Biophysical Chemistry 19 (1984) 355–362
Elsevier

BPC 00857

THE EFFECTS OF VARIOUS PEPTIDES ON THE THERMOTROPIC PROPERTIES OF PHOSPHATIDYLCHOLINE BILAYERS

Richard M. EPAND [a] and Julian M. STURTEVANT [b]

[a] *Department of Biochemistry, McMaster University Health Sciences Center, Hamilton, Ontario L8N 3Z5, Canada, and* [b] *Department of Chemistry and Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511, USA*

Received 1st November 1983
Accepted 13th December 1983

Key words: Glucagon; Pentagastrin; Lipid bilayer; Phase transition; Peptide-lipid interaction

The effects of an amino acid derivative (*N*-benzoyl-L-argininamide), four small peptides (Phe-Gly-Phe-Gly, gastrin-related peptide (Trp-Met-Arg-Phe-NH_2), tetragastrin (Trp-Met-Asp-Phe-NH_2), pentagastrin (Boc-βAla-Trp-Met-Asp-Phe-NH_2)) and one medium-sized peptide, glucagon (29 residues), on the gel-to-liquid crystalline transition of a multilamellar suspension of dimyristoylphosphatidylcholine have been studied by means of high-sensitivity differential scanning calorimetry. At low concentrations of added solutes, the temperature at which the excess apparent specific heat in the gel-to-liquid crystalline phase transition of the lipid is maximal is lowered by an amount proportional to the total concentration of the peptide, with proportionality constants ranging from -0.018 K mM^{-1} for Phe-Gly-Phe-Gly to -3.1 K mM^{-1} for the gastrin-related peptide. The lipid mixtures involving the first two solutes listed above exhibited approximately symmetrical curves of excess apparent specific heat vs. temperature. The curves for the other solutes were asymmetric, and could be well represented as the sum of either two or three two-state curves. The asymmetry, which was especially pronounced in the cases of pentagastrin and glucagon, thus appeared to be due to the presence of components having lower and/or higher transition temperatures than that of the lipid. Pentagastrin and glucagon (R.M. Epand and J.M. Sturtevant, Biochemistry 20 (1981) 4603) have much smaller effects on the gel-to-liquid crystalline phase transition of dipalmitoylphosphatidylcholine than on that of the dimyristoyl analog.

1. Introduction

Proteins are a major constituent of biological membranes, and the biological activity of a variety of peptides and proteins is dependent on their ability to bind to cell membranes. It is therefore of interest to study the nature of the interactions between peptides and lipids in model systems.

The ability of a substance to alter the phase transition characteristics of a phospholipid model membrane is indicative of its association with the lipid either by simple solution or by some more complicated means. In the work reported here we have employed high-sensitivity DSC to study the effects of several peptides on the gel-to-liquid crystalline, or main, transition of multilamellar DMPC. In the cases of two peptides, pentagastrin and glucagon, a comparison was made of the effects on bilayers of DMPC with those of DPPC.

Abbreviations: BAA, *N*-benzoyl-L-argininamide; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSC, differential scanning calorimeter(ry); Boc, *t*-butoxycarbonyl.

2. Materials and methods

DMPC (Sigma Chemical Co.) was recrystallized once from chloroform/hexane. DPPC (Avanti Polar Lipids, Inc.) was used without further purification. Both these lipids were adequately pure for

DSC applications as judged by their transition temperatures (temperatures of maximal excess heat capacity, t_m) and their transition widths at half-maximal excess heat capacity. BAA was purchased from Sigma Chemical Co. The following peptides were purchased from Bachem Co. (all optically active amino acid residues in the L-configuration): Phe-Gly-Phe-Gly, tetragastrin (Trp-Met-Asp-Phe-NH_2), gastrin-related peptide (Trp-Met-Arg-Phe-NH_2), pentagastrin (Boc-βAla-Trp-Met-Asp-Phe-NH_2). Glucagon was purchased from the Elanco Corp. All these compounds were used without further purification. Peptide solutions were prepared in 10 mM sodium phosphate buffer containing 0.5 mM EDTA, at pH 7.2 unless otherwise specified, and were clarified if necessary by centrifugation. Peptide concentrations were estimated spectrophotometrically, using extinction coefficients equal to the values given for the *N*-acetyl methyl esters of the aromatic amino acids [1], 394 M^{-1} cm^{-1} at 257.5 nm for Phe-Gly-Phe-Gly and 5600 M^{-1} cm^{-1} at 280 nm for the tryptophan-containing peptides. The absorption coefficient 2.38 l g^{-1} cm^{-1} at 278 nm [2] was used for glucagon. The concentration of BAA was determined by weight.

Samples for DSC were prepared by suspending 4 mg of lipid in 2 ml of buffer or peptide solution by vortex mixing for 30 s at about 35°C, allowing the mixture to stand at least 1 h at room temperature and then again vortex mixing at 35°C. No changes in phase transition behavior were observed with samples that were kept up to 8 h at room temperature or were vigorously mixed for longer periods of time up to 5 min. All suspensions were scanned at 0.5 K min^{-1} in a DASM-1M scanning microcalorimeter [3] as previously described. For the sharper transitions, with width at half height of less than 1.0 K, the precision of determinations of t_m on the same sample left in the calorimeter and rescanned was ± 0.03 K, while day-to-day determinations of the t_m values of different preparations were precise to ± 0.1 K. For all samples tested, the shape of the DSC curve was essentially the same on rescanning, and was independent of scan rate from 0.1 to 0.5 K min^{-1}. For deconvolution of the DSC curves, the observed scans were converted into plots of excess heat capacity vs. temperature by subtracting a baseline which was constructed by extending the linear portion of the observed scan occurring either before or after the transition. These constructed baselines were coincident, indicating a negligible ΔC_p for the transition. The noise levels of all scans were below 0.1 cal K^{-1} g^{-1} (see fig. 1).

The relatively complex transition curves observed with glucagon/DMPC and pentagastrin/DMPC mixtures were analyzed into component curves on the assumptions (a) that the component curves adhere to the van't Hoff equation, (b) that they represent physical components which undergo completely independent transitions, and (c) that the ratio of van't Hoff to calorimetric, or actual enthalpies need not be the same for each component curve. There are thus three independent parameters which are selected for each com-

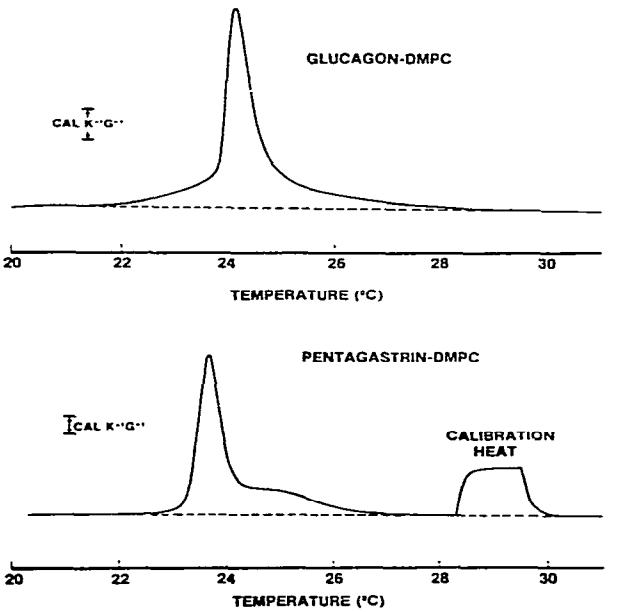

Fig. 1. Typical DSC output curves. The pentagastrin/DMPC sample contained 0.3 mM pentagastrin and 3.0 mM DMPC at pH 8.0. The glucagon/DMPC sample contained 0.02 mM glucagon and 3.0 mM DMPC at pH 7.2. Suspensions in 10 mM sodium phosphate, 0.5 mM EDTA. Scan rate, 0.5 K min^{-1}. The electrical calibration signal was produced by supplying 8.62 mcal of excess energy to the reference cell over a period of 150 s.

Table 1

Concentration dependence of t_m and enthalpies of transition

ΔH is given as the mean ± S.E., per mol lipid. a, b and c are constants in the equation $t_m = a + bc$, where c is the overall concentration (mM) of peptide; r, regression coefficient.

Solute	a (°C)	b (°C/mM)	r	ΔH (kcal mol^{-1})
N-Benzoyl-L-arginine amide	24.08	−0.034	–	5.5 ± 0.1
Phe-Gly-Phe-Gly	24.07 ± 0.03	−0.018 ± 0.004	0.98	5.7 ± 0.1
Tetragastrin	24.15 ± 0.06	−0.27 ± 0.09	0.94	5.1 ± 0.1
Gastrin-related peptide	24.10 ± 0.05	−3.1 ± 0.2	1.0	5.3 ± 0.1
Glucagon (lower transition)	24.75 ± 0.03	−16.9 ± 0.6	1.0	*
Glucagon (middle transition)	23.9 ± 0.2	12 ± 4	0.94	*
Glucagon (upper transition)	25 ± 1	14 ± 17	0.6	*
Pentagastrin (lower transition)	24.23 ± 0.04	−1.79 ± 0.06	1.0	5.7 ± 0.1 (total)
Pentagastrin (upper transition)	24.5 ± 0.1	0.81 ± 0.2	0.95	

* See table 2.

ponent to minimize the standard deviation of calculated from observed excess specific heats. With a total of as many as nine adjustable parameters, and experimental data of modest precision, it is to be expected that the parametric values obtained carry a large uncertainty. Nevertheless, it is possible to show that some of the observed curves require a minimum of three components for an adequate analysis, including tracing regularities in the variations of the parameters with changes in lipid-peptide ratios.

3. Results and discussion

3.1. Variation of t_m with solute concentration

All the solutes led to a lowering of t_m which was proportional to the overall concentration of solute, at least at low solute concentrations, with the proportionality constant varying widely for the various solutes (table 1). Although it is evident from the shapes of some of the transition curves that certain of the systems cannot be considered to be ideal, it is nevertheless of interest to compare the observed lowerings of t_m with those expected on the basis of ideal solution theory. In all the cases studied here, the observed lowering of t_m was considerably less than would be expected on this basis if the solute were insoluble in both aqueous and gel phases of the lipid. In the case of the gastrin-related peptide, which showed the strongest dependence of t_m on concentration, the lowering of t_m was only 1/3 of the maximal ideal value. It appears likely that the peptides are not completely partitioned into the lipid and/or that they have some solubility in the gel phase of the lipid [4]. *

In the cases of the gastrin-related peptide, tetragastrin and pentagastrin there was a prominent second component present with a higher melting temperature. This higher melting component was particularly evident with pentagastrin/DMPC mixtures, where it represented a large fraction of the total area and was well resolved from the lower

An assumption included in the ideal solution treatment for lipid-solute mixtures given in ref. 4 is that the partitioning of solute between aqueous and lipid phases does not change appreciably within the temperature range of the transition. This assumption cannot hold with respect to the total amount of solute partitioned into the lipid unless (a) the solute is essentially insoluble in the aqueous phase, or (b) the partition coefficient for the solute between the aqueous and gel phases of the lipid is the same as that for partitioning into the liquid-crystalline phase. In the latter case, the solute, if it behaves ideally, can have no effect on t_m or the transition shape at any concentration. It is thus evident that of the solutes considered in ref. 4, only dodecane and hexadecane fully satisfy the assumption mentioned above. A quantitative treatment including the effect of significant solubility of the added solute in the aqueous phase will be published elsehwere.

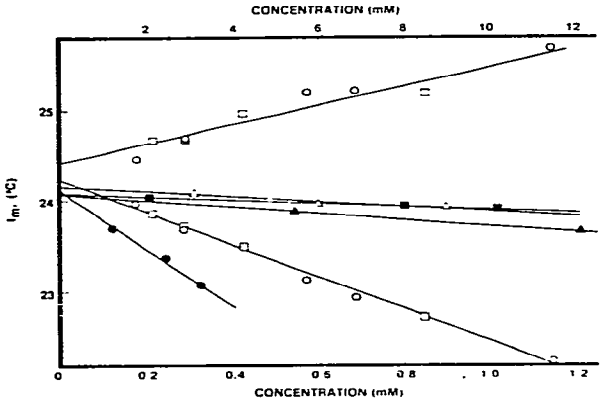

Fig. 2. Effect of added solutes on t_m, the temperature of maximal excess heat capacity, for the main transition of DMPC. t_m is plotted vs. the overall concentration of solute. Lipid concentration 3 mM in 10 mM sodium phosphate, 0.5 mM EDTA (pH 7.2), except as noted below. Upper concentration scale for benzoyl-L-arginine-amide (▲) and for Phe-Gly-Phe-Gly (■). Lower concentration scale for tetragastrin (△), gastrin-related peptide (●), pentagastrin (□), at pH 7.2 and (○) at pH 8.0. The two transition temperatures reported for each pentagastrin concentration were obtained by analyzing the observed transition into two van't Hoff components and are $t_{1/2}$ values (see text).

melting component. The DSC curves for mixtures containing pentagastrin were evaluated in detail by resolving the observed transition curves into van't Hoff components. A sum of two van't Hoff components fits the DSC curves very well (see below) and the temperature of the maximal excess heat capacity for each of the two components is shown in fig. 2. The parameters of the least-squared lines in fig. 2, along with the mean enthalpy of transition, are given in table 1.

3.2. The effect of charge

The structural difference between tetragastrin and the gastrin-related peptide is that the former peptide has aspartic acid as its third residue while the latter has an arginine residue in this position. It is interesting that this difference causes the gastrin-related peptide to have a 10-fold larger effect on the t_m of DMPC than does tetragastrin. This effect may be at least in part the result of a difference in partitioning of the two peptides between the aqueous and lipid phases. It could also arise from a difference in the charge states of the peptides within the bilayer. The carboxyl group of tetragastrin may be located in a region of low dielectric constant and may thus carry a proton, and have zero net charge [5], whereas under these conditions the arginine group of the gastrin-related peptide would retain its positive charge and have an associated gegenion. Although it would be expected that if carboxyl groups are incorporated into the bilayer in uncharged form, the extent of the incorporation would decrease with decreasing H^+ activity, in actuality it is found that the t_m values for pentagastrin/DMPC mixtures are the same at pH 7.2 and 8.0 (and also at pH 6.0). It is possible that the differences in structure between the two peptides could lead to their being positioned quite differently within the bilayer system.

3.3. Effect of lipid structure on the effects of pentagastrin

In contrast to the effects of pentagastrin on DMPC outlined above, the peptide has very little effect on the phase transition of DPPC. For example, a 0.85 mM solution of pentagastrin which lowers the melting point of DMPC by 1.3°C gives a lowering of only 0.07°C in the t_m of DPPC (3 mM) at pH 7.2. Glucagon [6] and serum apolipoprotein A-I [7], which readily form complexes with DMPC, also have much lower tendencies to form complexes with DPPC [8,9]. The causes of these pronounced differences arising from a small change in lipid structure remain to be elucidated.

3.4. Comparison of the effects of pentagastrin and glucagon on DMPC

The higher melting component found in the transition curves of pentagastrin/DMPC mixtures is also present in the DSC curves of the glucagon/DMPC-lipoprotein complex [6]. In addition, the association of both glucagon [10] and pentagastrin [11] with lipid has been found to occur more readily in the gel state than in the liquid-crystalline state. We have made a detailed comparison of the effects of pentagastrin and glucagon on the phase

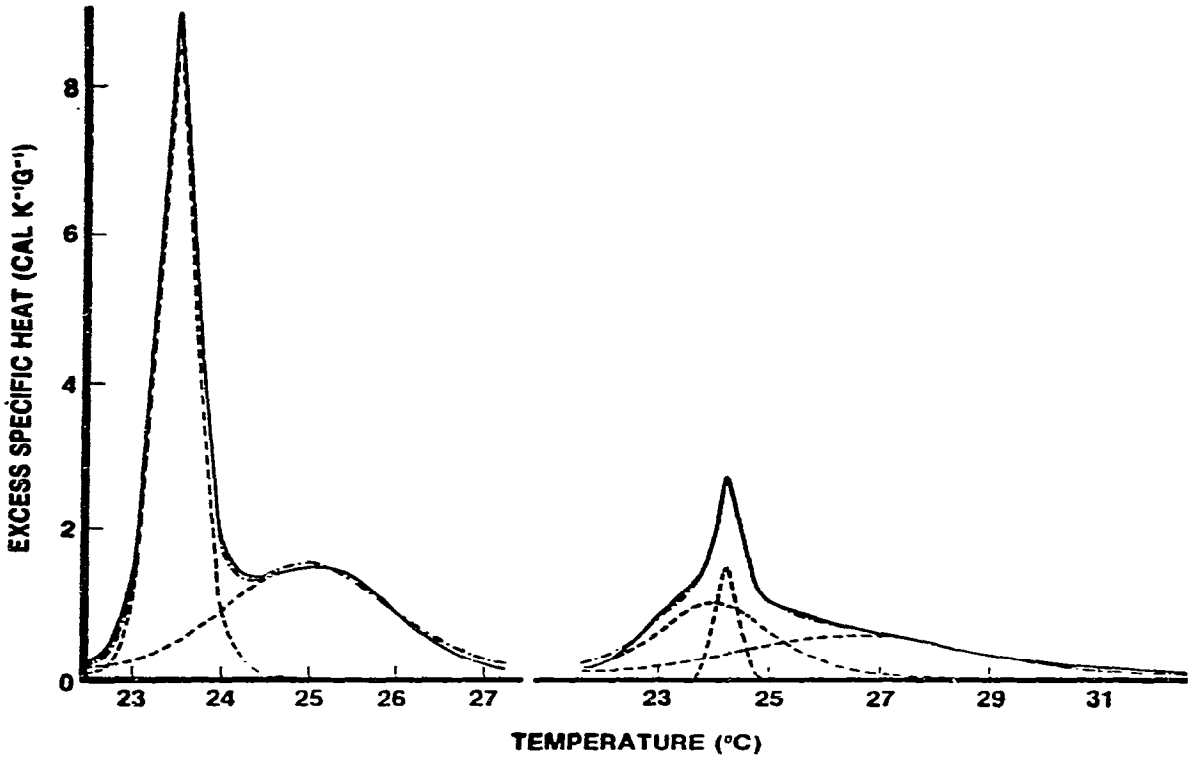

Fig. 3. Comparison of the DSC curves of pentagastrin (left) and of glucagon (right) with DMPC and their resolution into van't Hoff components. DMPC (3 mM) in 10 mM sodium phosphate, 0.5 mM EDTA (pH 7.2), containing either 0.44 mM pentagastrin (left) or 0.044 mM glucagon (right). Solid curves are the observed DSC scans, corrected for baseline. Dashed curves are the calculated individual van't Hoff components. The sum of these van't Hoff components for each example is represented by a dot-dash curve which appears in the figure only when it is not coincident with the observed scan.

transition properties of DMPC. Glucagon can cause a given alteration in the phase transition of DMPC at a much lower concentration than is required with pentagastrin. The resultant DSC curves of the peptide/DMPC mixtures are well described by only two van't Hoff components in the case of pentagastrin while for glucagon at least three components are required (see figs. 1 and 3). The melting temperatures and enthalpies for the calculated van't Hoff components of the observed DSC curves of DMPC with glucagon (table 2) and with pentagastrin (table 3) are presented along with the standard deviation of the observed fit and the total calculated and observed transition enthalpies.

Component 2 of the glucagon/DMPC mixtures has a t_m which is very close to that of the pure lipid and shows very little variation with changes in the peptide/lipid ratio. The van't Hoff enthalpy, ΔH_{vH}, of component 2 decreases markedly with increasing glucagon/lipid ratio (fig. 4), while those of components 1 and 3 show very little variation with peptide/lipid ratio. These results suggest that component 2 is a residual free lipid whose melting temperature is not affected greatly by the presence of glucagon. The magnitude of this component as measured by its calorimetric enthalpy, ΔH_{cal}, decreases markedly with increasing glucagon concentration, essentially disappearing at a peptide/lipid ratio of 0.03. It appears that

Table 2

Transition properties of van't Hoff components from DSC analysis of glucagon/DMPC mixtures at pH 7.2

P/L, overall peptide/DMPC molar ratio; S.D., standard deviation of calculated from observed curves expressed as percent of the maximal excess specific heat. ΔH_{cal} is given as kcal (mol DMPC)$^{-1}$. Values for the van't Hoff enthalpies of the components are given in fig. 4.

100 P/L	S.D. (%)	Component 1		Component 2		Component 3		Total ΔH_{cal} (kcal mol^{-1})	
		$t_{1/2}$ (°C)	ΔH_{cal} (kcal mol^{-1})	$t_{1/2}$ (°C)	ΔH_{cal} (kcal mol^{-1})	$t_{1/2}$ (°C)	ΔH_{cal} (kcal mol^{-1})	Calculated *	Observed
0.739	1.1	24.4	1.74	24.2	1.84	25.1	1.25	4.84	4.60
1.478	1.4	24.0	1.89	24.2	0.54	26.5	2.19	4.62	4.24
2.956	2.9	23.3	0.84	24.9	0.08	26.2	2.47	3.39	3.42

* Calculated as the sum of the two-state components.

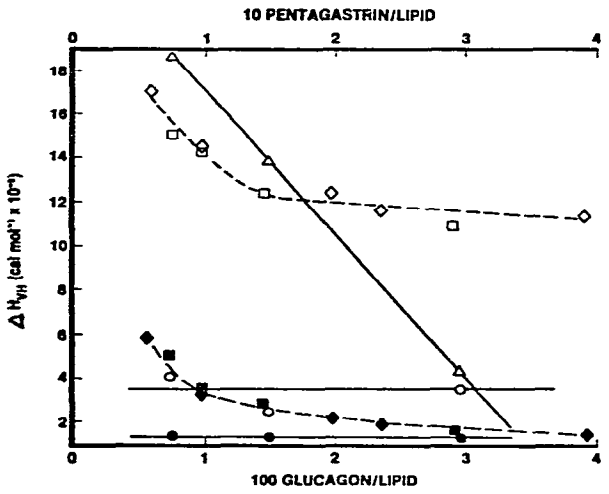

Fig. 4. Dependence of the van't Hoff enthalpies (ΔH_{vH}), for the component transitions described in tables 2 and 3, on the peptide/lipid ratio. Glucagon/DMPC samples (solid lines): component 1, ○, component 2 (▲), component 3 (●). Glucagon/DMPC molar ratio given on lower scale. Pentagastrin/DMPC samples (broken lines): component 1, pH 7.2 (□); component 1, pH 8 (◇); component 2, pH 7.2 (■); component 2, pH 8 (◆). Pentagastrin/DMPC molar ratio given on upper scale.

this residual free lipid is in equilibrium with a glucagon-lipid complex which is characterized by having a two-component phase transition [6], represented by components 1 and 3. The fact that the van't Hoff enthalpies of these two components are relatively insensitive to the ratio of glucagon to lipid (fig. 4) suggests that they may arise from a specific glucagon-lipid complex, similar to the one which has been previously isolated [12].

This behavior of glucagon is in marked contrast to that of pentagastrin, the transitions of which with DMPC do not show the presence of any component with a melting temperature which remains close to that of the pure lipid with increasing peptide concentrations (table 3). Thus, pentagastrin does not appear to form a specific complex with DMPC but rather combines with the lipid in variable proportions to produce two components whose t_m values become progressively further removed from that of pure DMPC with increasing peptide concentration (fig. 2).

The total transition enthalpy of DMPC is markedly reduced in the presence of glucagon (table 2) as has been previously reported [6]. In contrast, this quantity for the pentagastrin/DMPC

Table 3

Transition properties of van't Hoff components from DSC analysis of pentagastrin/DMPC mixtures at pH 7.2 and pH 8.0

P/L, overall peptide/DMPC molar ratio; S.D., standard deviation of calculated from observed curves expressed as percent of the maximal excess specific heat. ΔH_{cal} is given as kcal (mol DMPC)$^{-1}$. Values for the van't Hoff enthalpies of the components are given in fig. 4.

10 P/L	S.D. (%)	Component 1		Component 2		Total ΔH_{cal} (kcal mol^{-1})	
		$t_{1/2}$ (°C)	ΔH_{cal} (kcal mol^{-1})	$t_{1/2}$ (°C)	ΔH_{cal} (kcal mol^{-1})	Calculated *	Observed
pH 7.2							
0.725	2.8	23.9	3.60	24.7	2.15	5.75	5.57
0.967	0.8	23.7	3.08	24.7	2.44	5.52	5.68
1.45	0.8	23.5	3.34	25.0	2.68	6.02	6.21
2.90	1.5	22.7	2.66	25.2	3.55	6.21	5.88
pH 8.0							
0.587	2.1	24.0	3.41	24.5	1.97	5.39	5.41
0.978	1.1	23.7	3.11	24.7	2.57	5.68	5.82
1.96	1.0	23.1	2.90	25.2	2.74	5.64	5.43
2.35	1.3	22.9	2.83	25.2	3.53	6.36	5.72
3.91	1.5	22.2	2.59	25.7	4.07	6.67	5.93

* Calculated as the sum of the two-state components.

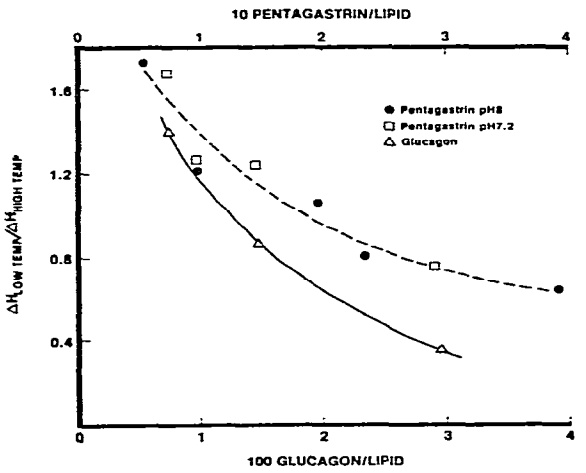

Fig. 5. Dependence on concentration of the ratio of the calorimetric enthalpy of the low-temperature transition to that of the high-temperature transition of glucagon and pentagastrin mixtures with DMPC. Enthalpies from the resolved van't Hoff components given in tables 2 and 3; components 1 and 3 for glucagon and 1 and 2 for pentagastrin. Molar ratios of peptide to lipid presented as in fig. 4. Pentagastrin/DMPC (broken line) pH 7.2 (□) pH 8 (●); glucagon/DMPC (solid line). pH 7.2 (△).

mixtures is slightly higher than that of the pure lipid (table 3). Both in the case of glucagon as well as of pentagastrin there is a trend for the ratio of the enthalpy of the lowest melting component to that of the highest melting component to decrease with increasing peptide/lipid ratio (fig. 5).

The results reported here show that peptides can exert a wide variety of effects on the phase transitions of phospholipid bilayers, and presumably on other properties of these structures. The effects are so strongly dependent on both peptide and lipid structure that it is impossible at present to give any basis for the prediction of such effects.

Acknowledgement

This research was aided by grants from the National Institutes of Health (GM04725), the National Science Foundation (PCM 7824107) and the Medical Research Council of Canada (MA 7654).

References

1 H.A. Sober, CRC handbook of biochemistry: Selected data for molecular biology, 2nd edn. (Chemical Rubber Co., 1970) p. B74.
2 W.B. Gratzer, G.H. Beaven and E. Bailey, Biochem. Biophys. Res. Commun. 28 (1967) 914.
3 P.O. Privalov, V.V. Plotnikov and V.V. Filimonov, J. Chem. Thermodyn. 7 (1975) 41.
4 J.M. Sturtevant, Proc. Natl. Acad. Sci. U.S.A. 79 (1982) 3963.
5 S. Mabrey and J.M. Sturtevant, Biochim. Biophys. Acta 486 (1977) 444.
6 R.M. Epand and J.M. Sturtevant, Biochemistry 20 (1981) 4603.
7 H.J. Pownall, J.B. Massey, S.K. Kusserow and A.M. Gotto, Biochemistry 17 (1978) 1183.
8 R. Mendelsohn, T. Taraschi and R.W. van Holten, J. Raman Spectrosc. 8 (1979) 279.
9 A. Jonas and W.R. Mason, Biochemistry 20 (1981) 3801.
10 R.M. Epand, A.J.S. Jones and S. Schreier, Biochim. Biophys. Acta 491 (1977) 269.
11 R.M. Epand, Biochim. Biophys. Acta 471 (1977) 412.
12 A.J.S. Jones, R.M., Epand, K.F. Lin, D. Walton and W.J. Vail, Biochemistry 17 (1978) 2301.