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Dielectric spectroscopy of L-α-lysolecithin-packaged gramicidin A

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The complex permittivities of L-α-lysolecithin in the absence and presence of the gramicidin A ion channel were measured over the temperature range 0–60 °C and over the frequency range 1–1000 MHz. One dielectric relaxation/loss has been observed. It is located at 103.3 MHz (1.54 ns) for a micellar 0.4 M L-α-lysolecithin solution at 20 °C, whereas it is shifted to 71.7 MHz (2.22 ns) for a lamellar L-α-lysolecithin-gramicidin A aqueous solution (0.4 M L-α-lysolecithin, 0.0308 M gramicidin A) at 20 °C. The dielectric relaxation decreases and the relaxation time increases when gramicidin A is incorporated into L-α-lysolecithin. These dielectric changes are related, in part, to the micellar-to-lamellar lipid phase transition induced by the incorporation of gramicidin A into lysolecithin. We suggest that the diffuse rotational motion of the polar head group of L-α-lysolecithin contributes to the dielectric relaxation/loss at around 100 MHz.

1. Introduction

Gramicidin A is a pentadecapeptide forming a dimer when incorporated into L-α-lysolecithin. This dimer forms a β-helical conformation associating amino end to amino end (head to head) by means of six hydrogen bonds to form a continuous lipid-spanning transmembrane channel about 26 Å in length [1–4].

This dimer makes lipid bilayer membranes permeable to small monovalent cations and water (for reviews, see refs. 5–7). Since the conformation of gramicidin A in phospholipid membranes and its molecular mechanism of ion transport are well known [8,9], it constitutes a useful model for studying the polypeptide-phospholipid interactions in biological membranes (for examples, see refs. 10–13).

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One point of particular interest is the effect of gramicidin A on the phase transition from the micellar to lamellar lipid structure. It has been demonstrated that thermal incubation of gramicidin A with lysolecithin micelles results in the formation of a lipid bilayer structure [14–16].

Some dynamic properties of lipids in the presence of gramicidin have been investigated by means of ^{31}P-NMR [17], ^{13}C-NMR [18] and ^{2}H-NMR [19]. The dynamic properties of gramicidin in phospholipid membranes have been recently investigated by means of ^{2}H-NMR spectroscopy [20]. In this respect, we could mention that dielectric relaxation spectroscopy may provide information on the dynamic properties of polar groups. The dielectric properties of micellar solutions [21] and bilayer solutions [22,23] have been reviewed. Recently, Kell and Harris [24], Pethig and Kell [25] and Foster and Schwan [26] have discussed the dielectric properties of membranes and biological systems.

In order to examine further the dynamic properties of polar groups in polypeptide-lipid systems, we have measured the dielectric relaxation of L-α-lysolecithin in the presence and absence of gramicidin A within the temperature range 0–60 °C and over the frequency range 1–1000 MHz. The temperature dependence of the dielectric spectra provides information on the activation energy of the mobility of the polar group and on the effect of the micellar-to-lamellar phase transition. Comparisons between the dielectric spectra of the L-α-lysolecithin and L-α-lysolecithin-gramicidin A solutions are discussed.

2. Materials and methods

2.1. Preparation of L-α-lysolecithin micelles

L-α-Lysolecithin (Avanti Polar Lipids, Birmingham, AL) was used without further purification. 400 mM L-α-lysolecithin was dissolved in ^2H_2O and sonicated three times for 3 min per cycle in a Heat Systems cell disruptor.

2.2. Preparation of lamellar L-α-lysolecithin packaging of gramicidin

The preparation of the lamellar solution of L-α-lysolecithin-packaged gramicidin ion channel has been described previously [27]. As an overview, gramicidin (naturally occurring gramicidin containing a mixture of gramicidin A, B and C from ICN Nutritional Biochemicals, Cleveland, OH) was used without further purification and was lyophilized. Gramicidin (40 mM) was added to the micellar L-α-lysolecithin solution (400 mM). The sample was vortex-mixed and sonicated twice for 6 min per cycle. The sample was incubated in a thermostatted bath at 70 °C for 36 h with sonication at intervals to disrupt the large particles formed during incubation. The sample was centrifuged at $3000 \times g_{max}$ for 30 min at room temperature to remove the denser particles. The supernatant was directly used in subsequent characterizations. The concentrations of gramicidin dimer was estimated by taking an aliquot of the supernatant, diluting it with methanol and conducting an ultraviolet spectral analysis. Using a molar extinction coefficient of 2.25×10^4 M^{-1} cm^{-1}, the dimer concentration was found to be 15.4 mM. The state of incorporation of each sample was checked by taking an aliquot of the solution, which was diluted with water followed by determination of the CD spectra from 300 to 185 nm. The CD spectrum characteristic of complete channel formation [28,29] was observed for all samples. Under these conditions of higher channel concentration, L-α-lysolecithin-packaged gramicidin A solution forms large multilamellar structures rather than micelles [14–16,27].

2.3. Dielectric relaxation measurements

Dielectric relaxation studies were carried out by the coaxial line/vector analyzer method [30,31]. The dielectric instrumentation used in this work has been described in a previous paper [32].

The phospholipid sample was injected into the dielectric cell and allowed to equilibrate at 60 °C. The cell temperature was decreased in 10 °C steps from 60 to 0 °C. After each temperature had been reached, the sample was allowed to equilibrate for 30 min before data collection began. 61 logarithmic scale data points were collected for the frequency range 1–1000 MHz. For the second run, the sample was allowed to equilibrate at 0 °C for 10 h. The cell temperature was then increased in 10 °C steps from 0 to 60 °C.

3. Results

The results are depicted in fig. 1. This shows the real part (top) and the imaginary part (bottom) of the dielectric permittivity of L-α-lysolecithin over the frequency range 1–1000 MHz and temperature range 0–60 °C. From the imaginary part of the dielectric permittivity a conductivity term has been subtracted. The conductivity term reflects the dielectric properties of the free ions (as impurities) in water. Table 1 lists the conductivity values. For the three-dimensional plots, linear segments connected each pair of experimental data points. Fig. 1 clearly shows the dispersion region and the corresponding dielectric loss. This dielec-

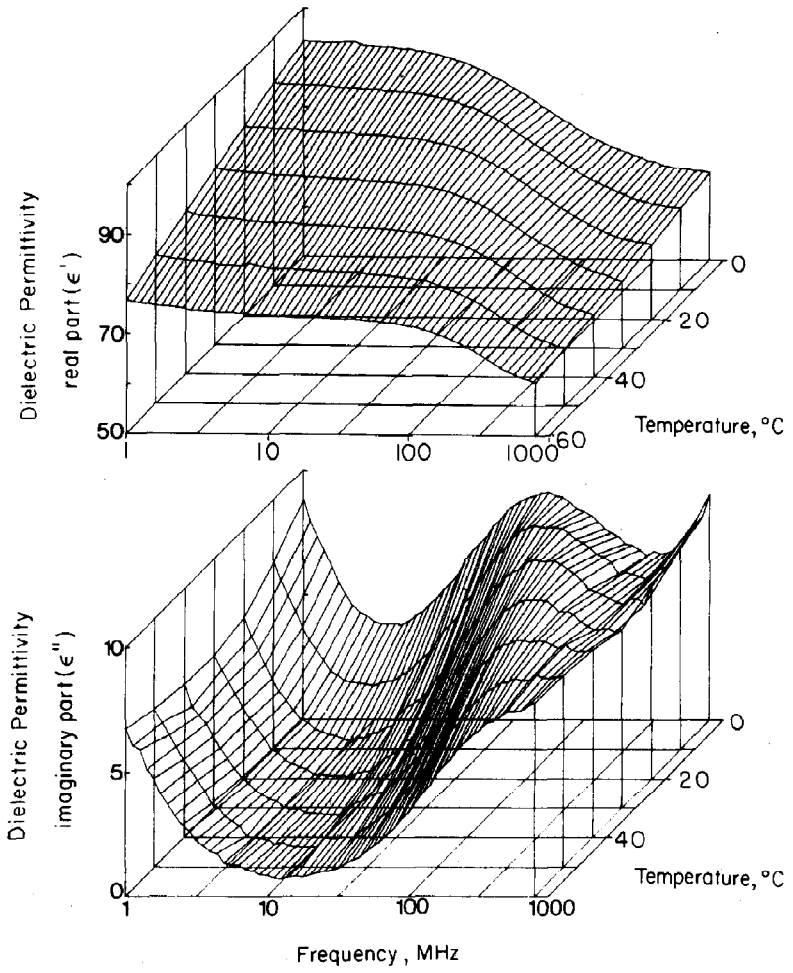

Fig. 1. The real part (top) and imaginary part (bottom) of the dielectric permittivity of 0.4 M L-α-lysolecithin in ^2H_2O solution over the temperature range 0–60 °C over the frequency range 1–1000 MHz. From the imaginary part of the dielectric permittivity the conductivity term (which represents mainly the dielectric properties of the free ions present in water) has been subtracted. The conductivity values are listed in table 1. The imaginary part of the dielectric spectrum contains a small contribution from the water spectrum, which appears within the gigahertz frequency range.

Table 1

Dielectric parameters of 0.4 M L-α-lysolecithin in ^2H_2O

Temperature (°C)	$\Delta\epsilon_{oz}$	τ_z (ns)	α	σ ($\times 10^{-3}$) (S/m)	ϵ_{sw}
0	25.7±1.2	3.0 ±0.2	0.16±0.03	1.71	67.8±0.8
10	23.7±1.2	2.1 ±0.2	0.11±0.03	2.44	66.6±0.7
20	21.7±1.2	1.54±0.1	0.07±0.04	3.23	65.0±1.0
30	19.9±1.2	1.09±0.08	0.05±0.03	3.89	63.7±0.9
40	17.2±1.0	0.88±0.09	0.01±0.01	4.65	63.0±0.9
50	15.2±0.9	0.74±0.07	0.01±0.01	5.35	62.7±1.1
60	12.9±1.2	0.65±0.04	0.01±0.01	5.89	60.9±1.1

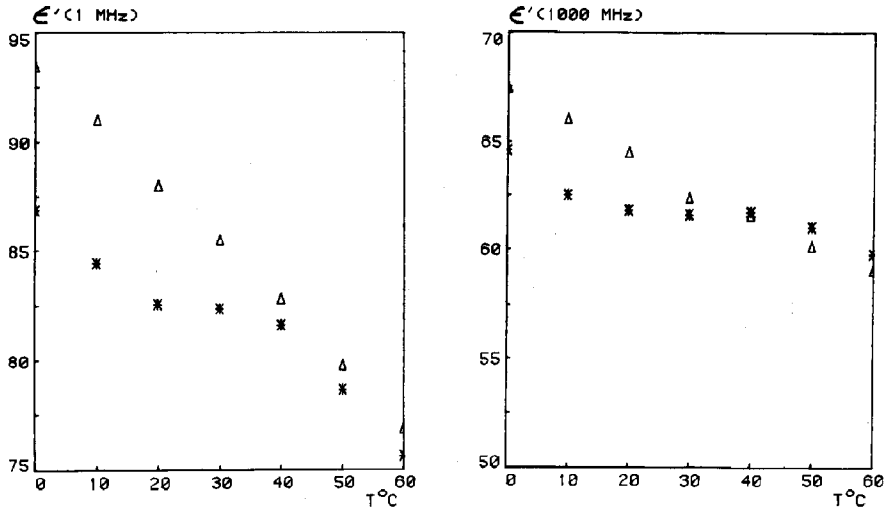

Fig. 2. Temperature dependence of the real part of the dielectric permittivity of a 0.4 M L-α-lysolecithin solution in ^2H_2O at 1 MHz (left) and 1000 MHz (right). The first run (triangles) corresponds to measurements of the dielectric permittivity of the solution from 60 to 0 °C. The second run (crosses) was begun after the solution had stood for 10 h at 0 °C and the temperature increased from 0 to 60 °C.

tric/relaxation loss is located at 103.3 MHz (20 °C). The origin of this dielectric relaxation/loss will be discussed below.

Decreasing the temperature leads to a nearly linear increase in magnitude of the dielectric relaxation. However, if the solution has been allowed to stand for 10 h at 0 °C, the magnitude of the dielectric relaxation decreases. Fig. 2 shows the temperature dependence of the real part of the permittivity at 1 MHz (left) and 1000 MHz (right). The first run (indicated by triangles) corresponds to measurement of the dielectric properties from 60 to 0 °C. The second (shown by asterisks) began after the solution had stood at 0 °C for 10 h and was subjected to temperature increases from 0 to 60 °C. The third run (not shown in fig. 2) is the same as the first, corresponding to measurements taken from 60 to 0 °C.

The high-frequency range (1000 MHz) of our dielectric relaxation measurements corresponds to the dielectric permittivity of water, since that of L-α-lysolecithin could be neglected over this frequency range. The decrease in the real part of the dielectric permittivity of the L-α-lysolecithin solution (at 1000 MHz) during freezing of the L-α-lysolecithin solution at 0 °C is attributed to a decrease in the fractional volume of water. This can be partly explained by the fact that freezing the L-α-lysolecithin solution at 0 °C induces a slow lipid phase transition from the micellar to lamellar conformation.

From our dielectric measurements, it can be seen that the fractional volume of water is smaller in the lamellar state than in the micellar state. Huang and Mason [33] have reviewed the micellar to lamellar transition of several mixed-chain phospholipids. In addition, Enders and Nimtz [34] and Grünert et al. [35] have measured the dielectric properties within the gigahertz range of dimyristoylphosphatidylcholine (DMPC) and of dipalmitoylphosphatidylcholine (DPPC), respectively. They observed a 'hysteresis effect' on the dielectric permittivities of these bilayer phospholipids. While the nature of the phase transitions and their temperature transition differ from those of our L-α-lysolecithin solution, it is interesting to note that the earlier findings also suggest the release of water (i.e., a decrease in fractional volume of water within the phospholipids) during the lipid phase transition.

The temperature dependence of the low-frequency range (1 MHz) of the real part of the dielectric permittivity (see fig. 2, left) also demonstrates the hysteresis effect, with a greater magni-

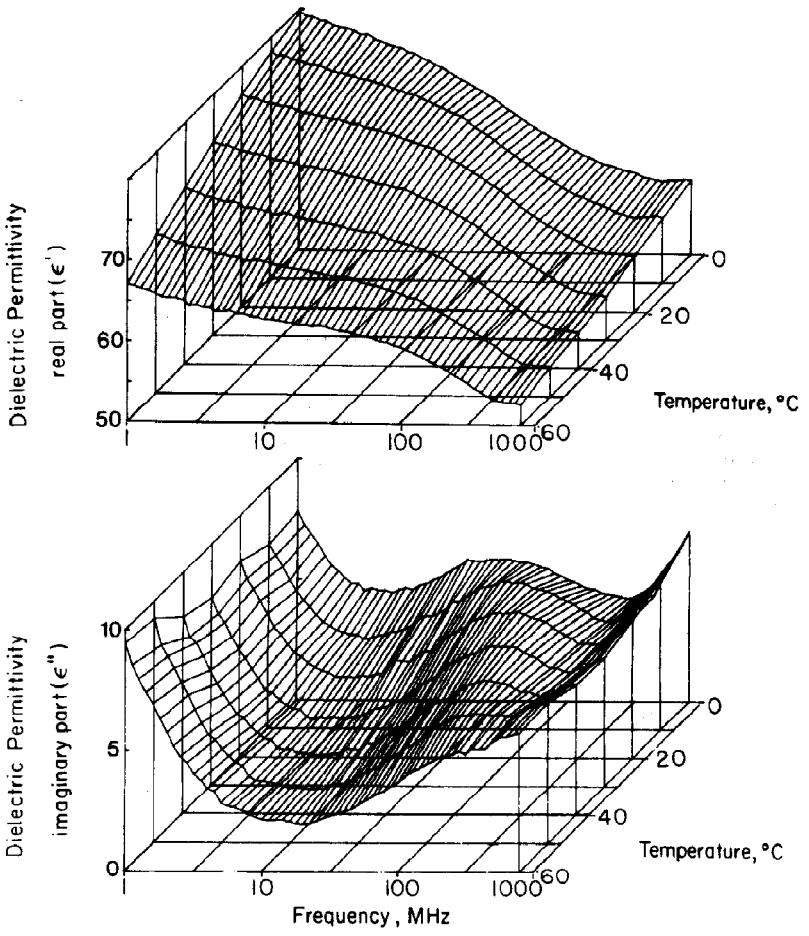

Fig. 3. The real part (top) and imaginary part (bottom) of the dielectric permittivity of gramicidin A (30.8 mM) packaged into L-α-lysolecithin (0.4 M) over the temperature range 0–60 °C and frequency range 1–1000 MHz. From the imaginary part of the dielectric permittivity, the conductivity term has been subtracted (conductivities are listed in table 2). The imaginary part of the dielectric permittivity also shows a small contribution from the water spectrum, which appears within the gigahertz frequency range.

Table 2

Dielectric parameters of the L-α-lysolecithin-gramicidin A solution in ^2H_2O (0.4 M L-α-lysolecithin; 0.0308 M gramicidin A)

Temperature (°C)	$\Delta\epsilon_{\alpha z}$	τ_z (ns)	α	σ ($\times 10^{-3}$) (S/m)	ϵ_{sw}
0	19.8±0.8	4.97±0.1	0.26±0.03	1.94	59.4±0.5
10	18.7±1.0	3.17±0.1	0.22±0.06	2.70	58.4±0.6
20	17.7±1.0	2.22±0.08	0.19±0.06	3.51	57.3±0.4
30	18.0±1.4	2.00±0.20	0.25±0.12	4.44	55.6±0.9
40	14.7±1.0	1.45±0.05	0.16±0.03	5.42	54.9±0.8
50	13.0±1.0	1.30±0.09	0.16±0.07	6.37	54.3±0.7
60	11.4±0.9	1.17±0.11	0.16±0.07	7.36	53.2±0.7

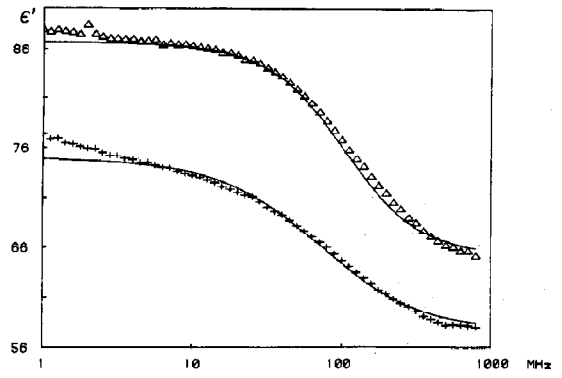

Fig. 4. The real part of the dielectric permittivities of L-α-lysolecithin (triangles) and of L-α-lysolecithin-gramicidin A (crosses) at 20 °C and over the frequency range 1–1000 MHz. The theoretical curve (full lines) was computed by using eq. 1. The respective Cole-Cole parameters are listed in tables 1 and 2.

tude than that observed at 1000 MHz (fig. 2, right). The low-frequency range of the dielectric relaxation spectrum contains a superposition of the water and L-α-lysolecithin dielectric relaxation spectra. Indeed, it is natural to suspect that a part of the decrease in the real part of the dielectric permittivity during freezing could be due to the phospholipid. During the freezing process at 0 °C, the decrease in fractional volume of water and the reduction of the dielectric permittivity of L-α-lysolecithin occur concomitantly in pure L-α-lysolecithin aqueous solution.

Fig. 3 shows the temperature dependence of the real part (top) and imaginary part (bottom) of the dielectric permittivity of the lysolecithin-packaged gramicidin channel over the frequency range 1–1000 MHz. The conductivity term has been subtracted from the imaginary part (table 2 lists the conductivity values). Fig. 3 is very similar to fig. 1. The dispersion region and the corresponding peak of dielectric loss are located at 71.7 MHz for the lamellar L-α-lysolecithin-gramicidin A solution at 20 °C. However, the magnitude of the dielectric relaxation, as well as the relaxation time and the shape of the dielectric dispersion/loss, are slightly different from that observed in the pure L-α-lysolecithin system. This is best seen in fig. 4 where the real parts of the dielectric permittivity of L-α-lysolecithin (triangles) and of L-α-lysolecithin-gramicidin A (crosses) are shown together. These differences are not due to a concentration effect, since the concentration of L-α-lysolecithin is the same in both systems. The real part of the dielectric permittivity of L-α-lysolecithin-gramicidin A solution (lamellar structure)

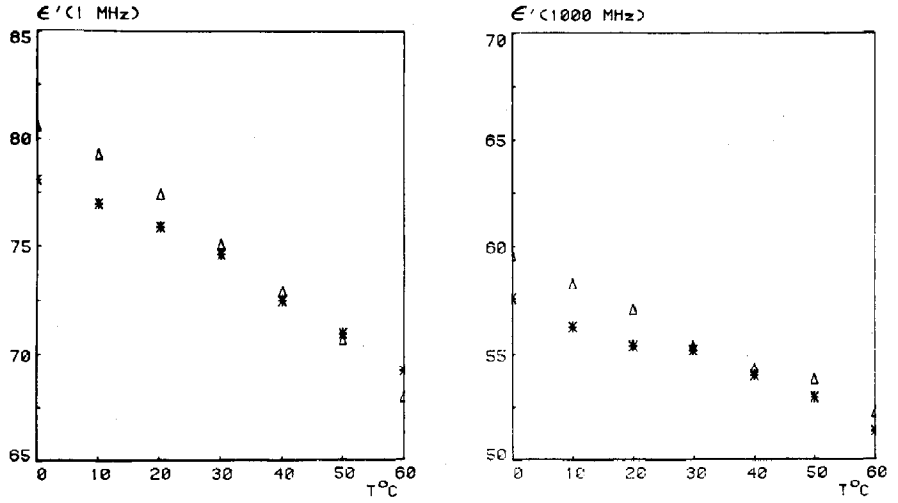

Fig. 5. Temperature dependence of the real part of the dielectric permittivity of L-α-lysolecithin-gramicidin A solution in ^2H_2O (0.4 M L-α-lysolecithin and 0.0308 M gramicidin A) at 1 MHz (left) and 1000 MHz (right). The first run (triangles) corresponds to measurements of the dielectric permittivity from 60 to 0 °C. The second run (crosses) was begun after the solution had stood for 10 h at 0 °C and reflects the increase in temperature from 0 to 60 °C.

is smaller than that of the pure L-α-lysolecithin solution (micellar structure).

These dielectric changes could be related to the micellar-to-lamellar transition induced by gramicidin A [14–16], or to the change in fractional volume of water (i.e., the fractional volume of water is smaller in the lamellar than in the micellar structure).

With respect to these suggestions, it is interesting to analyze the temperature dependence effect on the L-α-lysolecithin-gramicidin A solution. Fig. 5 illustrates the temperature dependence of the real part of the dielectric permittivity of the L-α-lysolecithin-gramicidin A solution at 1 MHz (left) and 1000 MHz (right). The first run (triangles) corresponds to measurements of the dielectric permittivity from 60 to 0°C. The second (crosses) commenced after the L-α-lysolecithin-gramicidin A solution had stood at 0°C for 10 h and corresponds to measurements of the dielectric permittivity from 0 to 60°C. Fig. 5 (L-α-lysolecithin-gramicidin A solution) shows a similar but smaller hysteresis to that in fig. 2 (L-α-lysolecithin solution). Since the solution of L-α-lysolecithin-gramicidin A corresponds to a lamellar structure, the phase transition from the micellar to lamellar conformation (which has already been induced by the addition of gramicidin A) does not occur when the solution is frozen.

4. Discussion

There are several mechanisms to explain the dielectric relaxation of L-α-lysolecithin and L-α-lysolecithin-gramicidin A solutions. One may wonder whether the dielectric relaxation/loss observed in fig. 1 for L-α-lysolecithin solution as well as in fig. 3 for L-α-lysolecithin-gramicidin A solution could be due to Maxwell-Wagner effects. Since the conductivities of these solutions are low (of the order of 10^{-3} S/m), these effects cannot contribute to the dielectric relaxation around 100 MHz (for similar accounts, see also refs. 21, 22 and 36).

The counterion dielectric relaxation could contribute to the dielectric spectrum in the low-frequency range (within the range 1 kHz–1 MHz, according to Schwan et al. [37]). The counterion dielectric relaxation originates from ionic impurities or from counterions within the phospholipids and is sensitive to the presence of salts as pointed out by several authors [22,37,38]. However, the dielectric relaxation/loss observed around 100 MHz is insensitive to the addition of monovalent ions (20 mM thallium acetate).

In our opinion, the Maxwell-Wagner and counterions effects may contribute to the dielectric spectrum at around 1 MHz and below but not around 100 MHz. One may consider whether polar groups such as the zwitterionic group of L-α-lysolecithin and water could contribute to this dielectric relaxation/loss around 100 MHz. It is well known that free or bulk water develops dielectric relaxation/loss centered at 22 GHz [39]. Several authors [22,23,40–43] have suggested that the dielectric relaxation/loss centered at 100 MHz originates from dipolar relaxation of the zwitterionic group of the phospholipids. As pointed out by Kaatze and co-workers [43], the possibility cannot be excluded that 'bound' water may show unusual dielectric behavior and may contribute to the dielectric relaxation/loss around 100 MHz.

As a summary, we suggest that the dielectric relaxation/loss centered at 100 MHz corresponds mainly to the rotational motion of zwitterionic groups of L-α-lysolecithin (in accordance with the suggestions of several authors [21–23,40–43]).

This dielectric relaxation/loss can be expressed approximately by the mean of the Cole-Cole equation [22,43]:

$$\epsilon = \epsilon' - i\epsilon'' = \epsilon_{sw} + \frac{\Delta\epsilon_{oz}}{1 + (i2\pi\nu\tau_z)^{1-\alpha}} + \frac{\sigma}{i2\pi\epsilon_0\nu} \quad (1)$$

Where ϵ is the complex permittivity, ϵ' and ϵ'' the real and imaginary parts, respectively, of the dielectric permittivity, ϵ_{sw} the permittivity of the solution (at 1000 MHz), $\Delta\epsilon_{oz}$ the amplitude of the dielectric relaxation, τ_z the relaxation time, α a parameter that describes the spread of the dielectric relaxation centered at frequency $1/2\pi\tau_z$, ν the frequency of the alternating electric field, ϵ_0 the permittivity of a vacuum and σ the conductivity term.

Since water (bulk or free water) develops dielectric relaxation with the gigahertz range and since the dielectric relaxation/loss falls within the 100 MHz range, we did not account for the water contribution by adding a second Cole-Cole term. In this frequency range, the water contribution is contained mainly in the ϵ_{sw} term. Table 1 details the parameters of eq. 1 obtained by curve-fitting analysis on the real part of the dielectric permittivity. Fig. 4 shows the quality of curve fitting on the L-α-lysolecithin solution at 20 °C, the experimental data being indicated by triangles and the full line corresponding to the best fit. Our dielectric parameters are of the same order of magnitude as those of Pottel et al. [41]. They determined the following values for a similar solution of 0.37 M L-α-lysolecithin at 25 °C: $\epsilon_{sw} = 56.8$; $\Delta\epsilon_{oz} = 24$; $\tau_z = 0.95$ ns and $\alpha = 0.09$. Our ϵ_{sw} value is slightly overestimated because our high-frequency limit of dielectric measurement is fixed at 1000 MHz, and perhaps ϵ_{sw} is better estimated within the range 1–3 GHz. The temperature dependence of the dielectric parameters, which has not been reported, provides some information on the mobility of the polar groups.

The Cole-Cole parameter α increases when the temperature is decreased, indicating a greater spread of dielectric relaxation. This could be interpreted from the formation of some conformational states, which differ slightly from each other, with decreasing temperature. This result is predictable in view of the micellar-to-lamellar transition at low temperatures.

The temperature dependence of the relaxation time could provide an estimate of the activation energy of the rotational motion of polar groups. Since we have attributed this dielectric relaxation/loss to the rotational mobility of zwitterionic groups of L-α-lysolecithin, we can estimate the activation energy of the zwitterionic groups rotational mobility. We obtained a value of 4.1 ± 0.2 kcal/mol or 17.2 ± 0.8 kJ/mol by using the Eyring equation. Fig. 6 shows the temperature dependence of the relaxation time and the best fit. Shepherd and Büldt [40] determined values of 2.6 and 4.0 kcal/mol below and above 42 °C, respectively, for the activation energy of the zwitterionic group of DPPC.

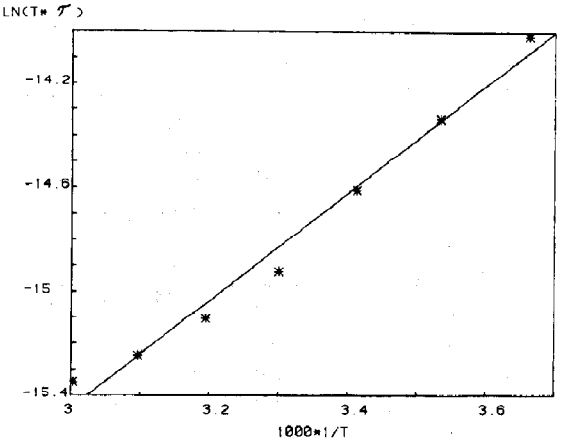

Fig. 6. Plot of $\ln(\tau T)$ vs. $1/T$ where T is the absolute temperature and τ the relaxation time (in s). The symbols represent the experimental data (0.4 M L-α-lysolecithin in ^2H_2O). The straight line corresponds to the best fit of the Eyring equation, yielding an activation energy of 4.1 ± 0.2 kcal/mol.

Table 2 presents the results of the curve-fitting analysis on the real part of L-α-lysolecithin-packaged gramicidin A. The dielectric permittivity was analyzed by means of one Cole-Cole function (see eq. 1). Fig. 4 shows the quality of curve fitting on the L-α-lysolecithin-gramicidin A solution, the experimental data being indicated by crosses and the full line corresponding to the best fit. Comparisons between the parameters of tables 1 and 2 show the difference that can be observed qualitatively in fig. 4. The dielectric relaxation of L-α-lysolecithin-gramicidin A is smaller than that of pure L-α-lysolecithin.

It could be expected that these dielectric parameters are sensitive to the micellar-to-lamellar phase transition and/or to the interaction between gramicidin A and L-α-lysolecithin. The decrease in the dielectric relaxation amplitude ($\Delta\epsilon_{oz}$) could be interpreted as a restriction of the rotational mobility of the phospholipid zwitterion.

The Cole-Cole parameter α of the L-α-lysolecithin-gramicidin A system is greater than that of L-α-lysolecithin. Since the parameter α is a measure of the spread of dielectric relaxations centered at the relaxation frequency, α also indicates the dispersion of states contributing to the dielectric relaxation. A greater α value corresponds to a

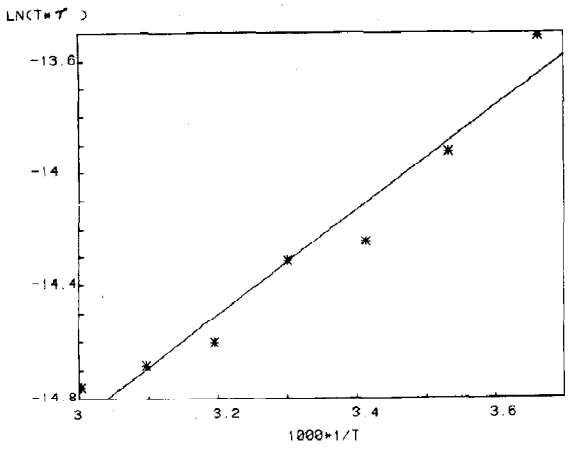

Fig. 7. Plot of $\ln(\tau T)$ vs. $1/T$ where T is the absolute temperature and τ the relaxation time (in s). The symbols represent the experimental data (0.4 M L-α-lysolecithin and 0.0308 M gramicidin A solution in ^2H_2O). The straight line corresponds to the best fit of the Eyring equation, yielding an activation energy of 3.7 ± 0.3 kcal/mol.

greater dispersion of conformational states. It is possible that gramicidin A induces small local heterogeneous conformational changes of L-α-lysolecithin, contributing to an increasing spread of dielectric relaxations.

The temperature dependence of the relaxation time of the L-α-lysolecithin-gramicidin A system could provide an estimate for the activation energy of the rotational mobility of the polar groups of L-α-lysolecithin. Fig. 7 presents the best fit, yielding an activation energy of 3.7 ± 0.3 kcal/mol or 15.4 ± 1.3 kJ/mol (the experimental data are shown as symbols and the theoretical line corresponds to the Eyring equation). Killian and De Kruijff [10] obtained a value of 17.3 kJ/mol for the mobility of the α segment of the head group of L-α-lysolecithin in a L-α-lysolecithin-gramicidin A mixture by using ^{2}H-NMR spectroscopy. This compares well with our data. The values of the activation energy resulting from the rotational motion of the polar groups of L-α-lysolecithin (4.1 ± 0.2 kcal/mol) and L-α-lysolecithin-gramicidin A (3.7 ± 0.3 kcal/mol) are similar within experimental error. The incorporation of gramicidin A into L-α-lysolecithin does not induce large changes in the enthalpy of rotational mobility of the zwitterionic group, but it does seem to decrease the mobility of the zwitterion as may be seen by collating the relaxation time and the dielectric relaxation amplitude in tables 1 and 2. For comparison, the activation energy (above the lipid phase transition temperature) of DMPC is 5.4 ± 0.2 kcal/mol and of gramicidin A packaged in DMPC, 5.3 ± 0.3 kcal/mol [44].

The dielectric permittivity of the solution at 1000 MHz (ϵ_{sw}) is greater in pure L-α-lysolecithin than in L-α-lysolecithin-gramicidin A solution (cf. tables 1 and 2). Qualitatively, this means that the fractional volume of water is greater in micellar solution (L-α-lysolecithin) than in lamellar solution (L-α-lysolecithin-gramicidin A).

5. Conclusions

The dielectric spectra of L-α-lysolecithin and L-α-lysolecithin-gramicidin A show dielectric relaxations which are centered at 103.3 MHz ($\tau_z = 1.54$ ns) at 20 °C and at 71.7 MHz ($\tau_z = 2.22$ ns) at 20 °C respectively. This dielectric relaxation has been attributed mainly to the rotational motion of the zwitterionic group according to Pottel et al. [22] and Shepherd and Büldt [40].

The hysteresis effect observed in the micellar aqueous solution of L-α-lysolecithin (fig. 2) results from a slow phase transition from the micellar to lamellar state. During freezing at 0 °C, the fractional volume of water decreases and the magnitude of dielectric relaxation at about 100 MHz decreases slightly.

A similar but smaller hysteresis effect was observed in the lamellar aqueous solution of L-α-lysolecithin-gramicidin A (fig. 5). Since the solution of L-α-lysolecithin-gramicidin A corresponds to a lamellar state, the phase transition from the micellar to lamellar conformation (which has already been induced by the addition of gramicidin A) does not occur when the solution is frozen.

The dielectric relaxation/loss of the lysolecithin solution is slightly different in the presence and absence of gramicidin A. The differences at 20 °C (see tables 1 and 2) consist of the magnitude of the dielectric relaxation ($\Delta\epsilon_{oz} = 21.7 \pm 1.2$ for L-α-lysolecithin and 17.7 ± 1 for L-α-lysolecithin-gramicidin A) and the relaxation time (τ_z

$= 1.54 \pm 0.1$ ns for L-α-lysolecithin and 2.22 ± 0.08 ns for L-α-lysolecithin-gramicidin A), as well as the permittivity of the solution (at 1000 MHz) ($\epsilon_{sw} = 65 \pm 1$ for L-α-lysolecithin and 57.3 ± 0.4 for L-α-lysolecithin-gramicidin A).

The difference in ϵ_{sw} values between the micellar solution of L-α-lysolecithin and the lamellar solution L-α-lysolecithin-gramicidin A may originate from the difference in the fractional volume of water (i.e., the fractional volume of water is smaller in the lamellar than the micellar structure).

We interpreted tentatively the small dielectric differences ($\Delta\epsilon_{oz}$, τ_{oz}) (see tables 1 and 2) as being due to restricted rotational motion of the zwitterionic group when gramicidin A is incorporated into L-α-lysolecithin. However, the activation energy for the rotational mobility of the zwitterionic group of the phospholipid is not affected by the presence of gramicidin A. The values of the activation energy of L-α-lysolecithin and L-α-lysolecithin-gramicidin A solutions are, respectively, 4.1 ± 0.2 and 3.7 ± 0.3 kcal/mol.

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