

Interaction of the antimicrobial peptide gramicidin S with dimyristoyl-phosphatidylcholine bilayer membranes: a densitometry and sound velocimetry study

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Abstract

We determined changes in the volume and adiabatic compressibility of large multi- and unilamellar vesicles composed of dimyristoylphosphatidylcholine containing various concentrations of the antimicrobial peptide gramicidin S (GS) by applying densitometry and sound velocimetry. Gramicidin S incorporation was found to progressively decrease the phase transition temperature of DMPC vesicles as well as to decrease the degree of cooperativity of the main phase transition and to increase the volume compressibility of the vesicles. GS probably enhanced thermal fluctuations at the region of main phase transition and provide more freedom of rotational movement for the phospholipid hydrocarbon chains. The ability of GS to increase the membrane compressibility and to decrease the phase transition temperature is evidence for regions of distorted membrane structure around incorporated gramicidin S molecules. At relatively high GS concentration (10 mol%), more significant changes of specific volume and compressibility appear. This might suggest changes in the integrity of the lipid bilayer upon interaction with high concentrations of GS. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Gramicidin S; Phosphatidylcholine bilayer; Antimicrobial peptide; Lipid-peptide interaction; Densitometry; Sound velocimetry; Volume compressibility

1. Introduction

The antimicrobial peptide gramicidin S (GS) is a

Abbreviations: GS, gramicidin S; MLV, multilamellar vesicle; LUV, large unilamellar vesicle; SUV, small unilamellar vesicle; FTIR, Fourier transform infrared; PC, phosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DMPG, dimyristoylphosphatidylglycerol; DSC, differential scanning calorimetry; T_m , gel to liquid-crystalline phase transition temperature; ULV, unilamellar vesicle

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cyclic decapeptide first isolated from *Bacillus brevis* [1]. This peptide exhibits appreciable antibiotic activity against a broad spectrum of Gram-negative and Gram-positive bacteria as well as against several pathogenic fungi [2,3]. In aqueous solution GS forms an amphiphilic, two-stranded, antiparallel β -sheet structure [2] (Fig. 1). In general, the antimicrobial activity of GS analogs increases with the degree of hydrophobicity and amphiphilicity of the peptide up to some optimal value [3]. However, GS is rather nonspecific in its action and exhibits appreciable hemolytic as well as antimicrobial activity [4–6]. The

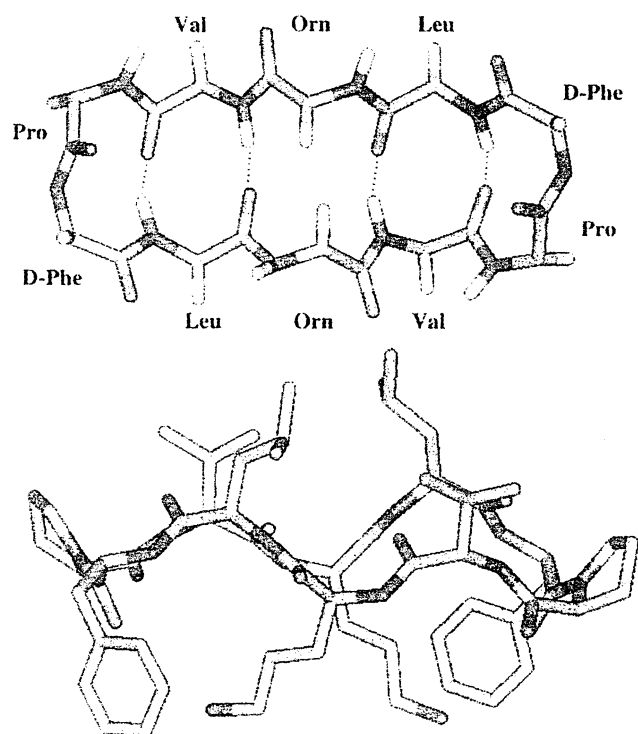


Fig. 1. Structure and conformation of GS. The upper panel is a view of the GS molecule perpendicular to the plane of the ring, illustrating the peptide backbone structure and the positions of the hydrogen bonds in the antiparallel β -sheet region. The lower panel is a view of the GS molecule in the plane of the ring, indicating the disposition in space of the hydrophobic Val and Leu residues (top) and the basic Orn residues (bottom) relative to the peptide ring. Adapted from [10].

therapeutic utilization of GS has therefore been limited to topical applications [5]. A major aim of current studies on the interaction of GS with lipid model and biological membranes is to provide fundamental knowledge of the mechanism of action of this peptide on lipid bilayers. This will support the design of GS analogs with enhanced activity for bacterial membranes and diminished activity against the plasma membranes of human and animal cells [3,7].

Considerable evidence exists that the primary target of GS is the lipid bilayer of cell surface membranes and that this peptide kills cells by destroying the structural integrity of the lipid bilayer [8]. GS partitions strongly into liquid-crystalline lipid bilayers in both model and biological membranes and seems to be located primarily in the glycerol backbone region below the polar headgroup and above the hydrocarbon chains [9]. In addition, GS binds more strongly to negatively charged lipids [9,10]

than to zwitterionic or uncharged phospho- and glycolipids [11]. Furthermore, its interaction with phospholipid bilayers is attenuated by cholesterol [12]. The presence of GS appears to perturb lipid packing in the liquid-crystalline state and to enhance the non-lamellar phase-forming propensities of certain lipid classes, resulting in the formation of inverted cubic phases in single lipid systems [13] as well as complex lipid extracts of *Escherichia coli* and *Acholeplasma laidlawii* [13,14]. Furthermore, at lower concentrations, GS increases the permeability of model and biological membranes and at higher concentrations causes membrane destabilization [2,9,15]. A recent review on the interaction of GS with lipid bilayer model and with biological membranes was published by McElhaney and coworkers [9].

Since GS interacts strongly with lipid bilayers and alters many of their physical properties [9], we would expect that GS should also induce changes in the dynamics and mechanical properties of lipid model and biological membranes. This assumption is based on previously reported results by Strom-Jensen et al. [16], who showed changes of the membrane dynamics of DMPC/DMPG vesicles following interaction with gramicidin A by measuring the absorption of ultrasound in a broad range of frequencies. Furthermore, Hianik et al. [17] showed that short analogs of the adrenocorticotrophic hormone peptide (ACTH₂₄) changed the adiabatic compressibility of PC vesicles. We can therefore expect that methods based on molecular acoustics could provide new insight on the mechanisms of interaction of GS with bilayer lipid membranes. In this work we studied the changes of density and sound velocity of multilamellar and unilamellar vesicles composed of DMPC containing various concentrations of GS upon heating through the phase transitions of the lipids and at different contents of GS.

2. Materials and methods

2.1. Chemicals and preparation of vesicles

DMPC was purchased from Sigma (St. Louis, MO) and used without further purification. Gramicidin S was obtained from Sigma, dissolved in pure ethanol and stored at -20°C . In order to remove

traces of water from DMPC, the samples were dried in vacuo at 90°C until constant weight was achieved (typically 3 h) [18]. Then the lipid was dissolved in chloroform/methanol (3:1, v/v) and mixed with appropriate amounts of peptide stock solution. These solutions were dried under N₂ and the lipid/peptide films were exposed to high vacuum for 3 h. The GS-containing MLVs were prepared by vortexing in an excess of double distilled water at temperature above the main phase transition temperature of the phospholipid. The GS-containing ULVs were prepared by the extrusion method of MacDonald et al. [19] using a LiposoFast-Basic Extruder (Avestin, Canada) and polycarbonate filters with pores of 100 nm in diameter. It has been shown that the average diameter of ULVs prepared at these conditions is ~100 nm [19]. Prior to measurements the aqueous vesicle suspension was degassed using a water vacuum pump. The lipid concentration for both preparations was 5 mg/ml.

2.2. Sound velocimetry

The measurement of ultrasound velocity allows us to evaluate the elastic properties of aqueous media and suspensions such as vesicles [20,21], lipoproteins [22] or cell surface proteins [23] based on a simple relationship:

$$\beta_s = 1/(\rho u^2) \quad (1)$$

Whereby β_s , ρ , and u are the adiabatic compressibility, the density, and the sound velocity of the suspension, respectively. Thus, by measuring the changes of sound velocity and density, one can determine the changes of adiabatic compressibility [24].

Ultrasound velocity was measured using a fixed-path differential velocimeter consisting of two almost identical acoustic cavity resonators [25] operated at frequencies ν around 7.2 MHz. The resonance frequencies and the width of the resonance peaks of the cells were measured with the aid of a computer-controlled phase-sensitive feedback circuit. The sample volume was 0.7 ml. The resonator cells were equipped with magnetic stirrers to ensure homogeneously dispersed samples during the measurements. One resonator contained the vesicle solution whereas the other one was filled with the reference liquid (double-distilled water). When starting a series of

measurements, the resonance frequencies of the resonators were always first compared with one another by filling both cells with the reference liquid. As the intensity of the sonic signal was small throughout (the pressure amplitude in the ultrasonic wave was less than 10³ Pa), any effects of the sound wave on the structural properties of the vesicles were avoided. In general, ultrasonic velocimetry allows the determination of the sound velocity [u] and the absorption per unit wavelength [$\alpha\lambda$], or rather their concentration increments [26], respectively, as defined by the equations:

$$[u] = (u - u_0)/u_0 c \quad (2)$$

$$[\alpha\lambda] = (\alpha\lambda - \alpha\lambda_0)/c \quad (3)$$

whereby c is the solute concentration in mg/ml and the subscript 0 refers to the solvent (distilled water). The value [u] can be directly determined from the changes of resonance frequencies f , f_0 of the resonators (f is resonance frequency of the sample and f_0 that of the reference – distilled water):

$$[u] = (u - u_0)/u_0 c = [(f - f_0)/f_0 c](1 + \gamma) \quad (4)$$

(the coefficient $\gamma \ll 1$ [27] and can be neglected in the calculations). The changes in sound absorption per unit wavelength are determined by the equation:

$$\Delta\alpha\lambda = (\alpha\lambda - \alpha_0\lambda_0) = \Omega(\delta f/f) \quad (5)$$

where δf is the half-power width of a resonator peak and Ω the calibration coefficient. For an ideal resonator, when the peak is measured at the half power level, $\Omega = \pi$. For real systems the value of Ω has to be determined by calibration, which was performed by using a 0.1 M MnSO₄ solution of known ultrasonic absorption [28].

2.3. Density measurements

A high precision densitometric system (DMA 60 with two DMA 602 M sample chambers, Anton Paar, Graz, Austria) operating according to the vibrating tube principle [29] has been used to determine the density ρ of the vesicle solution. Apparent specific partial volumes φ_v have been calculated from the density data using the relation

$$\varphi_v = [1 - (\rho - \rho_0)/c]/\rho_0 = 1/\rho_0 - [\rho] \quad (6)$$

whereby the subscript 0 again refers to the solvent and $[\rho] = (\rho - \rho_0)/(\rho_0 c)$ denotes the concentration increment of density.

2.4. Experimental errors

The uncertainty in the concentration of the phospholipid suspensions was smaller than 0.25%. The temperature of the cells was controlled to within 0.02°C with a Lauda RK 8 CS ultra-thermostat (Lauda, Germany). The accuracy of determination of the sound velocity increment, $[u]$, and the specific partial volume, φ_V , was better than 10^{-3} ml/g. The accuracy of the determination of the density was better than 10^{-3} g/ml. Each series of measurements was performed at least three times.

3. Results and discussion

3.1. Mechanics and thermodynamic characteristics of unmodified vesicles

Sound velocimetry and densitometry are sensitive tools to study the mechanics and thermodynamics of biocolloids. These methods, if used simultaneously to study peculiarities of the phase transition of lipid bilayer, allow one to determine the phase transition temperature and the degree of phase transition cooperativity. In addition to the thermodynamic properties of the lipid phase transition obtained by differential scanning calorimetry (DSC), these methods allow one to study the mechanical properties of lipid bilayer membranes. This advantage is most useful in the study protein–lipid interactions, since the determination of mechanical parameters is crucial for an evaluation of the size of distorted membrane structure around proteins [30]. The basic values of the parameters usually determined in sound velocity and density experiments are shown in Fig. 2 for suspensions of LUVs composed of DMPC as a function of temperature and in the range of the phase transition temperature ($T_m \sim 24^\circ\text{C}$).

The characteristic peculiarity of the concentration increment of sound velocity, $[u]$, is its sharp minimum at the T_m (Fig. 2a, curve 1). At temperatures below the T_m of DMPC, (i.e., $T < 24^\circ\text{C}$), the value of $[u]$ slowly decreases with increasing temperature,

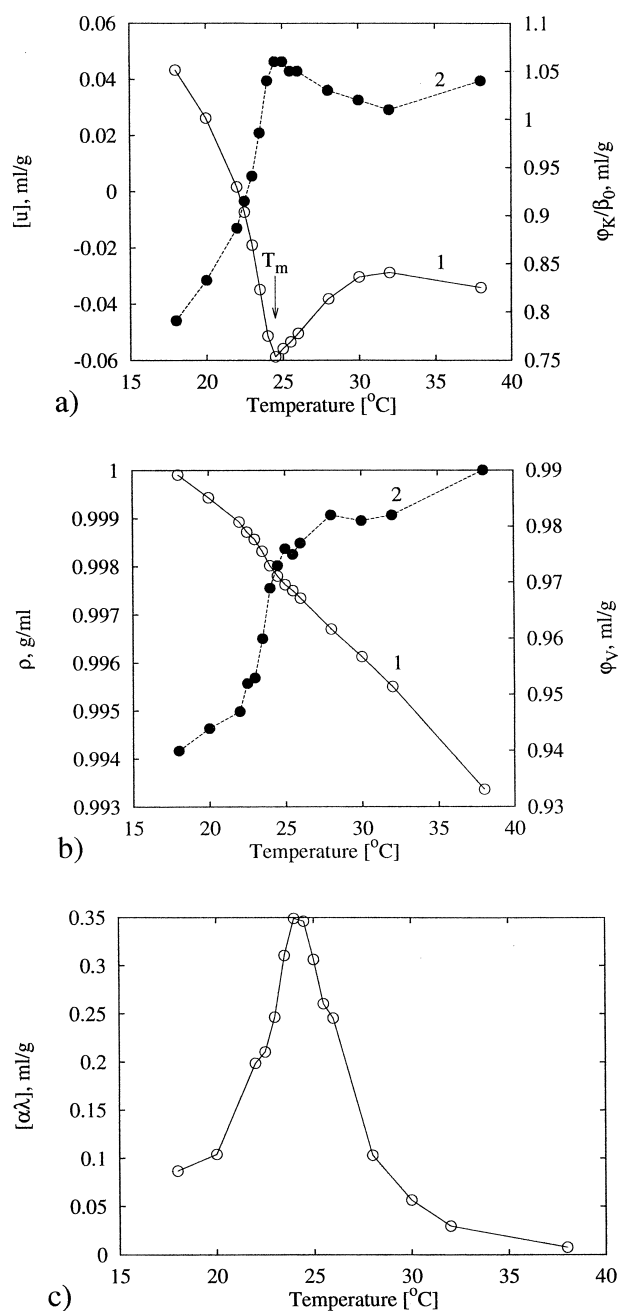


Fig. 2. Temperature dependence of the concentration increment of sound velocity, $[u]$, (panel a) (1), apparent specific partial compressibility φ_K/β_0 (2); (panel b) density (1), specific volume (2); (panel c) concentration increment of absorption, $[\alpha\lambda]$ for LUVs composed of DMPC. The arrow indicates the phase transition temperature.

whereas an increase of $[u]$ is characteristic for higher temperature regions above the T_m . This characteristic shape of the plot of $[u]$ versus temperature was described in a number of publications dealing with

sound velocimetry studies of phase transitions in vesicle suspensions composed of saturated PCs (see, e.g., [20,31,32]). The nature of the minimum in the plot of $[u]$ versus temperature can be explained as follows. According to [33], the adiabatic compressibility, β_S (see Eq. 1), is related to the isothermal compressibility, κ_T , and the heat capacities, c_V and c_P , at constant volume and constant pressure:

$$\beta_S = \beta_T c_V / c_P, \quad (7)$$

so that

$$u = [c_P / (c_V \rho \beta_T)]^{1/2} \quad (8)$$

Hence, the minimum in the $[u]$ value reflects the effects from both the increasing heat capacity, c_P , [34] and isothermal compressibility, β_T , upon approaching the T_m . The value β_T is an additional parameter compared to the T_m conventionally used in DSC.

Absorption of ultrasound is characterized by a maximum at the T_m [35]. This is shown in Fig. 2b, where the value of $[\alpha\lambda]$ is plotted as a function of temperature for LUVs composed of DMPC. The increased absorption per unit wavelength at the phase transition region is related to enhanced thermal fluctuations in the membrane. In this respect, the absorption of ultrasound is analogous to the increase of heat capacity as measured by DSC (see, e.g., [30]) in vesicle suspensions.

According to Eq. 1, in order to calculate the coefficient of adiabatic compressibility, β_S , it is necessary to determine the density of the vesicle solution, ρ , and consequently the specific volume, φ_V (see Eq. 6). In the case of phospholipids this parameter changes considerably at the T_m . The value of φ_V , as well as the behavior of its plot versus temperature, provides important information about the nature of the lipid phase transition of membranes, e.g., degree of cooperativity and expansion coefficient (see below) [18]. The typical plot of density, ρ , and specific volume, φ_V , for LUVs composed of DMPC is presented in Fig. 2c, curves 1 and 2, respectively. We can see that density monotonically decreases, while specific volume increases with increasing temperature.

The determination of the specific volume in addition to the sound velocity concentration increment allowed the estimation of changes of the specific adiabatic compressibility, φ_K/β_0 , of the vesicles during the phase transition, which is based on the fol-

lowing equation:

$$\varphi_K/\beta_0 = -2[u] - 1/\rho_0 + 2\varphi_V \quad (9)$$

whereby β_0 is the coefficient of adiabatic compressibility and ρ_0 is the density of the buffer [25]. The value of φ_K/β_0 indicates changes of the volume compressibility of the vesicles relative to the buffer. The plot of φ_K/β_0 as a function of temperature for LUVs composed of DMPC is presented in Fig. 2a, curve 2. We can see that below the phase transition temperature, the value of φ_K/β_0 monotonically increases with temperature, reaches a maximum at T_m , and then decreases at $T > 24^\circ\text{C}$. This is in agreement with previously reports [20] and can be explained as follows. The apparent specific compressibility can be expressed as:

$$\varphi_K/\beta_0 = \varphi_V + [\beta_S] \quad (10)$$

where $[\beta_S] = (\beta_S - \beta_{S0})/(\beta_{S0}c)$ is the concentration increment of the adiabatic compressibility (see [20]). Thus, the increase of the φ_K/β_0 value in the phase transition region is related both to an increasing specific volume, φ_V , and an increase of β_S . Quite remarkably, φ_K/β_0 decreases when going from 25°C , slightly above T_m , to 30°C . In this temperature range φ_V increases (see Fig. 2b, curve 2). We therefore conclude that the decreasing φ_K/β_0 values result from a decrease in β_S . The heat capacity, c_P , also decreases in this temperature range. According to Eq. 7, the temperature dependence in the φ_K/β_0 data above T_m thus seems to reflect the isothermal compressibility of lipid bilayers, which is related to the volume fluctuations of the samples [36] and which decreases with T (see also [20]).

$$\beta_T = \frac{1}{V} \left(\frac{\partial V}{\partial p} \right)_T = \frac{\overline{V^2} - \overline{V}^2}{\overline{V}RT} \quad (11)$$

Here R denotes the gas constant.

From results presented above, it is evident that sound velocimetry and densitometry measurements provide important information about mechanics and thermodynamics of phase transitions of lipid vesicles. These methods allow the characterization of physical properties of vesicles in more detail then, for example, traditional methods like DSC, by also providing information about the compressibility of the membrane. Additional advantages of the

sound velocimetry method, in comparison with so-called microscopic methods like ESR, NMR or fluorescence spectroscopy, is that the compressibility of the membrane derived from this technique is a macroscopic parameter, i.e., changes of membrane mechanics caused, for example, by the interaction of proteins, which as a rule influence large regions of lipid membrane [29].

Usually two sets of vesicles are used in study of protein-lipid interactions, unilamellar and multilamellar. It is known that the phase transition in MLVs is accompanied by sharper changes of thermodynamic parameters due to the increased cooperativity of the phase transition (see, e.g., [10]). This effect has been demonstrated also in sound velocimetry study of MLVs [31]. The specific volume of phospholipids, however, does not substantially differ for uni- and multilamellar vesicles [18]. Since in our work we used both LUVs and MLVs, it is interesting to compare their mechanical properties. The plot of sound velocity increment, $[u]$, as a function of temperature for LUVs (curve 1) and MLVs (curve 2) of DMPC is presented in Fig. 3a. We can see that for MLVs the changes of $[u]$ at phase transition region are sharper than that for LUVs. Analogous results have been reported previously on vesicles composed of dipalmitoylphosphatidylcholine [31]. Using the specific volume as derived from density measurements, we also determined the specific adiabatic compressibility for LUVs and MLVs. However, the density measurement have been performed only on LUVs since the experimental set up did not allow stirring of the solution and it was therefore impossible to avoid sedimentation of MLVs, which in turn could influence the accuracy in the density determination. However, as mentioned above, the apparent specific volumes of water suspensions of multilamellar [18] and unilamellar vesicles [20] are not significantly different. The plot of the values φ_K/β_0 as a function of temperature for LUVs (curve 1) and MLVs (curve 2) is shown in Fig. 3b. We can see that both functions have a similar shape, but the MLVs are characterized by lower values of apparent specific compressibility at the temperature range $T < 28^\circ\text{C}$, while at $T = 28^\circ\text{C}$ the adiabatic compressibility of both MLVs and ULVs is practically identical.

Two contributions could principally be involved in

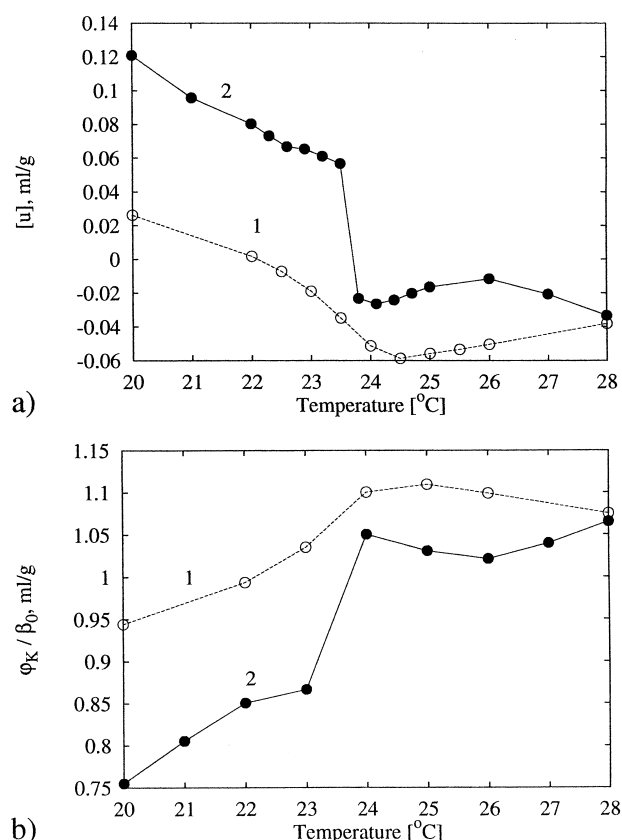


Fig. 3. (a) Plot of the concentration increment of sound velocity, $[u]$. (b) Apparent specific compressibility, φ_K/β_0 , as a function of temperature for LUVs (1) and MLVs (2) composed of DMPC.

the above-mentioned differences of vesicle compressibility: asymmetry of inner and outer monolayers of LUVs and hydration effects. The LUVs used in our work are sufficiently large (diameter ~ 100 nm) to avoid significant effects on bilayer ordering arising from asymmetry of inner and outer monolayers and this should consequently not influence the compressibility. Thus, the hydration effect is probably the main reason for the observed differences in adiabatic compressibility and can be represented as a sum of two terms at low vesicle concentrations

$$\varphi_K/\beta_0 = (\varphi_K/\beta_0)_V + \Delta(\varphi_K/\beta_0)_H \quad (12)$$

where $(\varphi_K/\beta_0)_V$ is the apparent adiabatic compressibility of the parts of vesicles, which are inaccessible for the surrounding water molecules, and $\Delta(\varphi_K/\beta_0)_H$ is the hydration term determined by the change of the water density in the hydration shell of the solute. The value $(\varphi_K/\beta_0)_V$ is positive, while the hydration

contribution $\Delta(\varphi_K/\beta_0)_H$ is negative at $T=20^\circ\text{C}$ [22,25]. With increasing temperature the compressibility of the hydrated shell increases. Thus, the hydration term considerably modifies the adiabatic compressibility of the solution. This contribution is even negative at lower temperatures. This means the adiabatic compressibility of the hydrated shell is lower than that of the surrounding solvent (unbounded water). Therefore the resulting overall compressibility of vesicles will depend on the degree of hydration of the lipid bilayer which is obviously higher for MLVs than for ULVs at the same phospholipid concentration. At higher temperatures, the compressibility of hydrated shell increases and becomes comparable with the compressibility of the solvent. Therefore, at these temperatures we should not expect differences between the adiabatic compressibility of MLVs and LUVs. At our experimental conditions this holds for $T=28^\circ\text{C}$ (see Fig. 3b).

3.2. Mechanics and thermodynamic characteristics of vesicles modified by gramicidin S

Fig. 4 illustrates the concentration increment of the sound velocity, $[u]$, of MLVs composed of DMPC and different molar ratios of DMPC-GS as a function of temperature between 19°C and 28°C . We can see that, except for the highest GS content (10 mol%), the curves have similar shapes. At temperatures below the T_m of DMPC (i.e., $T < 24^\circ\text{C}$), the value of $[u]$ slowly decreases with increasing tem-

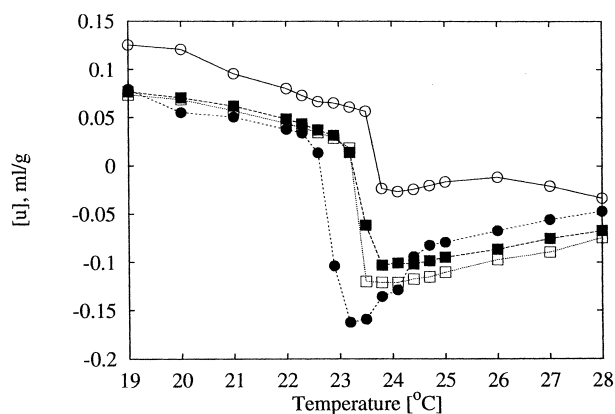


Fig. 4. Concentration increment of sound velocity $[u]$ versus temperature for aqueous suspension of MLVs from DMPC with different lipid/peptide molar ratios: ○, 1:0; □, 1:50; ■, 1:25; ●, 1:10.

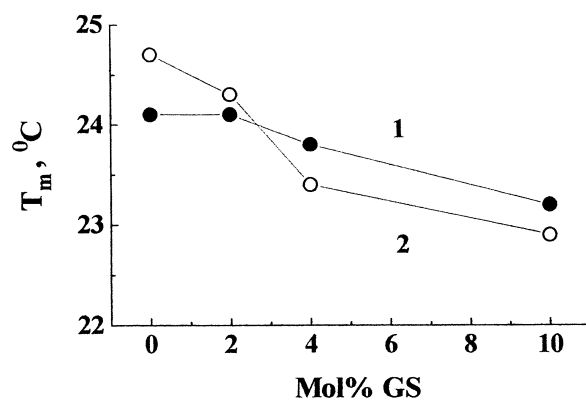


Fig. 5. Dependence of the temperature of main transition, T_m , of DMPC/GS MLVs on concentration of GS determined from the minimum of $[u]$ value (curve 1) and from DSC experiments (curve 2). Curve 2 was plotted using the data published in [11].

perature. A sharp decrease of $[u]$ and a local minimum is observed around the chain-melting temperature of the phospholipid, whereas an increase of $[u]$ is characteristic for higher temperature regions above the T_m [31]. For higher contents of GS, the local minimum is more pronounced and shifted toward lower temperatures. As discussed above, the minimum in the $[u]$ value reflects the effects from both the increasing heat capacity, c_p , and isothermal compressibility, β_T , upon approaching the T_m . We can also see in Fig. 4 that an increase in the GS content results in a lower $[u]$ value and that the T_m also shifts toward lower temperatures. Fig. 5 illustrates a comparison of these parameters in more detail. Curve 1 is a plot of the T_m derived from the minimal value of $[u]$ (i.e., at region of main phase transition) and curve 2 describes calorimetric data published previously [10] as a function of GS concentration. We can see that there is quite a good correlation between these two independent techniques in the measured decrease of the T_m with increasing GS content. In addition, we also observed an increase of the value of the sound absorption per unit wavelength and a shift of the absorption maximum toward lower temperatures with increased GS content (results not shown). We want to stress that the calorimetric measurements illustrate the changes in the heat capacity of the system, whereas the sound velocimetry data will be influenced in addition by compressibility parameters. The decrease of the $[u]$ value with increasing GS content clearly indicates an increase of the adiabatic

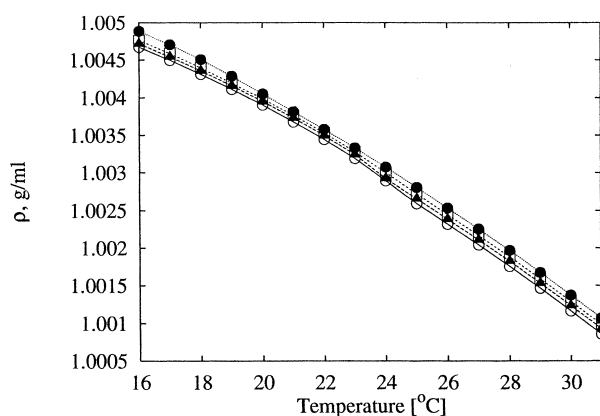


Fig. 6. Plot of density, ρ , versus temperature for aqueous suspension of MLVs from DMPC with different lipid/peptide molar ratios: \circ , 1:0; \blacktriangle , 1:100; \square , 1:50; \bullet , 1:10.

compressibility of the vesicles, suggesting a decrease in lipid bilayer order. The classification of membrane-associated proteins into three classes was suggested by Papahadjopoulos [37] and McElhaney [38]. GS was suggested to belong to class II and thus being located near the interface whereby it decreases the temperature, enthalpy and cooperativity of the main phase transition moderately (for a detailed discussion, see [10]).

The measurement of the density is crucial for the determination of the specific volume of the membrane and thus for evaluation of the specific adiabatic compressibility (see Eq. 9). In addition, as demonstrated earlier (see, e.g., [18]), from the plot of the specific volume versus temperature we can estimate the degree of cooperativity of the membrane phase transition and expansion coefficient of lipid bilayer which should be affected by GS. The density of the vesicle suspension of DMPC and samples containing different concentration of gramicidin S decreases with increasing temperature as shown in Fig. 6. This density decrease is related to an increased volume of the phospholipids. Using Eq. 6, we calculated the apparent specific partial volume, φ_V , of the DMPC/water system and plotted it as a function of temperature. From this plot, presented in Fig. 7, we can see that the value of φ_V generally increases with temperature as expected in all cases. However, φ_V exhibits a more rapid increase of $\sim 3\%$ over the temperature interval near the T_m , indicating an increase in the volume of the phospholipid molecules from 1.069 nm^3 at $T < T_m$ to 1.101 nm^3 at $T > T_m$. The

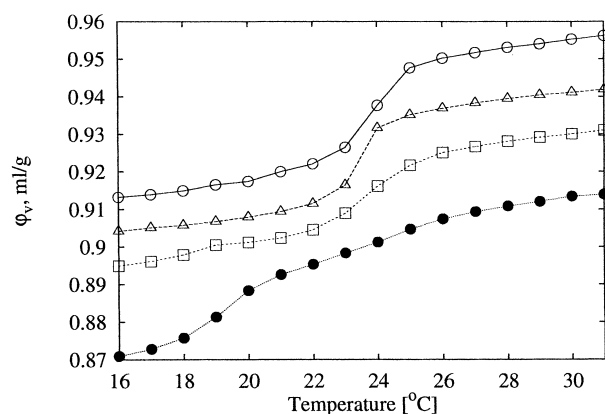


Fig. 7. Plot of apparent specific partial volume φ_V versus temperature for aqueous suspension of MLVs from DMPC with different lipid/peptide molar ratios: \circ , 1:0; \triangle , 1:100; \square , 1:50; \bullet , 1:10.

overall shapes of the φ_V versus temperature plots are similar, except that at the highest GS concentration a shift of the T_m toward lower values is observed (Fig. 7).

In order to analyze the peculiarities of the value φ_V , we plotted its change as a function of temperature $\Delta\varphi_V = (\varphi_V)_T - (\varphi_V)_{15}$, where $(\varphi_V)_T$ and $(\varphi_V)_{15}$ are the apparent specific volumes of vesicles at certain temperature from the starting value of 15°C , respectively. Several parameters can be derived from the plot of $\Delta\varphi_V$ versus temperature (Fig. 8): the temperature of the main transition, T_m , its tran-

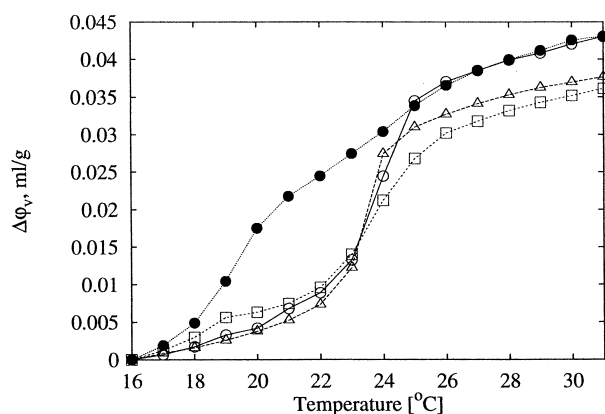


Fig. 8. Plot of the changes of apparent specific partial volume $\Delta\varphi_V$ ($\Delta\varphi_V = (\varphi_V)_T - (\varphi_V)_{15}$, where $(\varphi_V)_T$ and $(\varphi_V)_{15}$ are the apparent specific volumes of vesicle at certain temperature and at 15°C , respectively) versus temperature for aqueous suspension of MLVs of DMPC with different lipid/peptide molar ratios: \circ , 1:0; \triangle , 1:100; \square , 1:50; \blacksquare , 1:25; \bullet , 1:10.

sition half-width, ΔT_m , volume changes at this temperature, $\Delta\varphi_V$, and the expansion coefficient, α_M , at the main transition temperature (see [18] for more details). These parameters are summarized in Table 1. In addition to a decrease of the T_m , the width of phase transition region increases with increasing GS concentration (see also [10]). The expansion coefficient at the main transition has also clear tendency to decrease with increasing GS content, which is also evidence for a decrease in cooperativity of the phase transition in the presence of GS.

It is interesting to compare the results presented in Table 1 for pure DMPC MLVs with that obtained on MLVs of the same lipid composition reported in [18]. The volume change at the main transition has been found $\varphi_V = 2700 \times 10^{-5}$ ml/g for MLVs of DMPC [18]. A similar value of 2720×10^{-5} ml/g has been obtained in our experiments with LUVs. However, differences can be found by comparing the expansion coefficient values α_M at the main transition region. This value for MLVs of 9000×10^{-5} ml/g deg [18]) is considerably higher than that for LUVs obtained in our work (1786×10^{-5} ml/g deg). This comparison of the relative cooperativities of the phase transition in multi- and unilamellar vesicle properties indicates that the phase transition is more cooperative in multilamellar vesicles, i.e., the volume changes are more sharp in the transition region compared to LUVs, as found previously [18,20].

The determination of the specific volume in addition to the sound velocity concentration increment allowed the estimation of changes of the specific adiabatic compressibility, φ_K/β_0 , of the vesicles during the phase transition and as a function of the GS content (see Eq. 9). The plot of φ_K/β_0 as a function of temperature for MLVs of different GS content is presented in Fig. 9. The shape of the curves pre-

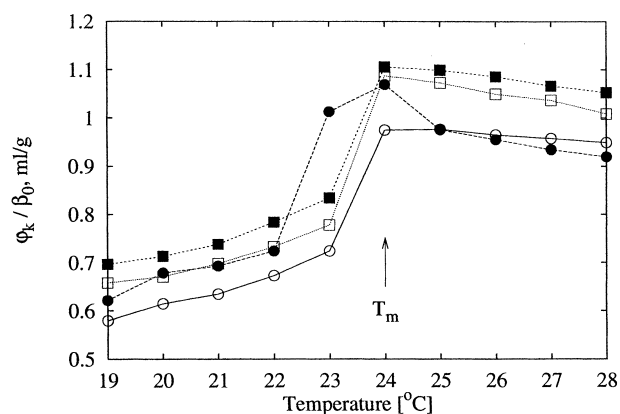


Fig. 9. Plot of apparent specific partial compressibility φ_K/β_0 versus temperature for aqueous suspension of MLVs of DMPC with different lipid/peptide molar ratios: \circ , 1:0; \square , 1:50; \blacksquare , 1:25; \bullet , 1:10. The arrow indicates the phase transition temperature.

sented are similar for relatively low GS content (below 4 mol%) and are in agreement with previously reported results for pure DMPC unilamellar vesicles [20]. The physical nature of the shape of the plot φ_K/β_0 , versus temperature for unmodified DMPC vesicles was discussed above. The shape of this dependence is similar for vesicles modified by GS; however a more pronounced decrease of the adiabatic compressibility in the high temperature region of the 10 mol% GS sample is evident. From Fig. 9 there is also evidence for increased adiabatic compressibility at relatively low GS concentrations (below 10 mol%), since the adiabatic compressibility in the low temperature region is still higher than that for pure DMPC; however, it does not significantly differ from that for 2 mol% GS. In the high temperature region ($T > 25^\circ\text{C}$), adiabatic compressibility for samples containing 10 mol% of GS is even lower compared to pure DMPC. This effect might be caused by

Table 1

The values of main transition temperature, T_m , volume changes, $\Delta\varphi_V$ at main transition, the transition half-width, ΔT_m , and expansion coefficient α_M , at main transition temperature derived from experimental data presented in Fig. 8 for suspensions of unilamellar vesicles with different DMPC/GS molar ration

DMPC/GS	T_m ($^\circ\text{C}$)	ΔT_m ($^\circ\text{C}$)	$\Delta\varphi_V$ ($10^5 \times \text{ml/g}$)	α_M ($10^5 \times \text{ml/g deg}$)
1:0	23.9	0.84	2720	1786
1:100	23.4	0.92	2643	1582
1:50	23.7	1.54	1846	660
1:25	23	1.62	2666	865
1:10	< 23	—	—	—

the ability of GS to disturb the integrity of the lamellar phase of the lipid bilayer at higher peptide concentrations [13].

The effect of GS on lipid ordering was a matter of debate in the literature for some time. Zidovetski et al. [39] utilized ^2H -spectroscopy to investigate the effect of GS on the hydrocarbon chain orientational order profiles of chain-perdeuterated DMPC MLVs in the gel and liquid-crystalline states. They reported no major changes in the gel state, suggesting that GS is excluded from the gel phase. However, above the T_m , the peptide appeared to disorder the hydrocarbon chains. Recent FTIR data [11] clearly show a partial penetration of GS into the gel phase of DMPC. Susi et al. [40], using Raman spectroscopy, reported that GS disordered the hydrocarbon chains below the broadened and reduced gel to liquid-crystalline phase transition but had no effect at temperatures above the phase transition. In contrast, Katsu et al. [41], using DPH fluorescence polarization spectroscopy, found no effect above or below the T_m , whereas Yagi et al. [42], using the same technique, reported a slight disordering of the liquid-crystalline phase. In contrast, Mihailescu and Horvath [43], using ESR spectroscopy, reported the existence of about six molecules of motionally restricted phospholipids (DMPC or dimyristoylphosphatidylserine) associated with each molecule of GS between 30–40°C. Our recent DSC data [10] show a destabilizing effect of GS on the gel phase of DMPC as indicated by the concentration dependent decrease of T_m .

The results obtained in our present work demonstrate that GS at contents up to 10 mol% increases the adiabatic compressibility of vesicles. The membrane compressibility is closely related to its ordering and to changes in the hydration of lipid bilayer. This has been shown previously on various planar and spherical membrane structures of different lipid compositions [11,21,30]. We should, however, note, that in our study we prepared liposomes together with peptide, i.e., we studied exclusively the thermodynamic behaviour of lipid bilayers with already incorporated peptide. Therefore, we suppose that the changes of hydration had no significant effect on membrane thermodynamics in comparison with the effect of peptide on the structural properties of lipid bilayer. We can therefore conclude that GS induces a disordering both in the gel and liquid-crystalline state

of the lipid bilayer. The ability of GS to increase the membrane compressibility and to decrease the phase transition temperature is evidence for regions of distorted membrane structure around GS molecules.

4. Summary

In this work we determined changes of the volume and adiabatic compressibility of vesicles composed of DMPC and containing various concentrations of GS by using sound velocimetry and density measurements. We showed a considerable influence of GS on thermodynamic, volume and mechanical properties of such vesicles. We demonstrate that the peptide progressively decreases the temperature as well as the cooperativity of the main transition. Furthermore, an increased concentration of GS results in an increased volume compressibility of the vesicles. This effect suggests a contribution to the possible mechanism of the action of gramicidin S on lipid bilayer, whereby the peptide induces the enhancement of thermal fluctuations in and above the region of main phase transition by providing more freedom of movement of phospholipid hydrocarbon chains. Changes in the membrane compressibility and the ability of GS to decrease the phase transition temperature indicate regions of distorted membrane structure around the GS molecules. In contrast to some previous studies, we clearly demonstrate that this peptide induces a disordering of the lipid bilayer both in the gel and liquid-crystalline state. At relatively high GS concentration (10 mol%), significantly stronger disordering of the membrane at phase transition region was found, which might be due to changes in the integrity of the bilayer matrix.

Finally, we have demonstrated that the concerted application of the techniques of density and sound velocimetry provide useful information about the macroscopic mechanical and thermodynamic properties of peptide model membrane systems.

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