The Influence of Cytotoxins from Central Asian Cobra Venom and Melittin from Bee Venom on the Thermodynamic Properties of Phospholipid Bilayer

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Abstract. The interaction of cytotoxin V_c1 and V_c5 from Central Asian cobra and melittin from the bee venom with multilayer liposomes prepared from dimiristoylphosphatidylcholine with an addition of phosphatidic acid have been studied by the method of differential scanning calorimetry. Incorporation of V_c1, V_c5 and melittin into the lipid resulted in pronounced changes in the thermodynamic properties of the lipid. Polypeptides studied induced lateral phase separation in the lipid. Interaction between molecules of the toxins and the lipid resulted in the formation of a new lipid phase characterized by a higher melting temperature and lower phase transition enthalpy.

Key words: Cytotoxin — Phospholipid — Bilayer membranes — Calorimetry — Enthalpy

Introduction

Cytotoxins V_c1 and V_c5 from Central Asian cobra venom (m.w. about 7000) and melittin from bee venom (m.w. 2840) are polypeptides. They are considered to be membrane-active substances (Grishin et al. 1974; Grishin et al. 1976; Dufourcq et al. 1978). The cytotoxic effect of these polypeptides has been shown to be related to the changes in the permeability of cell membranes (Condrea 1974; Dufourcq et al. 1977). However, the molecular mechanism of these toxins' action on cell membranes has not been sufficiently studied, particularly in relation to structural changes in lipid matrix of cell membranes induced by these toxins. The latter may be investigated on model lipid membrane systems. By studying the interaction of cytotoxin V_c5 and melittin with lipids using the method of spin

labels, the hydrophylic fraction of polypeptide molecule was shown to be exposed to aqueous medium, whereas the hydrophobic fraction is apparently submerged in the phospholipid bilayer, thus decreasing the mobility of its hydrocarbon chains (Salakhutdinov et al. 1981; Aripov et al. 1984; Williams et al. 1972). Such interaction is accompanied by the formation of an immobilized lipid (Salakhutdinov et al. 1981; Aripov et al. 1984; Williams et al. 1972; Megner et al. 1973; Ksenzhek et al. 1978).

The aim of the present study is to investigate the structural changes in membranes made of dimiristoylphosphatidylcholine (DMPC) induced by cytotoxins and melittin using the technique of scanning microcalorimetry.

As has been shown earlier, cytotoxins are the most active on negatively charged lipid bilayers (Aripov et al. 1984), the experiments have therefore been carried out on multi-layered liposomes made of the mixture of DMPC and phosphatidic acid (PA).

Materials and Methods

DMPC (Sigma) and PA of egg lecithin (Koch-Light Laboratories, LTD) were used for the experiments. Dispersion of multilamellar liposomes was achieved according to a method described earlier (Grachova et al. 1979) in 10 mmol/l Tris-HCl buffer (pH 7.5), 1 mmol/l EDTA at lipid concentration 5×10^{-4} mol/l. Cytotoxins were obtained by means of a whole cobra venom fractionation procedure (Grishin et al. 1974) and melittin was obtained according to the method of Habermann (1972). The purity of both substances was assessed by analysing N- and C-terminals by electrophoresis in 15% PAAG (Laemmli 1970), by elctrofocusing on PAAG plates with pH gradient 3.5–9.5 and by automatic titration to determine the presence of any phospholipase activity. Lipid dispersion containing polypeptides was prepared in the following way. Polypeptide solution was added to the predried in vacuum lipid solution in ethanol. The required volume was achieved by buffer addition. Before the experiment the dispersions prepared were kept for 1 hour at 30°C. As our experiments showed this time was quite enough for complete interaction of polypeptides with a bilayer. The determination of thermodynamic parameters was carried out using DASM-1M and DASM-4 (USSR) microcalorimeters. Deconvolution of melting peaks was achieved graphically. Enthalpy values of pretransition (H_{pt}) and those of the main phase transition (H_t) corresponded to the area under each peak. The area was determined by the weight of the corresponding paper cuttings by comparing to the weight of the cuttings obtained for the heating standards. The accuracy of enthalpy determination was $\pm 8\%$. The temperature of the main phase transition (T_t) and of pretransition (T_{pt}) were determined according to the position of the maximum of the coresponding peaks. The accuracy of temperature determination was $\pm 0.2\%$ C.

Results and Discussion

Figure 1 shows the endotherms of DMPC (curve 1) and of DMPC + PA mixtures (curves 2—5). The thermodynamic parameters (T_t, T_{pt}, ΔH_t, ΔH_{pt}) as well as the width of the main phase transition peak at its half-height ($t_{1/2}$) are

presented in Table 1. The results obtained show that a rise in PA concentration in the mixture from 1 to 6 mol/% leads to a slight increase in T_t, to the wider peak of a main transition (increase in $t_{1/2}$) and to some increase in ΔH_t. At the same time both T_{pt} and the pretransition enthalpy values (ΔH_{pt}) decrease markedly. Thus, pretransition at high PA content is not clearly marked, and the peak may totally disappear.

Table 1. Thermodynamic parameters of the mixture of dimyristoylphosphatidylcholine and phosphatidic acid (PA)

C_{PA} mol %	T_{pt}, °C	T_t, °C	H_t kcal/mole	H_{pt} kcal/mole	$t_{1/2}$, °C
0	14.7	23.9	5.40	0.55	0.8
1	13.2	23.9	6.25	0.22	0.9
2	12.7	24.2	6.10	0.18	1.0
3	11.9	24.2	6.13	0.15	1.1
6	—	24.2	6.17	—	1.3

Studying the changes in thermograms of DMPC + PA mixtures after addition of cytotoxin V_c5, the initially absent pretransition peak (Fig. 2) has been shown to appear and to be maintained within a concentration range of the given toxins of 1.25—5 mol/%. At a higher polypeptide content in the mixture the pretransition peak disappears again. The pretransition peak has not been observed in case where both cytotoxins were added to DMPC + PA 6 mol/% mixture (Fig. 3, 4) (as well as in the case of adding cytotoxin V_c1 to DMPC + PA 4 mol/%). Addition of melittin to DMPC + PA 6 mol/% mixture (Fig. 5) did not result in a reappearance of the pretransition peak.

There are several explanations for the nature of the endothermal pretransition peak occurring at the temperature of pseudocrystalline (gel) lipids state. The most simple of these appears to be the following: this thermal effect is related to a turn of the axis of aliphatic chains of lipid molecules, i.e. they turn to become perpendicular to the bilayer plane (Chapman 1974; Luzzati et al. 1974; Rand et al. 1975; Hui 1976). The tilted position of the chains ensures a match of the areas occupied by the polar groups of DMPC molecules in the bilayer plane with the areas of their hydrocarbon chains projection on the bilayer plane. This leads to more dense, i.e. (quazi-hexagonal) packing (an orthorhombic one) (Janiak et al. 1976). The change in the position of the chains, i.e. their transition to a position normal to bilayer plane is believed to lead to less dense (hexagonal) packing. There is a further explanation that relates pretransition to the rippling of bilayers (Luna et al. 1977), and there is also a possibility for pretransition to be determined to some extent by a reorientation

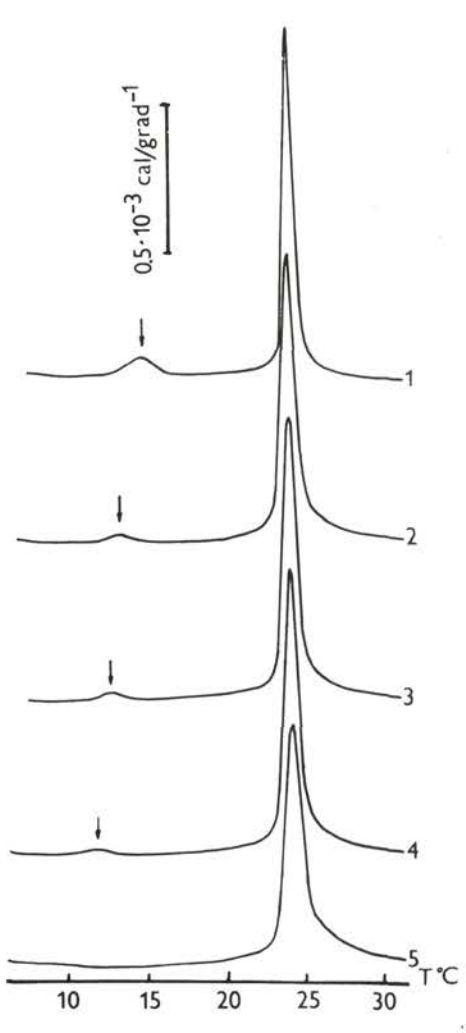

Fig. 1. Endotherms of the mixture of dimiristoylphosphatidylcholine and phosphatidic acid. DMPC concentration is 5×10^{-4} mol/l. PA concentration in mol% for the curves: 1—0; 2—1; 3—2; 4—3; 5—6.

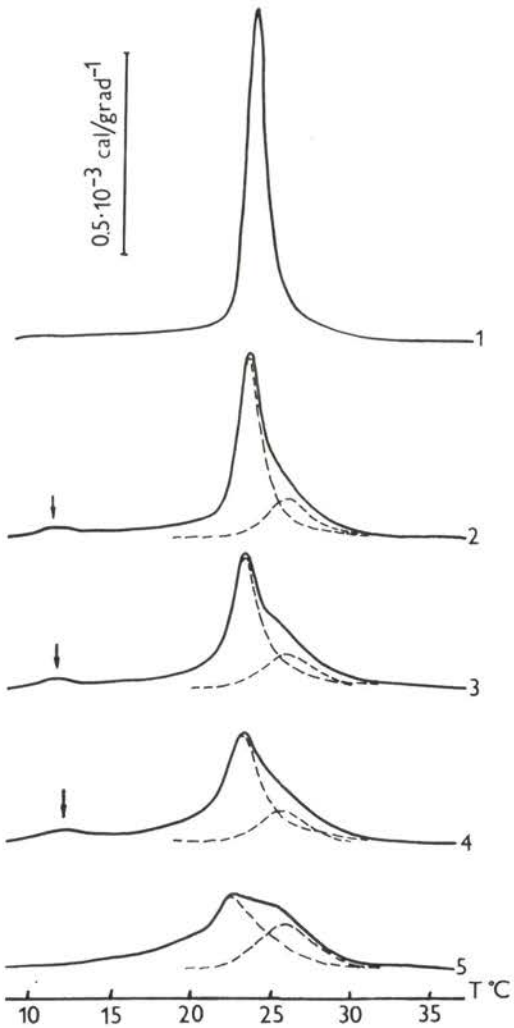

Fig. 2. Endotherms of the mixture of DMPC + PA 4 mol% with the addition of cytotoxin V_c5. Cytotoxin content in the sample is in mol%: 1—0;2—1; 25; 3—2.5; 4—5; 5—10.

of the polar groups in phospholipid bilayers (Lee 1977). It is notable that relatively weak thermal effects corresponding to pretransition are highly sensitive to the presence of any "foreign" components in an individual lipid bilayers.

Table 2. Thermodynamic parameters of the mixture of dimyristoylphosphatidylcholine and phosphatidic acid in response to the addition of cytotoxines V_c1, V_c5 and melittin

Mole ratio polypeptides/ /lipids	DMPC + PA (6 mol %) + V_c1							DMPC + PA (6 mol %) + V_c5		
	ΔH kcal/mole	$\Delta H/\Delta H_o$	S' cm^2	S'' cm^2	$\Delta H''$ kcal/mole	T_t' °C	T_t'' °C	ΔH_t kcal/mole	$\Delta H/\Delta H_o$	S' cm^2
0.0	5.95	1.0	16.65	—	—	23.9	—	5.95	1.00	16.65
0.002	5.20	0.87	12.32	2.16	3.03	24.0	25.6	5.95	0.94	13.04
0.004	5.12	0.86	11.92	2.33	3.00	23.8	25.7	5.45	0.92	11.95
0.01	4.8	0.81	10.65	2.75	2.78	23.5	25.6	5.04	0.85	10.14
0.0125	4.72	0.79	10.03	3.25	2.90	23.8	26.2	4.44	0.75	8.72
0.025	4.45	0.75	8.23	4.16	2.97	23.9	26.4	4.82	0.81	9.33
0.05	4.85	0.82	9.15	4.35	3.49	23.8	26.4	4.01	0.67	8.05
0.1	4.72	0.79	8.63	4.48	3.39	23.9	25.5	4.39	0.74	8.70
mean $\Delta H''$					3.08 ± 0.26					

Note: S' and S'' — the area of the peaks obtained by graphic separation (see Fig. 1—5)

Table 2. — continued

DMPC + PA (6 mol %) + V_c5				DMPC + PA (6 mol %) + melittin						
S'' cm^2	$\Delta H''$ kcal/mole	T_t' °C	T_t'' °C	ΔH_t kcal/mole	$\Delta H/\Delta H_o$	S' cm^2	S'' cm^2	$\Delta H''$ kcal/mole	T_t' °C	T_t'' °C
—	—	23.9	—	6.15	1.00	17.2	—	—	23.9	—
2.53	4.30	23.9	25.6	5.75	0.93	14.88	1.78	3.81	24.0	26.3
3.24	4.17	23.9	25.5	5.65	0.92	13.70	2.00	3.60	23.8	26.1
3.90	3.62	23.5	25.6	5.59	0.91	13.63	1.92	3.39	23.9	26.3
3.66	2.79	23.8	26.0	5.67	0.89	13.39	2.11	3.52	24.0	26.4
4.07	3.36	23.8	26.3	4.60	0.75	10.62	2.17	1.95	23.9	27.1
3.12	2.18	23.6	26.8	4.28	0.70	6.19	5.73	3.24	23.1	26.4
3.53	2.67	23.8	26.6	4.49	0.73	6.30	6.16	3.51	21.9	25.9
	3.30 ± 0.79							3.29 ± 0.20		

The decrease in pretransition enthalpy in response to increasing concentration of PA in the DMPC mixtures, as well as the pretransition disappearance at the 5—6 mol/% PA concentration range may reflect the fact that the presence of even a relatively small amount of this substance results in the loss of pseudohexagonal hydrocarbon chain packing of maximum density (or in bilayer folding), this excludes the transformations characteristic for the pretransition. It is also possible that PA addition sharply decreases cooperativity of orientation

changes of the DMPC chains of fatty acids. Thus, the temperature range of pretransitions expands and scanning calorimetry can not be used to monitor thermal effects. Data on the changes in packing reflex of X-rays scattering at large angles are needed to see which of the above hypotheses may be correct.

The reappearance of the pretransition peak in the case of addition of cytotoxin V_c5 to the DMPC + PA 4 mol/% mixture (Fig. 2) suggests a certain interaction of this peptide with PA. This interaction appears to be quite specific

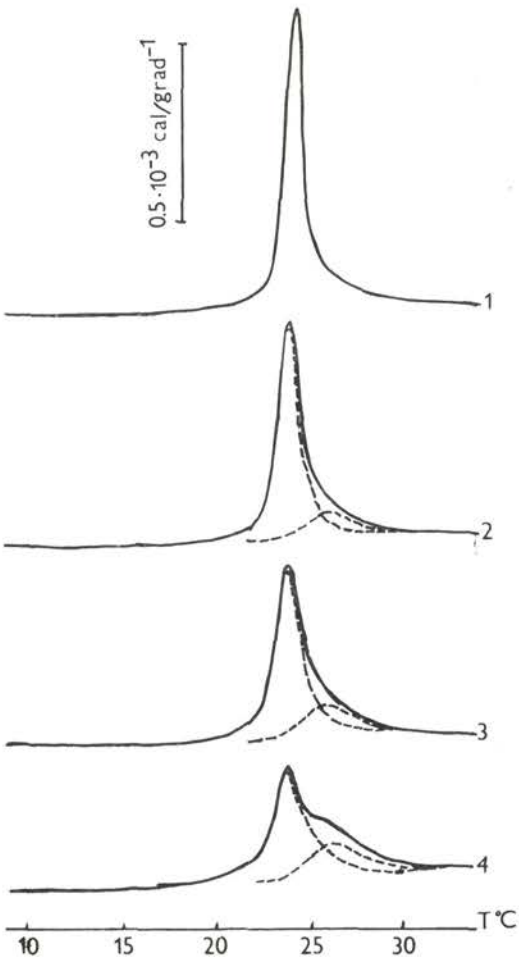

Fig. 3. Endotherms of DMPC + PA 6 mol% mixture with the addition of cytotoxin V_c1. Cytotoxin content in the sample is in mol%: 1—0; 2—0.4; 3—1; 4—10.

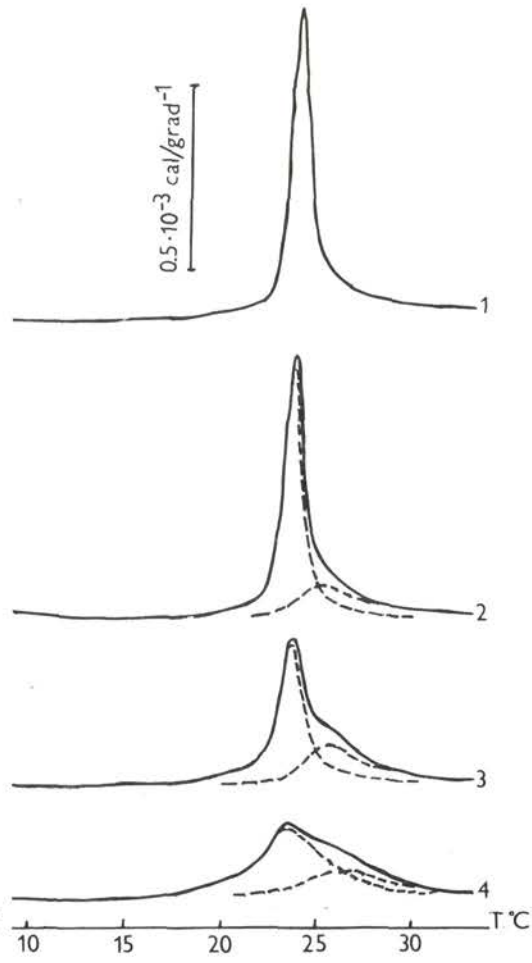

Fig. 4. Endotherms of DMPC + PA 6 mol% mixture with the addition of cytotoxin V_c5. Cytotoxin content in the sample is in mol%: 1—0; 2—0.4; 3—1; 4—10.

and can not be considered as a mere interaction between positively charged polypeptide molecules and negatively charged PA molecules, as the pretransition restoration has not been observed if positively charged cytotoxin V_c1 and melittin were also added to similar mixtures of lipids.

In relation to the effect of cytotoxins and melittin on main phase lipid transition, changes in thermogram patterns induced by the addition of these polypeptides to DMPC + PA 6 mol/% mixture are given in Figures 2—5. Table 2 summerizes the values of thermodynamic parameters of the main phase transition for the same systems.

The common feature for all three polypeptides studied appears to be the increasing changes in the shape of the main phase transition peak if their content in the mixture goes up. At a molar polypeptide/lipid ratio of less than 1/100 only an asymmetry of the main phase transition has been noticed: high polypeptide concentrations giving rise to a shoulder on the main descending transition branch, while at maximal polypeptide concentration there is an obvious separation of the peak into two components. The maximum position of one of them on the temperature scale (T_t') is slightly shifted towards lower temperatures compared to T_t values for DMPC + PA mixture in the absence of the polypeptides. This shift does not exceed 0.4°C for DMPC + PA 6 mol/% systems, containing cytotoxins V_c1 (Fig. 3) and V_c5 (Fig. 4), but it may go up to 0.8—2.0°C when a relatively large amount of melittin (5 to 10 mol/%) is added. A difference of T_t and T_t' of 1°C has been observed when the cytotoxin V_c5 (concentration 10 mol/%) was added to the DMPC + PA 4 mol/% mixture. Using the technique of graphic deconvolution for the main transition component (Figures 2—5) it is possible to determine T_t'' values, i.e. the maximum position of the second "high-temperature" peak component even for cases when only distortion of the descending branch of the peak is observed. The values of T_t'' (Table 2) are constant within the limits of the determination accuracy. The mean value of T_t'' (25.9 ± 0.3)°C (mean $\pm$ S. D ... $n = 21$) coincides for all three polypeptides. This observation indicates that the addition of cytotoxins and melittin leads to a lateral phase separation in the bilayer and to the formation of a specific lipid structure, probably identical for all three polypeptides studied.

Another effect of the addition of cytotoxins and melittin to the mixture was a decrease in main phase transition enthalpy. As ΔH_t values for the mixtures of 4 and 6 mol/% (in the absence of polypeptides) were different and in view of the fact that they did differ slightly for various series of experiments at constant PA concentration (Table 2), the appropriate way to present enthalpy changes in the case of polypeptide addition is that represented by Fig. 6*a*, i.e. in relation to the relative values of $\Delta H/\Delta H_o$, where ΔH is the main phase transition enthalpy at given molar polypeptide content and ΔH_o is the transition enthalpy in the absence of polypeptide for the given series of measurements.

As may be seen in Fig. 6*a*, an initial increase in the polypeptide molar ratio up to 0.02—0.05 induces a marked decrease in total enthalpy of the main phase transition, but a further increase in polypeptide concentration in the mixture is no longer accompanied by changes in enthalpy. The curves in Fig. 6*a* show the changes in ΔH values to be less significant at lower PA mixture content. At the 6 mol/% PA value certain difference in maximum values of $\Delta H/\Delta H_0$ for three peptides studied have been observed. The greatest changes correspond to the addition of cytotoxin V_c5 and melittin to the mixture, though the steepness of changes developing at small concentrations of polypeptides in the mixture is the highest for cytotoxin V_c1.

The curve $\Delta H/\Delta H_0$ which is characteristic for the samples containing melittin within the range of molar ratio polypeptide-lipid 0.002—0.0125 may be

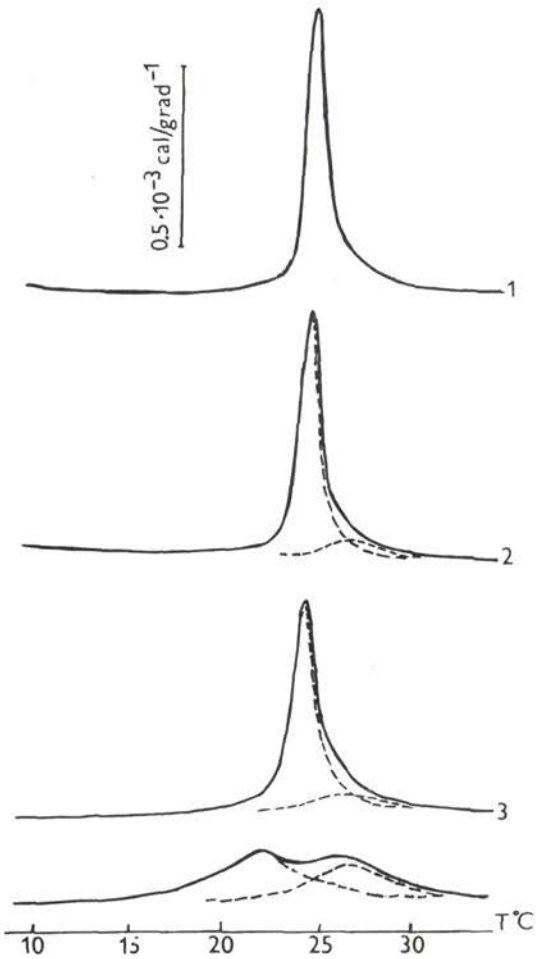

Fig. 5. Endotherms of DMPC + PA 6 mol% mixture with the addition of melittin. Melittin content in the sample is in mol%: 1—0; 2—0.2; 3—1; 4—10.

explained in terms of the oligomerization of melittin molecules (Ksenzhek et al. 1984), leading to an increase in membrane activity.

The decrease in the main transition enthalpy when the cytotoxins and melittin are added to lipid indicates that the new phase characterized by the transition temperature T_t'' shows small thermal effects during gel-liquid crystal phase transition. The decrease in ΔH may, however, also be the consequence of an exclusion of some lipid molecules adjoining the polypeptide molecules or of their aggregates incorporated into lipid bilayer from the transition process. Such lipid excluded from the phase transition process is often called "boundary lipid", "perturbed lipid" or "entrapped lipid".

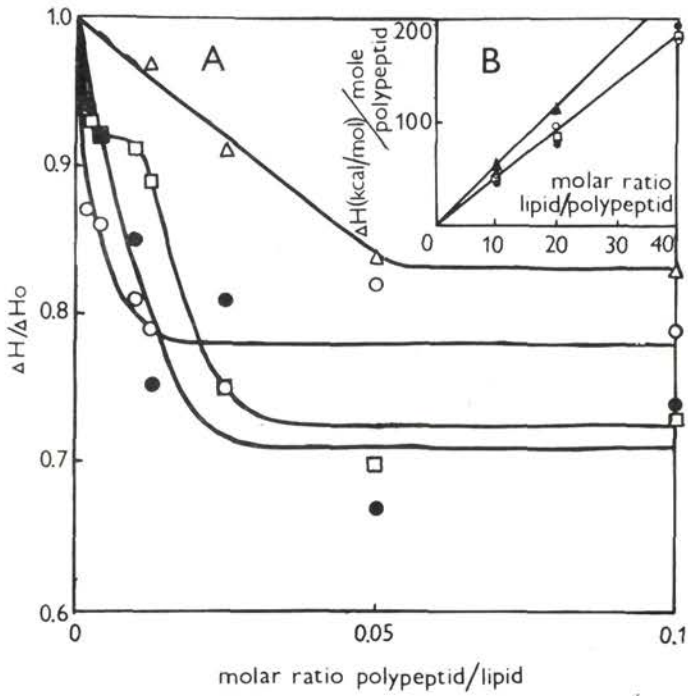

Fig. 6*a*. The dependence of ΔH enthalpy of the main phase transition on the concentration of polypeptides for DMPC + PA 4 mol% + cytotoxin V_c5 (△); DMPC + PA 6 mol% + cytotoxin V_c5 (●); DMPC + melittin + PA 6 mol% (□); DMPC + PA 6 mol% + cytotoxin V_c1 (○). **b.** The dependence of changes in the ratio ΔH/mole of polypeptides on the mole ratio lipid/polypeptide (details are in Fig. 6*a*). See the text for further explanation.

The fact that there are plateaus in the curves shown in Fig. 6*a* at molar polypeptide concentration greater than 0.01—0.05 (while $\Delta H/\Delta H_o$ values remain quite high) indicates clearly that an exclusion of the predominant lipid

fraction from the phase transition in the case of an infinite increase, i.e. increase in polypeptide mole fraction, can not take place. To ensure that the lipid has not been excluded from the process of phase transition and to estimate the value of lipid domain surrounding polypeptide molecules and characterized by the altered properties (particularly by low ΔH values), it is appropriate to plot total transition enthalpy against the lipid mole fraction in the mixture using the coordinates of Lentz and co-workers (Lentz et al. 1983). These coordinates differ from those in Fig. 6*a* in terms of inverse values of mole ratio lipid/polypeptide on the abscissa and in the values of ΔH/mole of polypeptide on the ordinate. Fig. 6*b* shows these curves for the region of relatively small values of lipid-polypeptide mole ratios. They demonstrate with a ratio of less than 40 the slope of the curves (for the mixtures containing 6 mol/% of PA) practically coincide and their value is in good agreement with the value of the total transition enthalpy, which is close to 4.5 cal/mole of peptide. This is considerably less than ΔH values determined from the slope of similar curves at lower polypeptide content in the mixture (5.3—5.8 kcal/mole of peptide). When the curves in Fig. 6*b* belonging to the region of low values of lipid/polypeptide ratio cross abscissa at its zero points, one can use this method of data analysis for the conclusion about the absence of "boundary lipid", the lipid excluded from the process of phase transition. This lipid can be clearly seen in case of the interaction with Ca^{2+} — Mg^{2+}-dependent ATP-ase with dimiristoylphosphatidylcholine (Lentz et al. 1983).

The experimental data presented above indicate that changes occurring in lipid in response to the addition of cobra venom cytotoxins and melittin exhibit similar relationships between the characteristics of their phase transition and the mole fraction of the polypeptides compared. Furthermore, the specific lipid phase induced by cytotoxins is similar to that induced by melittin. This phase in all cases is characterized by a phase transition temperature $T_t'' = 25.9°C$. Tentatively, it may be proposed that lateral phase separation leads to the formation of a system including only two components: lipid possessing the properties altered by the presence of polypeptide and unaltered lipid with the properties found in the absence of polypeptide.* The total phase transition enthalpy may therefore be presented by the following expression:

$$\Delta H_t = n \,.\, \Delta H_o' + (1 - n) \,.\, \Delta H''$$

where n and $(1 - n)$ are, respectively, mole ratios of the unaltered lipid with the main phase transition enthalpy ΔH_o and the lipid in the state altered by polypep-

*) This may be assumed except in cases when considerable change in T_t' values relative to T_t has been observed.

tide incorporation with the transition enthalpy value $\Delta H''$. In this case $\Delta H''$ can be easily determined by comparing the thermograms obtained for the system with varied polypeptide content (Table 2). In spite of the variability of these data there is a degree of similarity in the mean values of $\Delta H''$ for the three polypeptides. None of these, however, shows the relationship between H'' and the mole fraction of polypeptide in the system. Taking into consideration the identity of T_t'' values for the peptide studied, it is reasonable to assume the presence of the common features for the interaction of these substances with lipid. As the correspondence of the above mentioned peculiarities of the interaction of cytotoxins and melittin is unlikely to be random, it is worth considering experimental data concerning melittin while discussing the nature of the changes occurring in lipids. The molecules of this substance are known to carry 6 positive charges, they appear to be monomers at low concentrations in monolayers on the water-air interface. Under these conditions one can see abnormally high values of the product of surface pressure (π) and the area occupied by the molecule (πA): $\pi A \gg kT$. The latter points to the considerable electrostatic repulsion of melittin molecules which is apparent in cases whereby these interactions occur through media with a low dielectric constant (Ksenzhek et al. 1978; Schoch et al. 1980; Tosteson and Tosteson 1981). In relation to a consideration of monolayers at the water-air interface as a model of a "half" of biomolecular lipid membrane (i.e. one of the two monolayers forming the bilayer), then Coulombic interactions may be assumed to occur mainly through the hydrophobic bilayer zone to meet the conditions of the interactions between the monomers of melittin molecules incorporated in bilayers structure. This may take place only in the case that the charged group (most probably Lys^7) is submerged in the hydrocarbon chain zone of the bilayer, thus influencing the lipid state. This leads to a disappearance of pretransition and to a decrease in the total enthalpy of the main transition. This is clearly shown by the data presented in Figures 5 and 6 and in Table 2.

The increase in melittin concentration in monolayers results in oligomerization of this substance. The formation of melittin tetramers is characterized by a lower degree of ionization of its charged groups and by such changes in molecular arrangement that render these groups perpendicular to the plane of the phase boundary (Ksenzhek et al. 1984; Tosteson and Tosteson 1981). The factors mentioned above determine the most probable location of the polypeptide molecules in hydrophobic zone of the bilayer (Schoch et al. 1980; Tosteson and Tosteson 1981). Melittin molecules incorporated in lipid bilayer most probably exist in a tetrameric form (Gevod et al. 1984). The changes in the position of melittin molecules relative to the bilayer plane are accompanied by the penetration of tetramers into the zone occupied by hydrocarbon lipid chains. According to the literature on these monolayers such changes in the position of

melittin molecules take place in a narrow range of surface concentrations of the substance (Birdi et al. 1983; Ksenzhek et al. 1984), they are clearly characterized by a high degree of cooperativity (one of the collapses seen on the π-A curves). The penetration of melittin deep into the bilayer should induce more profound perturbations in lipid structure. This is probably observed in the case of lateral phase separation in the presence of melittin. Moreover, the appearance of new lipid state (DMPC + PA) is apparent. It is characterized by a phase transition temperature $T_t'' = 25.9°C$ and by transition enthalpy with an average value of 3.3 kcal/mole of lipid. It is also known that in the presence of electrolytes melittin tetramers form ion channels in lipid bilayers that are characterized by high ion selectivity (Tosteson and Tosteson 1981; Ksenzhek et al. 1984). The latter points to the presence of positively charged groups in the structure of these channels. The charged groups of the polypeptide are supposed to be mainly located in the region of bilyer polar groups. However, the ionization of Lys^7 amino group leads to charge transfer into the hydrophobic zone and if this charge is not properly compensated by anions coming from aqueous phase, predictable considerable Coulomb repulsion of tetramers is due to charge location in media with a low dielectric constant. In this case a limitation in tetramer accumulation in the bilayers can be easily explained, hence, the limited changes in lipid state with increasing polypeptide concentration. These considerations are in good agreement with the data shown in Fig. 6*a*.

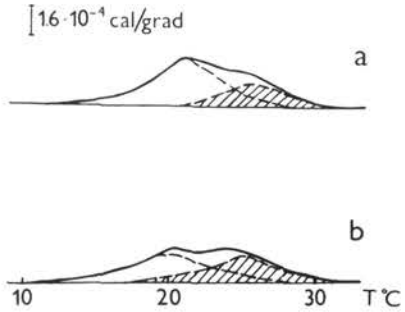

Fig. 7. Endotherms of DMPC + PA 6 mol% mixture in the presence of melittin (2.5 mol%). *A*) ionic strength of the medium 10^{-2} equiv. *B*) the same at ionic strength 6×10^{-2} equiv. (0.05 mol/l NaCl was added to the medium). Cross-hatched area is the thermal effect of "altered" lipid melting. The portion of the thermal effect of the "altered" lipid melting increased 1.7 times in response to higher ionic strength of the dispersion medium.

The increase in the fraction of endotherms related to the lipid altered by the presence of melittin, with an increase of the ionic strength of the dispersion medium presented in Fig. 7, indicates that the screening effect of electrolyte (NaCl) considerably enhances melittin induced alterations in the lipid probably by diminishing electrostatic repulsion and thus facilitating the incorporation of an additional amount of this polypeptide into the lipid bilayers. Obviously, the screening effect is possible only if the charged groups of polypeptide molecule are accessible to counter ions, i.e. this is evidence against the dislocation of these charges deep in the hydrophobic zone bilayer.

The data obtained in the present study show the similarity in lipid changes induced by melittin and cobra venom cytotoxins, in particular, a prominent saturation of the curves representing the total enthalpy dependence on the concentration of these polypeptides in the systems (Fig. 6*a*) indicates that in these cases strong electrostatic interaction between fixed charges of polypeptides takes place. Such a conclusion is in good agreement with the results of investigations of the interaction of cytotoxin V_c5 with bilayers using the spin-probe technique (Aripov et al. 1984).

At present there is no unified classification of changes in the thermodynamic characteristics of lipid phase transitions associated with the properties of proteins and polypeptides incorporated into bilayers. According to a comparatively simple scheme proposed by Papahadjopoulos and co-workers (1975) the incorporation of hydrophobic polypeptides and proteins (gramicidin A and apoprotein N-2 of human myelin) is considered to lead to the pretransition disappearance, the main transition peak widening and to a decrease of the main transition enthalpy occurring without a change in T_t values. The incorporation of comparatively hydrophilic polypeptides and proteins bearing uncompensated charges (i.e. polylysine and pancreatic ribonuclease) results in pretransition disappearance, together with an increase in main transition enthalpy and unaltered or slightly increased T_t*). According to this scheme pretransition disappearance as well as the decrease in the main transition enthalpy and the decrease in T_t should be observed for the cases of the interaction of lipids with proteins of intermidiate hydrophobicity (e.g. basic myelim protein-AI and cytochrome C). The complex of characteristic changes in the lipid phase transitions described in the present paper can be easily seen not to agree with any of the tenets of the scheme given above. The elements of the detected changes belong to different groups. Thus, the characteristic effect of the incorporation of cytotoxins and melittin into lipids (i.e. lateral phase separation providing a separation of a component of the main phase transition possessing a higher temperature compared to T_t of pure lipid) has been detected in the work cited (Papahadjopoulos et al. 1975) in the case of polylysine interaction with DMPC, although in the latter case a decrease in total transition enthalpy has not been registered. The changes described in the present paper on the one hand agree with the effects induced by the incorporation of a charged polypeptide and, on the other, agree with transformation conditioned by typical hydrophobic polypeptide-gramicidin A and apoprotein N-2. Nevertheless, the incorporation of

*) Judging by the thermograms in (Papahadjopoulos et al. 1975) the interaction of polylysine with DMPC leads to a distinct lateral phase separation and the new component of the main transition possesses T_t values much higher than those in the absence of polylysine.

the latter into DMPC leads to the appearance of the component in thermograms that has T_t coinciding with $T_t'' = 25.9°C$ established in the present work.

Surewicz and Epand (1985) showed that the substitution of even one amino acid residue in pentagastrin leads to considerable changes in the interaction with DMPC bilayer. Hydrophobic pentagastrin analogues do not cause pronounced changes in T_t and widen the transition peak simulteneously with the enthalpy decrease. Charged pentagastrin analogues alter DMPC phase behaviour, induce the transition components appearance at a temperature higher and lower than T_t. These effects are most vividly seen in case of arginine-containing analogue. The authors think that amino acid sequence is of importance for understanding the nature of peptide/lipid interaction.

In the present work not only the common features of the two studied toxins and melittin concerning their effect on lipids have been observed but also certain quantitative differences. The nature of these differences is presently unclear. More precise knowledge of the three-dimensional structure of the cytotoxins V_c1 and V_c5 and of their physico-chemical properties will help us to understand further the nature of protein/lipid interactions.

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