room temperature. Peptide concentration was obtained from the absorbance at 280 nm for hPTH-(1–34) using an extinction coefficient of 5600 $M^{-1}\,cm^{-1}$ calculated from the amino acid composition [8]; at 278 nm for glucagon using an extinction coefficient of 8300 $M^{-1}\,cm^{-1}$ [9] and at 275 nm for sCT and hCT using extinction coefficients of 1515 and 1531 $M^{-1}\,cm^{-1}$, respectively [6].

2.3. Lipid suspensions and solutions of lipid-peptide complexes

Weighed amounts of dried phospholipid samples were suspended in buffer or in peptide solution by vortex-mixing at room temperature. The mixtures were heated and cooled with tap water several times between 10 and 40°C.

2.4. Circular dichroism

CD measurements were made with a Cary 61 spectropolarimeter. The sample was maintained in a cell holder at constant temperature. The CD is expressed as the mean residue ellipticity, $[\theta]$.

2.5. Differential scanning calorimetry

An MC-2 high-sensitivity differential scanning calorimeter (Microcal Inc., Amherst, MA) was used. Cells contained 1.29 ml of solution. The scan rate used was generally 0.6 K min^{-1}. Electrical calibration was used to measure transition enthalpies.

2.6. Phase partitioning

Buffer (0.25 ml) containing 50 nmol peptide was extracted with 1 ml $CHCl_3/CH_3OH$ (2:1, v/v). Duplicate samples of each of the two phases were taken for Folin-Lowry assays. Tests were performed in quadriplicate and the means and standard deviations are presented.

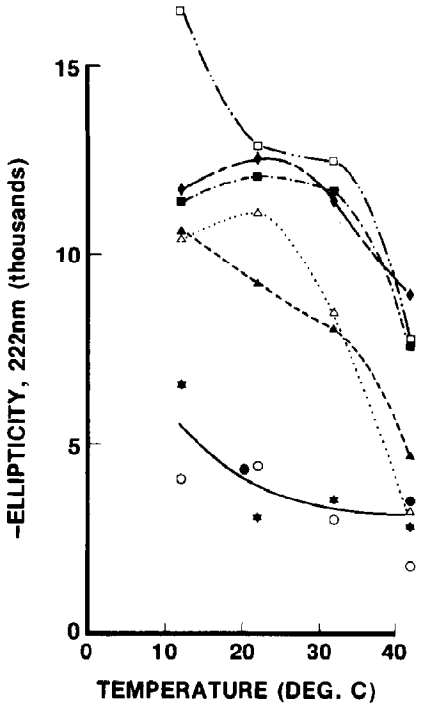

Fig. 1. Temperature dependence of the CD of glucagon-lipid complexes. (□-···-□) DMPG-glucagon, 43:1 molar ratio, pH 5; (◆---◆) DMPG-glucagon, 27:1 molar ratio, pH 7; (■-·-■) DMPG-glucagon, 32:1 molar ratio, pH 9; (△·····△) DMPC-glucagon, 39:1 molar ratio, pH 5; (▲---▲) DMPC-glucagon, 34:1 molar ratio, pH 9; (———) glucagon alone at pH 5 (●), 7 (○) or 9 (*). Values plotted are the mean residue ellipticity measured from a solution containing 50–100 μM glucagon.

3. Results

3.1. Effect of lipid on the helical content of the peptide hormones

3.1.1. Glucagon

Glucagon in dilute aqueous solution has little secondary structure but it self-associates to structures of higher helical content at increased concentrations [10,11]. There should be little self-association at the low glucagon concentrations used in this work and consequently there is little effect of temperature on the CD of glucagon in the absence of lipid, although the magnitude at 222 nm is somewhat higher at 12°C, pH 9 and somewhat lower at 42°C, pH 7 (fig. 1). Factors such as the self-association of glucagon or absorption to glass could cause these aberrant values but this was not investigated further.

The helical content of glucagon is increased in the presence of DMPC at pH 7.4 [5]. This increase in helicity is markedly dependent on temperature and is manifested only at or below the phase transition temperature of the lipid [5]. The entire CD spectrum of glucagon-DMPC complexes has been reported [12]. In this work we have summarized the data from a large number of spectra, by reporting only the ellipticity at 222 nm. As has been reported for glucagon-DMPC complexes at pH 7.4 [5] there is also a decrease with temperature in the magnitude of the ellipticity of glucagon-DMPC mixtures at pH 5 and 9 (fig. 1). The amount of secondary structure of glucagon in the presence of DMPG also decreases with temperature but to a much smaller extent than with the

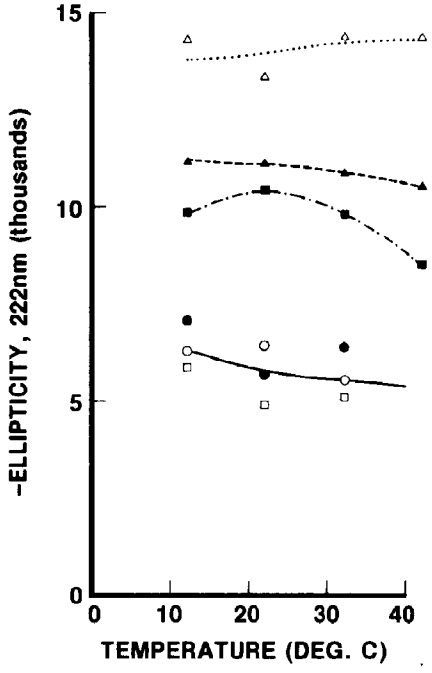

Fig. 2. Temperature dependence of the CD of hPTH(1–34)-lipid complexes. (△·····△) DMPG-hPTH(1–34), 30:1 molar ratio, pH 5; (▲------▲) DMPG-hPTH(1–34), 40:1 molar ratio, pH 7; (■-·-■) DMPG-hPTH(1–34), 42:1 molar ratio, pH 9; (———) hPTH(1–34) alone at pH 5 (□), 7 (●) and 9 (○). Mean residue ellipticity, 100 μM hPTH(1–34).

Table 1

DSC transitions of several DMPG-peptide complexes

Peptide	pH	Lipid/peptide molar ratio	Temperature of maximum ΔC_p (°C)	ΔH (kcal/mol lipid) (±S.E.)
None	5	–	22.4	5.8 ± 0.1
Glucagon	5	37	35	14.4 ± 0.3
hPTH(1–34)	5	30	22, 32, 38.5	13.3 ± 0.3
sCT	5	20	16.4, 21.9	3.9 ± 0.4
Myelin basic protein	5	74	16.3, 22.4	4.5
Metorphinamide	5	7.9	20.0	5.2 ± 0.3
Molluscan cardioexcitatory neuropeptide	5	2.1	16.8	6.8 ± 0.2
None [a]	7.4	–	22.5	4.2
Glucagon	7	23	21.6, 22.6, 36.6	5.1 ± 0.5
hPTH(1–34)	7	40	21.3	3.3 ± 0.2
sCT [a]	7.4	30	23.2	2.5
None	9	–	21.8	4.4 ± 0.1
Glucagon	9	27	24.3, 35.9	3.9 ± 0.5
hPTH(1–34)	9	42	23.8	4.1 ± 0.2

[a] Data from ref. 5.

DMPC complexes (fig. 1). The apparent helical content of glucagon is somewhat greater in the presence of DMPG at pH 7 or 9 at 12°C than it is with DMPC under these conditions. The greatest effect of DMPG on the structure of glucagon is at pH 5, 10°C (fig. 1). The magnitude of the ellipticity is greater in the presence of lipid than in its absence, except with DMPC at 42°C. This latter result may be caused by a light scattering artifact. The results presented for the glucagon-lipid complexes in fig. 1 represent samples containing close to saturating concentrations of lipid and are insensitive to changes in the lipid/peptide ratio (data not shown).

3.1.2. hPTH-(1–34)

There is little effect of temperature on the conformation of hPTH-(1–34) in the presence or absence of DMPG (fig. 2). The CD spectra of hPTH-(1–34) in the presence and absence of DMPC have been reported [12]. The change in the conformation of hPTH-(1–34) in the presence of DMPG is

Table 2

Effect of calcitonin on the thermotropic phase transition properties of DMPC

Buffer: 20 mM Pipes, 1 mM EDTA, and 0.15 M NaCl containing 0.02 mg/ml NaN_3, pH 7.40. Scan rate 0.6 K/min.

Calcitonin		DMPC/calcitonin (molar ratio)	Premelt transition ΔH (kcal/mol)	Main transition	
Species	Concentration (mM)			ΔH (kcal/mol)	$C_{p,max}$ (cal/K per g)
			1.0	5.4	22
sCT	0.51	7.6	0.9	5.6	6.3
sCT	0.51	1.7	0.3	5.1	3.3
sCT	0.64	1.5	0	3.9	1.4
sCT	0.76	1.3	0	4.3	1.1
hCT	0.28	3.4	0.9	5.7	22
hCT	0.72	1.3	0	5.8	10

much greater than has been observed with DMPC at pH 7.4 [13] or with DMPC at pH 5, 7 or 9 (this work, data not presented). The maximal effect with DMPG occurs at low lipid/peptide molar ratios and is similar for all pH values studied (data not shown). Thus, the observed changes of ellipticity with temperature were measured using samples which had a high enough lipid/peptide ratio to give a maximal conformational change.

3.1.3. sCT

The increase in the helical content of sCT in the presence of anionic DMPG contrasts with the apparent lack of interaction with DMPC [6]. However, using a high sCT concentration of 0.51 mM and a DMPC/peptide molar ratio of 1.7 the resulting solution at pH 7.4 is visually transparent. This indicates that some interaction occurs between sCT and DMPC. The DMPC does not affect the CD spectrum of sCT at any temperature from 5 to 40°C. The interaction of calcitonins with the zwitterionic DMPC occurs only in the presence of a high concentration of peptide. It is possible that no conformational change in the peptide is observed under these conditions because of the presence of a large excess of free peptide over lipid-peptide complex. The interaction between sCT and DMPC can be measured by observing the effect of the peptide on the thermotropic phase transition properties of the lipid (see below). At pH 5, sCT does not solubilize DMPC or DMPG and alkaline pH was avoided because of the instability of the disulfide bond under these conditions.

3.2. Differential scanning calorimetry

The parameters of the thermotropic transitions of DMPG (table 1) or DMPC (table 2) in the presence or absence of several proteins and peptides are summarized. A pretransition was observed only in the case of the pure lipid and in the case of hCT or sCT-DMPC mixtures at lipid/peptide molar ratios of 2 : 1 or greater (table 2). The lipid/peptide molar ratio used is larger for the myelin basic protein and smaller for metorphinamide and molluscan cardioexcitatory neuropeptide (table 1) so as to maintain similar lipid/peptide weight ratios for all of the samples of about 5. The lipid concentration was about 2 mM in all cases. The large increase in transition temperature and transition enthalpy observed for DMPG at pH 5 in the presence of glucagon or of hPTH-(1–34) is very pronounced (table 1). The shape of the DSC curve was generally very reproducible on rescanning except for the samples of DMPG at pH 5 in the presence of either glucagon or of hPTH-(1–34). In these latter two cases the shape of the curve depended markedly on the history of the sample, suggesting that the sample contains a mixture of phase domains which interconvert slowly. If either of these peptide-DMPG samples at pH 5 was rescanned without removing the samples from the calorimeter, the transition temperature decreased to about 16°C and the transition enthalpy was also lower. When the sample was removed from the calorimeter, the lipid

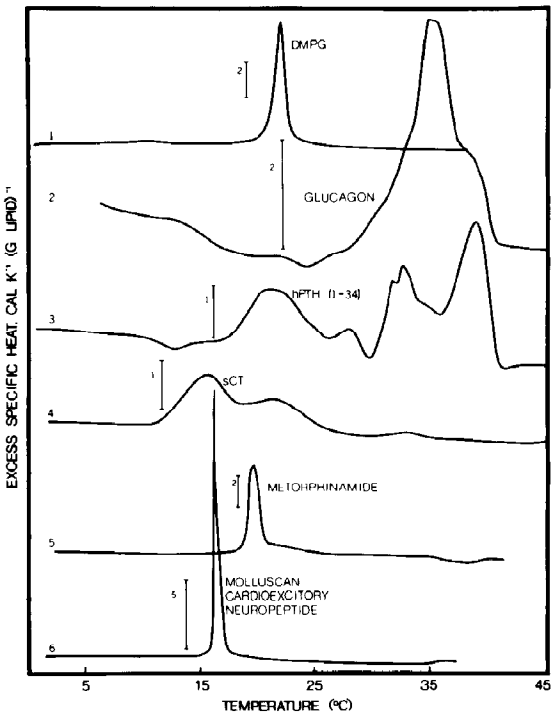

Fig. 3. DSC of peptide-DMPG complexes at pH 5. Scan rate of 0.6 K min^{-1}. Calibration bars in units of cal K^{-1} (g lipid)$^{-1}$. DMPG represents scan of lipid alone. DMPG concentration about 2 mM in all samples. Lipid/peptide ratios for each sample can be obtained from table 1.

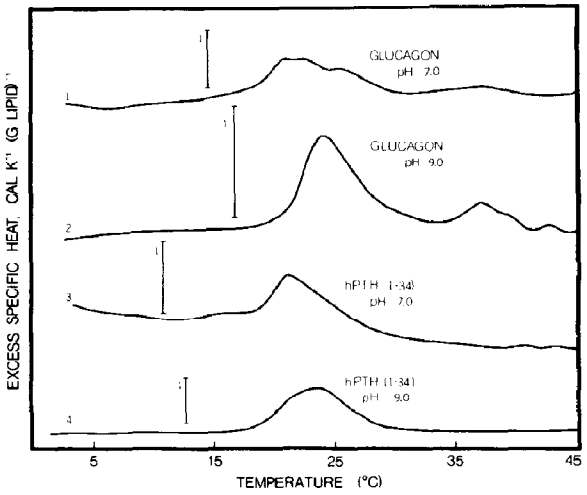

Fig. 4. DSC of glucagon or hPTH-(1–34) with DMPG at pH 7 or 9. Scan rate 0.6 K min^{-1}. Calibration bars in units of cal K^{-1} (g lipid)$^{-1}$. DMPG concentration about 2 mM. Lipid/peptide ratios for each sample can be obtained from table 1.

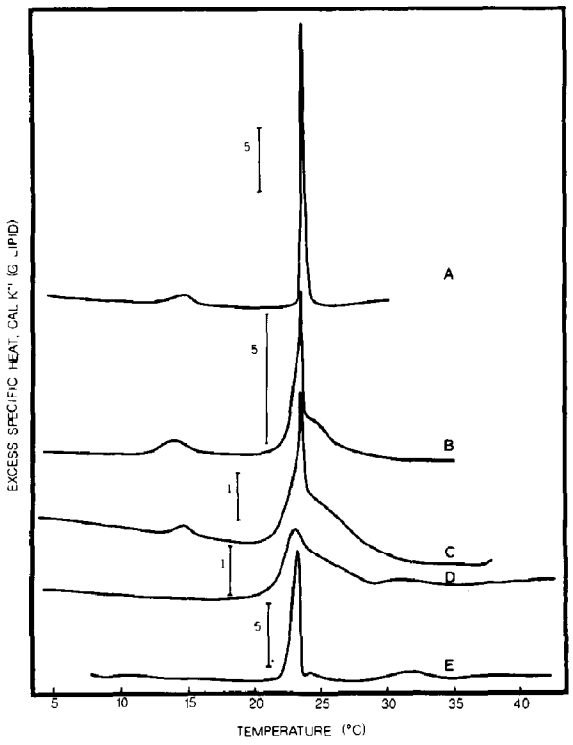

Fig. 5. DSC of DMPC in the absence or presence of sCT or hCT. 20 mM Pipes, 1 mM EDTA, and 0.15 M NaCl containing 0.02 mg/ml NaN_3, pH 7.40. Scan rate 0.6 K min^{-1}. Calibration bars in units of cal K^{-1} (g lipid)$^{-1}$. (A) 1 mM DMPC, (B) 3.9 mM DMPC and 0.51 sCT, (C) 0.87 mM DMPC and 0.51 mM sCT, (D) 1.0 mM DMPC and 0.64 mM sCT, (E) 0.94 mM DMPC and 0.72 mM hCT.

was found to be in the form of a flocculent precipitate. We have found that these DMPG-peptide samples aggregate at temperatures near the phase transition temperature. During this aggregation process it is likely that some of the peptide is removed from the water-soluble lipoprotein complex resulting in the precipitation of the lipid, especially during the slow cooling of the calorimeter cells during reequilibration. Alternatively, the sample can be withdrawn from the calorimeter after the first scan and maintained in a soluble form by rapid cooling and then loaded into a precooled, empty calorimeter cell. In such a case the high transition enthalpies and transition temperatures are again observed, although the detailed shape of the transition is not very reproducible. Because the high enthalpy transitions were so

Table 3

Aqueous-organic phase partitioning of calcitonins

Buffer [a]	Percent of total peptide transferred to organic phase			
	sCT	hCT	Glucagon	hPTH-(1–34)
20 mM Mops, pH 7.4	0.35 ± 0.03	42 ± 4	5 ± 2	3 ± 2
10 mM sodium phosphate, pH 7.4	0.3 ± 0.1	46 ± 4	4 ± 2	2 ± 1
500 mM sodium phosphate, pH 7.4	0.6 ± 0.5	50 ± 8	< 0.5	[b]
20 mM sodium acetate, pH 5	3.3 ± 0.4	15 ± 1	3 ± 2	6 ± 4

[a] All buffers contain 0.15 M NaCl and 1 mM EDTA in addition to the buffer salts.

[b] Not determined because of the formation of a precipitate at the solvent interface.

Table 4

Amino acid sequences of peptide hormones and their expected state of protonation

pH	Hormone	Sequence																														Overall charge
						5					10					15					20					25							
	Glucagon	His	Ser	Gln	Gly	Thr	Phe	Thr	Ser	Asp	Tyr	Ser	Lys	Tyr	Leu	Asp	Ser	Arg	Arg	Ala	Gln	Asp	Phe	Val	Gln	Trp	Leu	Met	Asn	Thr			
5		++								-1/2			+			-1/2		+	+			-1/2								–			$+2\frac{1}{2}$
7		+								–			+			–		+	+			–								–			0
9										–			+			–		+	+			–								–			-1
	hPTH-(1-34)	Ser	Val	Ser	Glu	Ile	Gln	Leu	Met	His	Asn	Leu	Gly	Lys	His	Leu	Asn	Ser	Met	Glu	Arg	Val	Glu	Trp	Leu	Arg	Lys	Lys	Leu	Gln	Asp	Val	His	Asn	Phe	
5		+			-1/2					+				+	+					-1/2	+		-1/2			+	+	+			-1/2		+		–	+6
7		+			–									+						–	+		–			+	+	+			–				–	+1
9					–									+						–	+		–			+	+	+			–				–	0
	sCT	Cys	Ser	Asn	Leu	Ser	Thr	Cys	Val	Leu	Gly	Lys	Leu	Ser	Gln	Glu	Leu	His	Lys	Leu	Gln	Thr	Tyr	Pro	Arg	Thr	Asn	Thr	Gly	Ser	Gly	Thr	Pro-NH_2		
5		+										+				-1/2	+	+							+									$+4\frac{1}{2}$
7		+										+				–		+							+									+3
9												+				–		+							+									+2
	hCT	Cys	Gly	Asn	Leu	Ser	Thr	Cys	Met	Leu	Gly	Thr	Tyr	Thr	Gln	Asp	Phe	Asn	Lys	Phe	His	Thr	Phe	Pro	Gln	Thr	Ala	Ile	Gly	Val	Gly	Ala	Pro-NH_2		
5		+														-1/2			+		+													$+2\frac{1}{2}$
7		+														–			+															+1
9																–			+															0

markedly dependent on the history of the sample, it is possible that the observed enthalpy is not a true thermodynamic property of a reversible equilibrium. The effect of the lipid/glucagon ratio on the thermotropic properties of DMPG at pH 5 was investigated. Scans of samples with a lower DMPG/glucagon ratio of 15 showed similar transitions to those presented for glucagon in table 1, although they could not be quantitatively compared because of the lack of exact reproducibility between successive scans. At a DMPG/glucagon ratio of 50, the lipid was still 'solubilized', i.e., the sample was not visually turbid. This sample exhibited a broader transition but the transition temperature and enthalpy were similar to those of the pure lipid.

The higher temperature transition is not observed with sCT, myelin basic protein, metorphinamide or molluscan cardioexcitatory neuropeptide at pH 5 (fig. 3). There is, however, an increase in the transition enthalpy in the presence of molluscan cardioexcitatory neuropeptide (table 1). The transition curve for the DMPG complex with this peptide is also remarkably sharp despite the marked lowering of the transition temperature (fig. 3). At pH 7 or 9 with DMPG, there is still a small high-temperature transition component observable with glucagon but not with hPTH-(1–34) (fig. 4). However, even with glucagon, the enthalpy of the high-temperature transition is markedly reduced at higher pH.

Glucagon forms water-soluble complexes with DMPC and their DSC properties have been studied at pH 7.4 [14,15]. We find that transition curves of similar shape are also observed at pH 5 and 9 (data not presented). Glucagon lowers the transition enthalpy of DMPC from 4.8 to 3.4 kcal/mol at pH 7.2 at a lipid/peptide ratio of 34 [15] while at pH 5 the transition enthalpy is raised from 5.2 to 6.4 kcal/mol DMPC. This is a very small change compared with that observed with DMPG in the presence of glucagon or hPTH-(1–34) at pH 5.

DSC demonstrates that sCT has a much more marked effect on the phase transition properties of DMPC than does hCT (fig. 5 and table 2). However, hCT is more effective than sCT in abolishing the pretransition. Thus, when $C_{p,\max}$ is reduced by only a factor of 2 there is no longer a detectable pretransition in the presence of hCT while it requires about a 16-fold reduction of $C_{p,\max}$ in the case of sCT before the premelt transition is abolished (table 2 taken from the data of fig. 7 and other scans not shown).

3.3. Phase partitioning

The only peptide to exhibit substantial partitioning into the organic phase is hCT (table 3). The amount transferred at pH 7.4 was not affected by the nature of the buffer or the ionic strength but was reduced by lowering the pH to 5. No significant amounts of sCT, glucagon or hPTH-(1–34) were extracted under the conditions employed. The small amounts of apparent transfer observed in these latter cases could be due to the presence of contaminants or to cross-contamination of phases through microdroplets or during sampling. The greater partitioning of hCT into the organic phase is probably due to its greater content of hydrophobic amino acids, especially near the carboxyl terminus and its smaller number of charged groups as well as a smaller overall charge of +1 at pH 7.4, compared with the other peptides studied (table 4). The effect of peptide charge is probably also responsible for the decreased extractability of hCT at pH 5 where it gains a greater positive charge.

4. Discussion

It is often difficult to specify the nature of the non-covalent interactions which stabilize lipid-protein complexes. The stability of these complexes depends not only on lipid-protein interactions but also on the strength of lipid-lipid and protein-protein interactions. In the present study we have used low peptide concentrations, where peptide-peptide interactions should be minimal and have restricted the range of pH to minimize pH-induced changes in lipid-lipid interactions.

One of the purposes of varying the pH was to study the effect of changes in the electrostatic charge of the peptides on peptide-lipid interactions. The sequences of the peptide hormones used

in this work and their approximate expected charges in an aqueous environment at pH 5, 7 and 9 are given in table 4. Because of the small size of these hormones it is unlikely that the side chains are completely sequestered from the aqueous environment and therefore the estimated charge on the peptides in water is likely to be reasonably accurate. There is no simple correlation between the overall charge of the peptide and its ability to interact with anionic vs. zwitterionic lipids. Thus, hPTH-(1–34) with no overall charge at pH 9 interacts more with the anionic DMPG than with the zwitterionic DMPC, while glucagon at pH 5 with a charge of $+2\frac{1}{2}$ interacts with both DMPG and DMPC. In addition, even negatively charged glucagon at pH 9 can interact with negatively charged DMPG. Although the overall charge of the peptide does not provide a good index of the type of lipid with which the peptide interacts, electrostatic interactions are likely to play a role in the interaction of these peptide hormones with lipid. Electrostatic repulsion among the negatively charged DMPG molecules would be decreased by the incorporation of another molecule into the bilayer. Furthermore, even though the overall charge on the peptide might not be positive, there may be specific sites which are positively charged and account for the preference of the peptide to associate with negatively charged lipid. One such positively charged site is made up by the three positively charged residues 25–27 in hPTH-(1–34).

Electrostatic interactions contribute to the thermal stability of the lipid-peptide complexes. Thus, hPTH-(1–34) and sCT which are able to solubilize more DMPG than DMPC and which undergo a large conformational change in the presence of the anionic lipid probably interact, at least in part, by electrostatic attraction (refs. 6 and 13 and this work). These peptides form complexes with DMPG which are stable over a wide range of temperatures. Glucagon, which interacts equally well with DMPC and DMPG, forms complexes which dissociate at higher temperatures. This effect is most pronounced for the complex formed between glucagon and DMPC which is less likely to be stabilized by electrostatic charges. The weak interaction between hPTH-(1–34) and DMPC is also temperature dependent, decreasing above the phase transition temperature of the phospholipid [13].

It is well known that protonation of anionic phospholipids or their interaction with divalent cations causes a marked increase in their transition temperature and transition enthalpy. In the case of DMPG, for example, protonation of lipid raises the phase transition temperature from 23 to above 40°C [16–18]. Addition of Mg^{2+} shifts the transition temperature to 65°C with an increase in the transition enthalpy to 10 kcal mol^{-1} [19]. In the present work we demonstrate that peptides such as glucagon and hPTH-(1–34) can also markedly increase the phase transition temperature and transition enthalpy of DMPG. Because the effects of the peptides resemble those of protons and divalent cations and because they occur at low pH and only with acidic phospholipid, they probably arise from charge interactions between cationic sites on the peptide and the negatively charged phosphate group of the lipid. At higher pH, when the peptide becomes less positively charged this interaction becomes less likely. However, not all positively charged peptides can induce this marked alteration in the phase behaviour of DMPG. Although sCT, myelin basic protein [20] and metorphinamide are positively charged at pH 5 they may not have sufficient overall hydrophobicity to exhibit this effect. At higher pH the effects are diminished because lysine residues would not retain their positive charge in a membrane environment at those pH values. In addition, at higher pH the increased negative charge on the carboxyl groups would lead to electrostatic repulsion with anionic lipids and to greater water solubility. A pentagastrin-related peptide N^{α}-*t*-Boc-β-Ala-Trp-Met-Arg-Phe-NH_2, exhibits effects on a lipid phase transition similar to those of glucagon or hPTH-(1–34) at pH 5 [21]. Our studies demonstrate that certain hydrophobic and positively charged peptide molecules can affect the phase transition properties of acidic phospholipids in a manner similar to divalent cations.

In general, the premelt transition is very sensitive to the presence of contaminants. The fact that, in the presence of increasing concentrations of sCT, the gel-to-liquid crystalline transition of DMPC is affected more than the premelt transition (fig. 5) suggests that the peptide is accommodated into regions of bilayer defects, at least in the

gel state [22], rather than dissolving into the membrane as do more hydrophobic substances. Comparing the effects of sCT and hCT on the phase transitions of DMPC (fig. 5) demonstrates that the interaction of calcitonins with lipids is not simply a result of the hydrophobic side chains of the peptide binding to a hydrophobic membrane. Thus, the less hydrophobic sCT binds DMPC better than hCT does. In addition, glucagon which interacts most readily with DMPC, of all of the peptide hormones studied, is also much less hydrophobic than hCT (table 3). Hydrophobic forces probably contribute to the lipid binding but as with electrostatic forces, they do so in a manner which is not completely non-specific. Another demonstration of this is the greater binding of pentagastrin than N^{α}-*t*-Boc-β-Ala-Trp-Met-Phe-Asp-NH_2 to DMPC even though both peptides have identical amino acid compositions and therefore hydrophobicities [23]. In the case of the calcitonins, factors such as the greater probability of sCT to form helical structures [24] or the greater hydrophobic moment of the helical segment of sCT [6] may contribute to its greater ability to affect the lipid phase transition of DMPC. The ability of the calcitonins to bind to lipid is related to their biological potency [25].

Acknowledgement

This work was supported by the Medical Research Council of Canada (Grant MA-7654).

References

1 R.M. Epand, Mol. Cell. Biochem. 57 (1983) 41.
2 E.T. Kaiser and F.J. Kézdy, Science 223 (1984) 249.
3 H.-U. Gremlich, U.-P. Fringeli and R. Schwyzer, Biochemistry 23 (1984) 1808.
4 D. Eisenberg, R.M. Weiss and T.C. Terwilliger, Proc. Natl. Acad. Sci. U.S.A. 81 (1984) 140.
5 R.M. Epand, A.J.S. Jones and S. Schreier, Biochim. Biophys. Acta 491 (1977) 296.
6 R.M. Epand, R.F. Epand, R.C. Orlowski, R.J. Schuleuter, L.T. Boni and S.W. Hui, Biochemistry 22 (1983) 5074.
7 J.P. Segrest, R.L. Jackson, J.D. Morrisett and A.M. Gotto, Jr, FEBS Lett. 38 (1974) 247.
8 H.A. Sober, CRC handbook of biochemistry: Selected data for molecular biology, 2nd edn. (Chemical Rubber Co., 1970) p. B74.
9 W.B. Gratzer, G.H. Beaven and E. Bailey, Biochem. Biophys. Res. Commun. 28 (1967) 914.
10 W.B. Gratzer and G.H. Beaven, J. Biol. Chem. 244 (1969) 6675.
11 J.C. Swann and G.G. Hammes, Biochemistry 8 (1969) 1.
12 R.M. Epand, A.J.S. Jones and B. Sayer, Biochemistry 16 (1977) 4360.
13 R.M. Epand, R.F. Epand, S.W. Hui, N.B. He and M. Rosenblatt, Int. J. Peptide Protein Chem. 25 (1985) 594.
14 R.M. Epand and J.M. Sturtevant, Biochemistry 20 (1981) 4603.
15 R.M. Epand and J.M. Sturtevant, Biophys. Chem. 19 (1984) 355.
16 P.W.M. van Dijck, B. de Kruijff, A.J. Verkleij, L.L.M. van Deenen and J. de Gier, Biochim. Biophys. Acta 512 (1978) 84.
17 E.J. Findlay and P.G. Barton, Biochemistry 17 (1978) 2400.
18 A. Watts, K. Harlos, W. Maschke and D. Marsh, Biochim. Biophys. Acta 510 (1978) 63.
19 J.M. Boggs and G. Rangaraj, Biochemistry 22 (1983) 5425.
20 J.M. Boggs, D. Stamp and M.A. Moscarello, Biochemistry 21 (1982) 1208.
21 W.K. Surewicz and R.M. Epand, Biochemistry 24 (1985) 3135.
22 R.M. Epand and W.K. Surewicz, Can. J. Biochem. Cell. Biol. 62 (1984) 1167.
23 W.K. Surewicz and R.M. Epand, Biochemistry 23 (1984) 6072.
24 M. Merle, G. Lefevre and G. Milhaud, Biochem. Biophys. Res. Commun. 87 (1979) 455.
25 R.M. Epand, R.F. Epand and R.C. Orlowski, Int. J. Peptide Protein Res. 25 (1985) 105.