EFFECT OF MELITTIN ON PHASE TRANSITION AND FLUIDITY OF A PHOSPHOLIPID MODEL MEMBRANE

Feride Severcan
Department of Electrical and Electronic Engineering
Dokuz Eylül University, Bornova, Izmir, Turkey

ABSTRACT. The interaction of melittin, the major protein component of bee venom, with dimyristoyl phosphatidylcholine (DMPC) liposomes was investigated by Electron Spin Resonance (ESR) spectroscopy using high resolution amphiphilic spin probe perdeutero-di-t-butyl nitroxide (PDDTBN). For pure DMPC liposomes, ESR signal due to the probe dissolved in the fluid hydrophobic environment provided by the interior of the phospholipid bilayer (lipid site) is observed around 24°C which corresponds to the main gel to liquid crystalline phase transition of pure DMPC liposomes. Howewer with the addition of melittin, onset temperatures of lipid lines shift towards higher temperatures. For lipid:melittin molar ratios of 32:1 and 19:1, lipid line appears at 28°C, and for lipid:melittin molar ratios of 14:1 and 10:1, lipid site appears around 30.5°C. The results are discussed in terms of boundary lipids.

Variation with temperature of both partition coefficient and peak to peak linewidth of high field lipid lines obtained by spectral deconvolution, indicate that in the liquid crystalline phase fluidity of the DMPC bilayers decreases when melittin concentration is increased.

INTRODUCTION

Structural complexity of the membrane proteins raises an obstacle in understanding the lipid-protein interactions within the membrane. In order to decrease this difficulty, small polypeptids such as melittin, have served as a model system for protein-lipid interactions. Melittin constitutes about 50 % of the dry weight of bee venom, whose amino acid sequence starts in the N-terminal part with 20 predominantly hydrophobic amino acid residues and finishes with 6 charged and hydrophilic residues in the C-terminal part [1]. The primary structure of melittin is (+)Gly-Ile-Gly-Ala-Val-Leu- Lys(+)-Val-Leu- -Thr-Thr-Gly-Leu-Pro- Ala-Leu-Ile-Ser- Trp-Ile-Lys(+)-Arg(+)-Lys(+)-Arg(+)-Gln-Gln-NH_2.Its main property is direct membrane lysis[2].This property also makes melittin research interesting and several techniques have been applied to study the interaction of melittin with membranes.

The conformation and state of aggregation of membrane-bound melittin remain a challenging problem in several respects, since many different possibilities have been inferred. Several models for the conformation of melittin have been considered, including the wedge [3-5], the transmembrane α-helix [4], α- helix with its axis parallel to the bilayer surface [6],which all assume that the protein exists as a monomer when bound to the membrane. On the other hand, another model proposed assumes that a tetrameric pore complex of these monomers is formed [7]. Concerning the state of aggregation of melittin in lipid membranes three different conclusions appear in the literature. Some authors present evidence for a monomeric state [5,8-11], some authors present evidence for a tetrameric state [7, 12-14]. However, some authors show that both monomeric and associated species can be observed depending on experimental conditions [15,16]. Despite intensive studies on conformation and state of aggregation of melittin in lipid membranes, only a limited number of study has been performed to gain information on the dynamical aspect of the interaction between melittin and lipid membranes [17-19]. Phase transition behaviour of melittin- containing PC liposomes also

has led to somewhat confusing and conflicting results [20-25].

In the present ESR communication, in order to obtain more information about melittin-phospholipid membrane interactions, the ability of the high resolution amphiphilic spin probe PDDTBN to resolve well both in aqueous and hydrophobic fluid interior of the bilayer has been exploited [26]. Such an enhanced spectral resolution allowes one to obtain information about the effect of melittin on phase transition and fluidity of the phospholipid membranes.

MATERIALS AND METHODS

DMPC and Melittin were purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. PDDTBN was kindly provided by Prof. W. Plachy of San Francisco State University.

Multilamellar phospholipid dispersions, containing excess water (10% lipid by weight), were prepared by heating mechanically mixed lipid-water samples to 15°C above the main phase transition temperature. PDDTBN dissolved in 10 mM sodium phophate buffer at pH 7.1 (5.10^{-4}M) was used for hydration. Melittin was added to aqueous dispersion of DMPC to yield final lipid: protein molar ratios of 32:1 (3 mol%melittin), 25:1 (5 mol%melittin), 14:1 (7 mol%melittin) and 10:1 (9 mol%melittin). These mixtures were mechanically shaken for 10 min and then kept at 40°C for 1 h to allow the polypeptide to interact completely with the multilamellar liposomes.

A gas permeable teflon sample holder was used to exclude the dissolved oxygen [26].

ESR experiments were carried out on a X-band Varian E109 spectrometer. A 100-kHz modulation frequency was used for conventional, first harmonic, absorption ESR spectra. The temperature was controlled with a Varian variable temperature accessory and measured with a thermocouple in thermal contact with the sample.

RESULTS AND DISCUSSION

The ESR spectrum of a rapidly tumbling nitroxide spin probe consists of three lines corresponding to the nitrogen quantum numbers M=-1, 0, +1 and the center of these lines are separated by the nitrogen hyperfine splitting constant a_N. Most nitroxide spin probes contain twelve or more protons. This gives rise to thir-

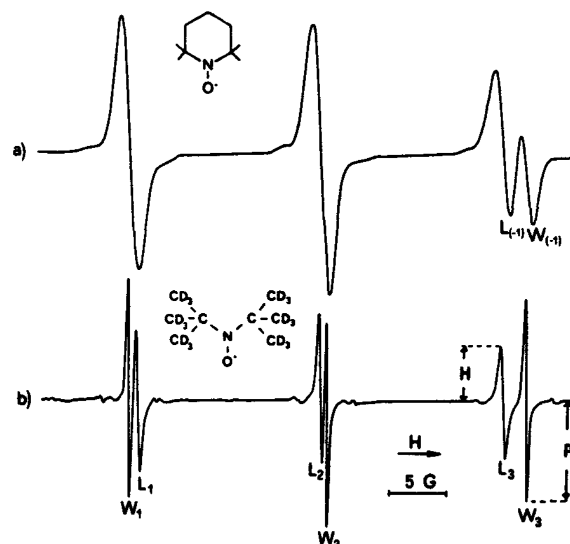

Fig.1. ESR spectrum of;Tempo(a) and PDDTBN (b) in PC multilamellar liposomes above the main phase transition temperature.

teen or more lines almost always unresolved, in each of three nitrogen hyperfine lines. The envelope of these proton hyperfine lines provides the main contribution to the experimentally determined linewidth as inhomogeneous broadening [27,28]. The effect of this unresolved structure is prevented by deuteration of the probe. Perdeuteration of the radical decreases the inhomogeneous broadening due to the smaller magnetic dipole moment of the deuteron. As a result a significant reduction in the observed linewidth can be achieved [29]. Fig.1 illustrates this effect. Pattern a and b of Fig.1 show the ESR spectrum of Tempo and PDDTBN, respectively, in PC liposomes. As seen from the figure only the high field component of Tempo can be partially resolved to Lipid (L_{-1}) and water (W_{-1}) signals. On the other hand, if PDDTBN is used, linewidth reduction may result in a higher resolution of the superimposed spectra in all three resonance lines; one ($L_{+}1$, L_O, L_{-1}) arising from PDDTBN dissolved in the fluid hydrophobic environment provided by the interior of the phospholipid bilayer and the other (W_{+1}, W_O, $W_{-}1$) arising from the spin probe dissolved in the surrounding aqueous environment. The narrow linewidth also increases the spectral sensitivity so that the mole fraction of probe can be less than 0.001 and therefore any perturbing effect is minimized. Advantage and use of PDDTBN in membrane studies have been reported in ref.[30, 31], in detail.

Fig.2 shows the ESR spectrum of PDDTBN in DMPC multilamellar liposomes just below and

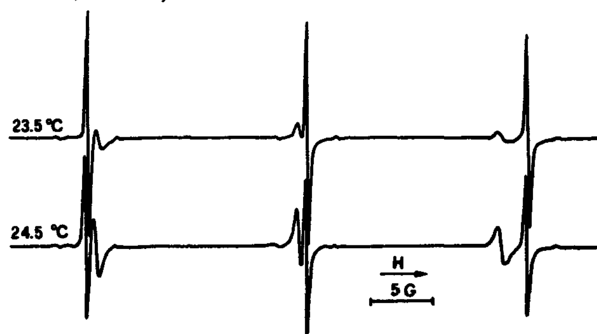

Fig.2. ESR spectra of PDDTBN in DMPC liposomes just below and above the main phase transition temperature.

above the main phase transition temperature. Lipid line gradually becomes visible at around pretransition temperature of pure DMPC bilayers which is associated with tilting of the hydrocarbon chains with respect to the plane of the bilayer (not shown). The abrupt increase in the amplitude of the ESR lipid line is observed around 24°C which monitors the main gel-to-liquid crystalline phase transition occuring in DMPC liposomes.

Fig.3 shows the ESR spectra of PDDTBN in DMPC membranes containing 3 mol% melittin (lipid:protein molar ratio of 32:1) at different temperature. When melittin is added to the DMPC liposomes lipid line started to appear around 28°C (not shown). As an example, in the figure the ESR spectrum of PDDTBN for 30°C is shown for which lipid lines are clearly observed. Inspection of the spectra shows that the amplitude of the lipid lines increase as temperature is increased.

Results of the experiments indicate that for lipid:melittin molar ratio of 19:1 lipid line also become visible at approximately 28°C and for lipid:melittin molar ratios of 14:1 and 10:1, lipid lines appear at approximately 30.5°C.

According to the behaviour of this probe in lipid membranes [30, 31], these results should indicate that with the addition of melittin, pretransition temperature disappears and main phase transition temperature shifts to higher degrees. Although there are previous studies supporting this conclusion [21], this explanation might not be correct and existance of boundary lipids should be considered.

Previously, by vibrational Raman Spectroscopy, for a lipid:melittin molar ratio of 14:1 Lavialle et al. [22] and Levin et al. [23] observed two order-disorder transition: One was centered

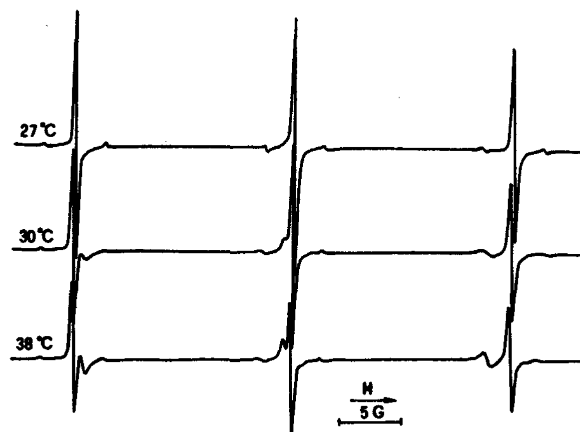

Fig.3. ESR spectra of PDDTBN in DMPC liposomes containing 3 mol % melittin at different temperatures.

at 17°C and was reported as a depression of the main lipid phase transition of DMPC, the other occured at around 29°C and reported to be associated with the melting behaviour of approximately seven immobilized boundary lipids which surround the inserted hydrophobic segment of the polypeptide. For a lipid:melittin molar ratio of 10:1 two thermal transitions were also observed at 11°C and 30°C which were reported as main gel to liquid crystalline phase transition and the melting behavior of approximated four boundary lipids attached to melittin, respectively. According to Dufourc et al. [24] it is known that melittin added DMPC membranes are in the form of fused unilamellar vesicles of diameter ~3000-6000 Å. Instead of using the "penetration model" proposed by Lavialle et al. [22], if it is assumed that membrane bound melittin is monomeric [5,8-11] and the wedge like conformation model is used in which both helices penetrate partially into the outher monolayer, while the N-terminal and the C-terminal parts are still accessible to the aqueous phase, one can speculate that there are boundary lipids attached to melittin and these boundary lipids behave like a barrier and do not let the spin probe partition to hydrophobic interior of the bilayer up to the temperature at which the fluidization of the boundary lipids occur. This situation may be analogous to a percolation process. Appoximately for temperatures T< 28°C, there is no spanning path for the probe across the boundary lipid layers and for T> 28°C there is a spanning path. In other words, the probability that the probe finds a spanning path is changing with temperature, for approximately T< 28°C it

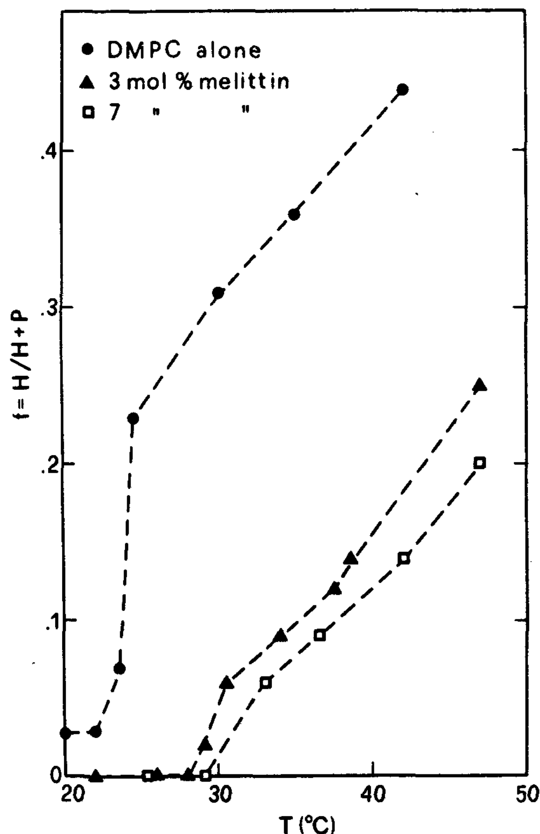

Fig.4. Plots of the f parameter as a function of temperature calculated from the high-field lines of PDDTBN dissolved in DMPC membranes containing different mole fractions of melittin

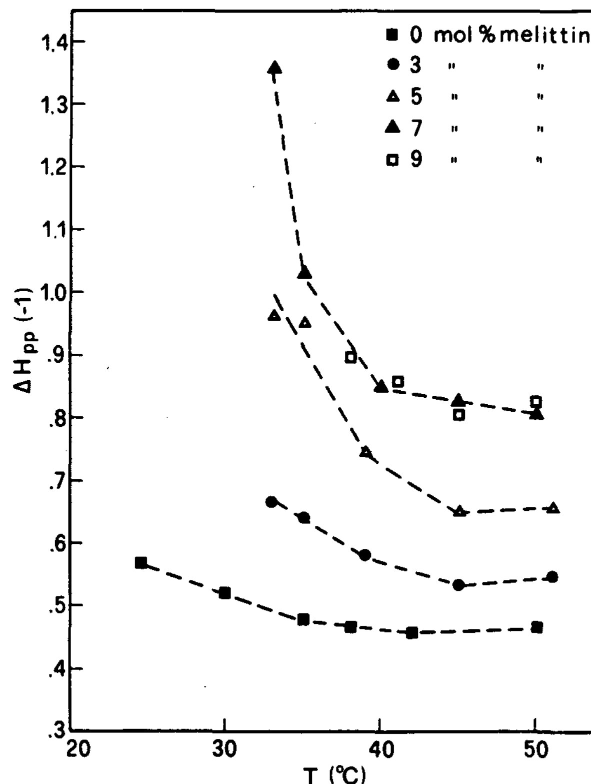

Fig.5. Plots of the peak to peak linewidth of high field lipid line of PDDTBN dissolved in DMPC membranes containing different mole fractions of melittin, as a function of temperature.

is zero, for T> 28°C it is one. Recently Bradrick et al. [25] also have postulated the existance of boundary lipids by differential scanning calorimetry and stated that protein would presumable prevent 10 lipids molecules (boundary lipids) from participating in the phase transition in low-salt solution where melittin is monomeric.

Finally the effect of melittin on fluidity of DMPC membranes has been investigated. Although the fluidty concept in membrane is under debate, PDDTBN may correctly be used to monitor the fluidity of the membrane since it is a very small (r = 2 Å) [32] and almost spherical molecule. As mentioned in ref.[31] high field line of PDDTBN can be used to obtain information about the fluidty of the membrane. For high field line, variation in the partition coefficient defined as f=H/H+P is a combined result of changes in spin probe partitioning and in linewidth, both of which are indicative of a variation in lipid fluidity. Fig.4 shows the variation of f calculated from the high field lines, as a function of temperature, for aqueous dispersion of DMPC in the presence and in the absence of melittin. The parameter f undergoes a gradual increase with increasing temperature. For pure DMPC liposomes abrupt increase is observed about 40°C which corresponds to the well-known main transition of fully hydrated DMPC from the gel to the liquid crystalline phase. With the addition of melittin, the f values of Fig.4 taken at the same temperature, decrease almost regularly as the melittin concentration is increased. These results indicates that fluidty of the membrane decreases in the liquid crystalline phases, with the addition of melittin. This result is supported by Fig.5 which shows the variation of peak to peak line width of high field lipid line ,($\Delta H_{pp}(-1)$, obtained by spectral deconvolution using standart regression methods which fit hybrid Gaussian-Lorentzian lineshapes and by taking into account the effect of ^{13}C lines, with the temperature for different melittin concentrations. As seen from the figure, with the addition of melittin , line width of high field lipid line increases. This increase in linewidth with the addition of melittin is indicative of the decreasing rate of tumbling of the spin probe molecules . These results on the lipid dynamics are in agreement with previous studies [17-19].

In view of these data one can conclude that dynamics of the probe and hence the fluidity of the membrane decreases with the addition of

melittin. ESR data presented in this communication also provide further evidence of monomeric state of membrane bound melittin and wedge like conformation, and of boundary lipids which surround the inserted hydrophobic segment of the polypeptide. However one should not rule out the possibility that phospholipases A_2 in commercial Sigma melittin also may induce some effect too. Although there are other studies using the same melittin [11,33] without further purification it will be interesting to investigate the effect of phospholipases A_2 on lipid phase transition and dynamics by using phospholipases A_2 free melittin.

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