EFFECT OF MELITTIN AND MELITTIN FRAGMENTS ON THE THERMOTROPIC PHASE TRANSITION OF DIPALMITOYLLECITHIN AND ON THE AMOUNT OF LIPID-BOUND WATER

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Received 20 February 1976

1. Introduction

Melittin is the main cytolytic component of bee venom [1]. The first twenty amino acids of this peptide are mostly apolar whereas the C-terminal hexapeptide is polar and highly basic. The biological activity of melittin has been studied by biochemical and biophysical methods [2–9].

Changes in the physical state of membrane phospholipids have been postulated to cause the biological effects of melittin such as increased susceptibility of lipids towards phospholipases A_2 [3,7,8], and at higher peptide concentrations, complete lysis of cells [2]. Melittin interacts with phospholipids both in liposomes [6] and in biomembranes [6,8]. According to recent evidence, this interaction causes a decrease in the mobility of the fatty acid chains and a rearrangement of the headgroup of phosphatides [5].

The hydrophobic segment of melittin, corresponding to the first nineteen residues (designated mel_{1-19}) and the basic heptapeptide (mel_{20-26}) can be isolated. The effects of melittin and of these characteristic fragments on the gel to liquid crystalline phase-transition of dipalmitoylphosphatidylcholine and the amount of lipid bound water are described in this paper.

Abbreviations: DPPC: 1,2-Dipalmitoyl-L-3-glycerylphosphorylcholine; DSC: Differential Scanning Calorimeter.

2. Materials and methods

1,2-Dipalmitoyl-L-3-glycerylphosphorylcholine from Koch-Light (England) was found to be chromatographically pure and used without further purification. Melittin was prepared from whole bee venom (Mack, Illertissen, Germany) as described recently [8]; the fragments of melittin, mel_{1-19} and mel_{20-26}, by digestion with chymotrypsin (0.4 mg enzyme per 75 mg melittin, [9]). Mel_{1-19} was purified by chromatography (butanol–acetic acid–water, 4:1:2, v/v/v, Whatman 3MM paper) and mel_{20-26} by high voltage paper electrophoresis (1% pyridine acetate buffer, pH 4.8, 50 V/cm, 90 min, Whatman 3MM paper).

Per sample pan, 2 μmol DPPC were dried from chloroform–methanol (2:1, v/v) in a desiccator. To the dry lipid 5 μl water or an appropriate peptide solution were added. The sealed sample pans were kept for 1 h at 45°C to allow melittin to interact with DPPC. The fluorescence emission maximum of the single tryptophan residue of melittin is shifted to the blue upon binding of melittin to phospholipids [6]. This transfer of the residue to an apolar environment can only be observed with lipids of the liquid-crystalline state. However, it was found that on cooling the melittin phospholipid complex below the phase transition temperature, no red shift takes place, indicating that the peptide remains bound to the lipid.

Scans were performed at a speed of 4 degrees per minute. The range setting for full scale deflection

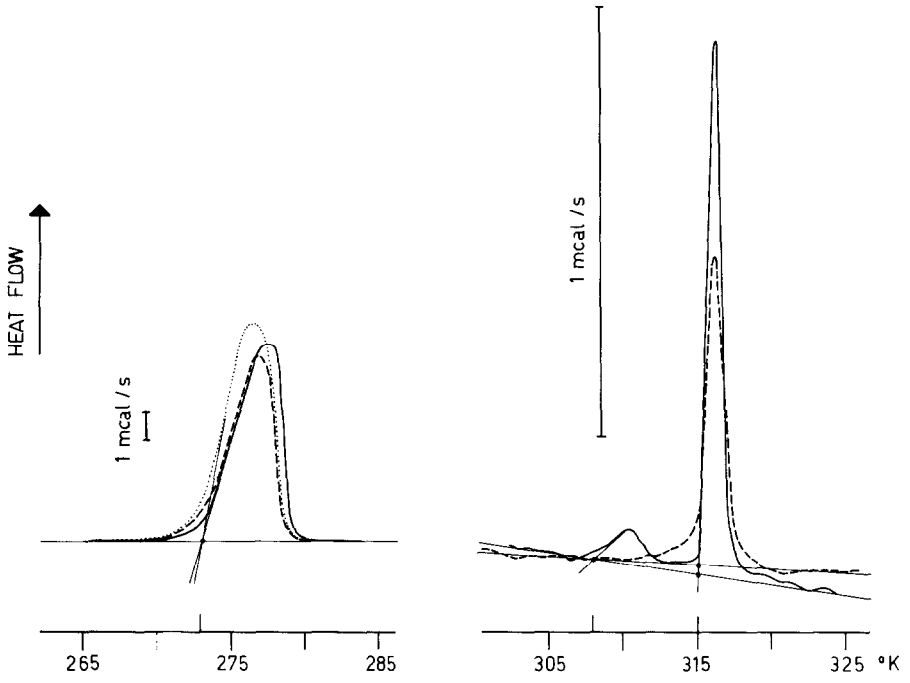

Fig.1. DSC scans of water, DPPC in excess water and DPPC plus melittin in excess water. 5 μl water (. . . . .); 2 μmole DPPC in 5 μl water (———) and 2 μmol DPPC plus 0.04 μmol melittin in 5 μl water (- - - - -).

was 2 and 32 mcal/s for phospholipid and water transitions, respectively. The onset temperature of a peak was defined as the temperature of transition [10]. To calculate the enthalpy of transition, peak areas were measured with a planimeter. Values presented here are derived from second scans only. The instrument used was a Perkin-Elmer DSC-1B.

3. Results

DSC scans of DPPC in excess water in the presence and absence of melittin are shown in fig.1.

In the presence of melittin, the pretransition at 308°K disappears; only at low peptide concentrations (1 mol peptide per 250 mol DPPC) a flat peak can be seen occasionally. Melittin fragments have been used to test whether this effect could be correlated with the interaction of any particular region of the peptide with the phospholipid. It is, however, difficult to quantify the pretransition because of the unstable baseline. Nevertheless, it seems to be quite clear that the hydrophobic fragment mel_{1-19} decreases the area of the pretransition by some 30% while the basic fragment mel_{20-26} has no detectable effect.

The main transition from the gel to the liquid crystalline state takes place at 315°K. In the presence of melittin the transition proceeds over a wider temperature range but the onset temperature remains unchanged. However, the area of the peak is reduced continuously with increasing peptide concentrations (fig.2). It is reasonable to assume that the observed enthalpy represents the mole fraction of DPPC, which is not influenced by melittin. From the difference between the enthalpy found in the presence and absence of the peptide it can be calculated that per melittin molecule about ten lecithin molecules are prevented from participating in the cooperative phasetransition. The fragment mel_{20-26} also decreases the transition peak. On the other hand, in the presence of the fragment mel_{1-19}, the heat of the transition corresponds to the enthalpy of pure DPPC (see fig.2).

It is known that phosphatides bind water [11–13]

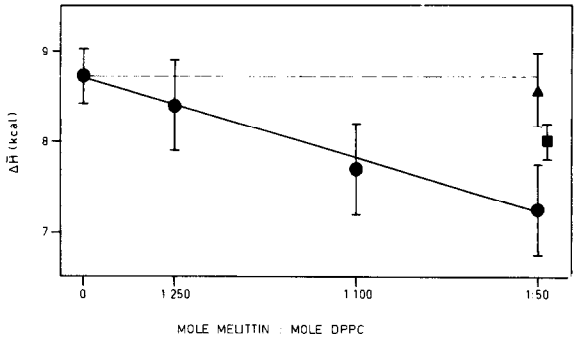

Fig.2. The enthalpy of the gel to liquid crystalline phase-transition apparent for 1 mol DPPC. $\Delta\bar{H}$ found in the presence of increasing amounts of melittin (●); the hydrophobic fragment mel_{1-19} (▲) and the basic fragment mel_{20-26} (■).

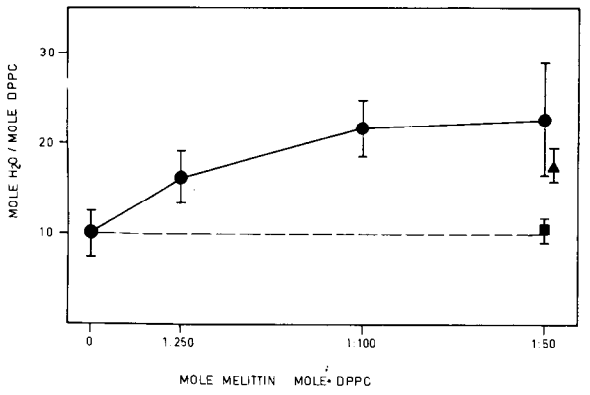

Fig.3. The amount of water bound to DPPC. Moles non-freezing water found per mole DPPC in the presence of melittin (●); the hydrophobic fragment mel_{1-19} (▲) and the basic fragment mel_{20-26} (■).

and that this water will not freeze [11]. From the differences between the transition peak areas of pure water and the same amount of water in a lipid–water or a lipid–peptide–water system, the amount of non-freezing water can be calculated. Melittin itself has no measurable effect on the size of this peak. In the presence of DPPC, the peak area decreases to an extent amounting to ten molecules of bound water per lipid molecule [11,12]. Upon addition of melittin, the area of the peak decreases still further. Fig.3 summarizes the change in the amount of lipid bound water with increasing melittin concentrations. A maximum value of 20–23 mol water per lipid molecule is found at a molar ratio of one melittin per hundred DPPC. Again, it was tested whether this increase of lipid-bound water could be obtained with a fragment of melittin. These experiments have shown that the hydrophobic segment mel_{1-19} can to a certain extent mimic this effect, whereas the basic fragment mel_{20-26} is inactive in this respect (see fig.3).

4. Discussion

The biological activity of melittin is based on its ability to interact with phospholipids [2–8]. The microcalorimetric data presented in this communication have shown that melittin has a pronounced effect on the pre- and main transitions of DPPC and on the amount of lipid-bound water. By using fragments of melittin it could be shown that different regions of the peptide are responsible for these effects.

Melittin and its basic, C-terminal fragment mel_{20-26} were found to decrease the heat content of the main transition of DPPC. Therefore, the polar, rather than the hydrophobic segment of melittin, causes an apparent 'immobilization' of a number of phospholipids [5], which no longer participate in the cooperative melting of the bulk lipid. Probably, this effect is a consequence of lipid headgroup rearrangement, which is known to occur in the presence of melittin [5] and which can conceivably be caused by an electrostatic binding of melittin to the head group.

Melittin abolishes the pretransition and increases the amount of water bound to DPPC. Both these effects are apparently based on the interaction of the hydrophobic region of melittin with the lipid, since only the fragment mel_{1-19} causes similar changes. The pretransition is now understood to represent a configurational change in the lipid structure from – in respect to the bilayer – tilted to vertically oriented hydrocarbon chains [14]. The absence of a pretransition peak in the presence of melittin may therefore indicate that the insertion of the hydrophobic part of melittin forces the fatty acid chains into the vertical configuration. As in the case of the lecithin–cholesterol–water system [11], it is likely that the increased hydration is a direct consequence of a configurational change.

These findings also provide a physical basis to

explain the stimulatory effect of melittin on the activity of phospholipases A_2 with either model or natural membranes as substrate [3,7,8]. The available data suggest that a change in the configuration and/or the hydration of the lipid molecules is a prerequisite for optimal activity of these enzymes. In this context it should be mentioned that the maxima for lipid hydration and for the stimulation of phospholipases A_2 [7,8] coincide at a melittin to lipid ratio of about 1:100 (mol/mol). The activity of bee venom phospholipase A_2, furthermore, can be stimulated by only one of the melittin fragments, that is mel_{1-19} (unpublished results), which similar to the entire peptide diminishes the pretransition and increases the amount of water bound to DPPC. This interpretation is also supported by the recent finding that lipids in the vertical configuration are the favoured substrate for phospholipase A_2 from the venom of *Crotalus atrox* [14]. Moreover, it has frequently been suggested that lipid hydration may be of relevance for the structure and function of biomembranes [11,15,16].

Acknowledgements

The Perkin-Elmer DSC-1B was generously made available by the Paracelsus Institut, Bad Hall, Austria and I am grateful to Drs H. Hellauer and R. Winkler for their advice and kind hospitality. Furthermore, I like to thank Dr G. Kreil for his encouragement throughout this work.

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