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Cholesterol attenuates the interaction of the antimicrobial peptide gramicidin S with phospholipid bilayer membranes

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Abstract

We have investigated the effect of the presence of 25 mol percent cholesterol on the interactions of the antimicrobial peptide gramicidin S (GS) with phosphatidylcholine and phosphatidylethanolamine model membrane systems using a variety of methods. Our circular dichroism spectroscopic measurements indicate that the incorporation of cholesterol into egg phosphatidylcholine vesicles has no significant effect on the conformation of the GS molecule but that this peptide resides in a range of intermediate polarity as compared to aqueous solution or an organic solvent. Our Fourier transform infrared spectroscopic measurements confirm these findings and demonstrate that in both cholesterol-containing and cholesterol-free dimyristoylphosphatidylcholine liquid-crystalline bilayers, GS is located in a region of intermediate polarity at the polar–nonpolar interfacial region of the lipid bilayer. However, GS appears to be located in a more polar environment nearer the bilayer surface when cholesterol is present. Our ^{31}P-nuclear magnetic resonance studies demonstrate that the presence of cholesterol markedly reduces the tendency of GS to induce the formation of inverted nonlamellar phases in model membranes composed of an unsaturated phosphatidylethanolamine. Finally, fluorescence dye leakage experiments indicate that cholesterol inhibits the GS-induced permeabilization of phosphatidylcholine vesicles. Thus in all respects the presence of cholesterol attenuates but does not abolish the interactions of GS with, and the characteristic effects of GS on, phospholipid bilayers. These findings may explain why it is more potent at disrupting cholesterol-free bacterial than cholesterol-containing eukaryotic membranes while nevertheless disrupting the integrity of the latter at higher peptide concentrations. This additional example of the lipid specificity of GS may aid in the rational design of GS analogs with increased antibacterial but reduced hemolytic activities.

Keywords: Cholesterol; Gramicidin S; Antimicrobial peptide; Lipid–peptide interaction; Circular dichroism; Fourier transform infrared spectroscopy; ^{31}P-nuclear magnetic resonance spectroscopy; Calcein leakage

Abbreviations: GS, gramicidin S; MLV, large multilamellar vesicle; LUV, large unilamellar vesicle; SUV, small unilamellar vesicle; CD, circular dichroism; FTIR, Fourier transform infrared; NMR, nuclear magnetic resonance; TFE, trifluoroethanol; PC, phosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; POPC, 1-palmitoyl,2-oleoyl-phosphatidylcholine; DPEPE, dipalmitelaidoylphosphatidylethanolamine

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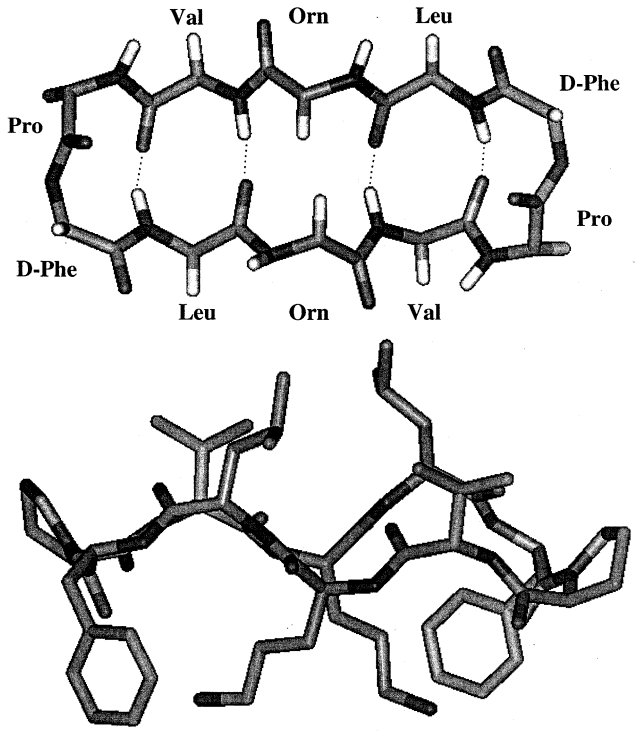

Fig. 1. Structure and conformation of GS. (Upper panel) Top view of the backbone structure of GS indicating the positions of the β-sheet interstrand hydrogen bonds. (Lower panel) Side view of GS indicating the disposition of the hydrophobic (top) and basic (bottom) residues relative to the peptide ring.

1. Introduction

Gramicidin S (GS) is a cyclic decapeptide of primary structure (cyclo-(Val-Orn-Leu-D-Phe-Pro)$_2$) secreted by *Bacillus brevis*. It is a powerful antimicrobial agent with potent cidal action on a wide variety of Gram-negative and Gram-positive bacteria as well as on several pathogenic fungi. Unfortunately, however, GS is rather non-specific in its actions and also exhibits a relatively high hemolytic activity, limiting its use as an antibiotic to topical applications. The GS molecule exists as a very stable amphiphilic antiparallel β-sheet structure in a wide variety of environments, with a polar and a non-polar surface (see Fig. 1). Moreover, the large number of structure–activity studies of GS analogs which have been carried out indicate that this 'sidedness' structure is required for its antimicrobial action (for reviews, see [1–3]). There is good evidence from studies of the interaction of GS with bacterial cells that the destruction of the integrity of the lipid bilayer of the inner membrane is the primary mode of the antimicrobial action of this peptide. Moreover, 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 headgroups and above the hydrocarbon chains. GS also binds more strongly to anionic than to zwitterionic or uncharged phospho- and glycolipids. The presence of GS appears to perturb lipid packing in liquid-crystalline bilayers and GS can induce the formation of inverted cubic phases at lower temperatures in lipids capable of forming such phases at higher temperature in the absence of peptide. The presence of GS at lower concentrations also increases the permeability of model and biological membranes and at higher concentrations causes membrane destabilization (see [1–3]). The considerable lipid specificity of GS for binding to and destabilization of lipid bilayer model membranes indicates that the design of GS analogs with an improved antimicrobial potency and a markedly decreased toxicity for eukaryotic cell plasma membranes should be possible (see [1–5])

A major difference between the lipid compositions of bacterial and animal cell surface membranes is the presence of cholesterol in the latter but not in the former membrane systems [6,7]. In fact, cholesterol is a major component of animal cell plasma membranes, comprising up to almost 50 mol percent of the total membrane lipid. However, although a number of biophysical studies of the interactions of GS with phospholipid model membranes have been carried out, none of these previous studies have addressed the effect of the presence of cholesterol on the nature and strength on GS–phospholipid interactions (see [1–3]). In order to rectify this situation, we have carried out the present study with the goal of determining the effect of cholesterol on the binding and penetration of GS into phosphatidylcholine (PC) bilayers and on the ability of GS to induce inverted nonlamellar phases in phosphatidylethanolamine dispersions. We have also investigated the effect of cholesterol on the ability of GS to permeabilize liposomes composed of PC. In all cases the presence of cholesterol was found to attenuate the characteristic effects of GS on the target phospholipid model membrane.

2. Materials and methods

Phospholipids and cholesterol were obtained from Avanti Polar Lipids Inc. (Alabaster, AL, USA) and used without further purification. Calcein was supplied by Molecular Probes (Eugene, OR, USA). GS was obtained from Sigma (St. Louis, MO, USA) and purified using the methodology described by Kondejewski et al. [4]. The peptide was subsequently converted to the hydrochloride salt by two cycles of lyophilization from 10 mM hydrochloric acid in order to remove the trifluoroacetate (TFA) counter ions which interfere with the monitoring of the phospholipid C=O stretching vibrations by Fourier transform infrared (FTIR) spectroscopy.

To prepare GS-containing large multilamellar vesicles (MLVs), the appropriate phospholipid or phospholipid/cholesterol mixture was co-dissolved in methanol with GS and the solvent was removed by slow evaporation under nitrogen such that the lipid/peptide mixture would be deposited as a thin film in a clean glass test tube. Subsequently, the last traces of solvent were removed by overnight evacuation and the samples hydrated with appropriate buffer by vigorous vortexing at temperatures well above the gel/liquid crystalline phase transition temperature of the phospholipid or phospholipid/cholesterol mixtures. For the circular dichroism (CD) experiments, small unilamellar vesicle (SUVs) were prepared from large MLVs by sonication with a probe sonicator for 15–20 min, followed by centrifugation for 20 min at $14\,000 \times g$ to remove titanium particles.

CD spectra were measured on a Jasco 720 spectropolarimeter (Tokyo, Japan). Ellipticities are reported as the mean residue ellipticity (θ) of GS. Spectra were measured in buffer solutions (100 μM peptide) composed of final concentrations of Tris (10 mM), NaF (150 mM) and EDTA (0.1 mM) at pH 7.4. All of the measurements were in quartz cells with 0.1 cm pathlength at 25°C. The total lipid concentration was 1 mM. Peptide concentrations between 10 and 100 μM did not reveal major differences in the shape of the spectra.

FTIR spectra were recorded with a Digilab FTS-40 Fourier transform instrument (Digilab, Cambridge, MA, USA) using procedures for sample preparation, data acquisition and subsequent data processing similar to those previously used in this laboratory [8]. Samples for such experiments were prepared by dispersing 2–3 mg of lipid (or lipid/peptide) material in 50 μl of a D_2O-based buffer composed of 50 mM phosphate, 100 mM NaCl, 1 mM NaN_3, pH 7.4.

^{31}P-nuclear magnetic resonance (NMR) spectra were recorded with a Varian Unit-300 instrument (Varian Instruments, Palo Alto, CA, USA) operating at 124.42 MHz for ^{31}P. The sample preparation methodology and data acquisition and analysis were the same as previously used in this laboratory [9]. Lipid and lipid/peptide samples were dispersed in 50 mM Tris, 100 mM NaCl, 1 mM NaN_3, 5 mM EDTA, pH 7.4.

For the fluorescence dye leakage experiments, calcein was dissolved in the same Tris buffer used for the ^{31}P-NMR experiments at self-quenching concentrations (70 mM). The lipid or lipid/peptide mixture was dried as described above and hydrated with the Calcein-containing Tris buffer. After 4 or 5 freeze/thaw cycles, large unilamellar vesicles (LUVs) were prepared by extrusion through 200 nm filters (extruder and filters were obtained from Avestin, Ottawa, Ont., Canada). Excess dye was removed on prepacked columns of PD-10 containing Sephadex G-25M (Amersham Pharmacia, Baie d'Urfé, Que., Canada) and the lipid content determined according to Broekhuyse [10]. Calcein leakage experiments were recorded with a Perkin Elmer LS-50B spectrofluorimeter (Beaconsfield, UK). The excitation wavelength was 496 nm, the emission was 517 nm and slit widths of 5 nm were used. The temperature (37°C) was controlled by an external waterbath (Neslab, Portsmouth, NH, USA).

3. Results

The CD spectra of GS dissolved in aqueous buffer or trifluoroethanol (TFE)/H_2O (1:1, v/v), or incorporated into SUV's of egg PC or egg PC/Cholesterol (4:1) mol ratio), are presented in Fig. 2. The CD spectrum in aqueous buffer exhibits two minima at 208 and 217 nm of almost comparable molar ellipticity. In TFE/H_2O, the overall shape of the CD spectrum of GS is maintained, but the two minima in the molar ellipticity values are shifted to shorter

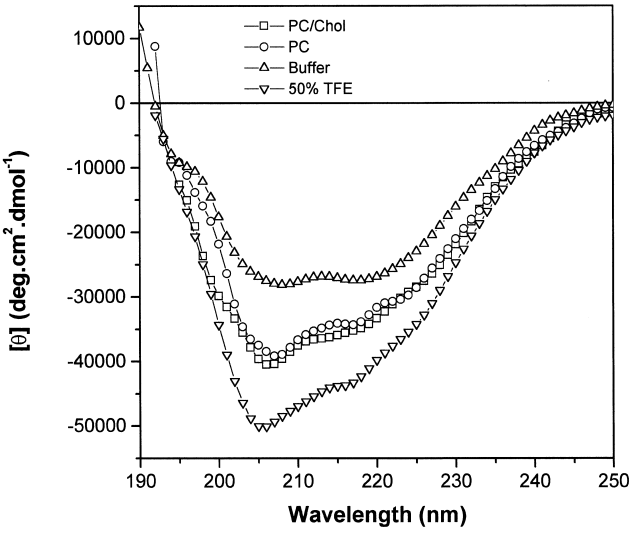

Fig. 2. CD spectra for GS dissolved in aqueous buffer (-△-△-) or TFE:H_2O (1:1, by volume) (-▽-▽-) or associated with egg PC SUVs containing (-□-□-) or not containing (-○-○-) 20 mol percent cholesterol. The total lipid concentration is 1 mM.

wavelengths, the ratio of the ellipticity minimum at the shorter to that of the longer wavelength increases, and the molar ellipticity curve in general is shifted to more negative values. The CD spectra of GS incorporated into egg PC or egg PC/cholesterol SUV's exhibits behavior intermediate between that in buffered H_2O and TFE/H_2O, although the cholesterol-containing vesicles exhibit an ellipticity minimum at slightly higher wavelength and the spectrum as a whole is slightly less negative. Although a more detailed interpretation of these results will be given in Section 4, for now we simply note these CD spectra indicate that the characteristic β-sheet/β-turn conformation of GS is maintained in environments of widely different polarity and hydrogen-bonding potential, in agreement with extensive previous work (see [1–3]) and with the FTIR spectroscopic results presented previously [11] and below.

A large number of studies have established that the characteristic conformation of the GS molecule is maintained in water, in protic and aprotic solvents of widely varying polarity, and in detergent micelles and phospholipid bilayers, over a wide range of temperature (see [1–3]). We have recently taken advantage of the conformational invariance of this peptide to relate changes in the frequency of the infrared amide I absorption band of the β-sheet region of the GS molecule exclusively to the polarity and hydrogen-bonding potential of its environment, since such frequency changes should not arise from alterations in the secondary or tertiary structure of the GS molecule itself. This approach has permitted us to determine the degree of association of the GS molecule with the gel and liquid-crystalline states of phospho- and glycolipid bilayers and their location in such model membrane systems [11]. We apply the same approach here to determine the effect of the presence of cholesterol on the interaction of GS with dimyristoylphosphatidylcholine (DMPC) MLVs above and below their gel/liquid-crystalline phase transition temperatures.

The C=O stretching absorption band of the phospholipid molecules and the amide 1 absorption band of the GS molecules, at temperatures below (0°C) and above (50°C) the gel/liquid-crystalline phase transition temperature range of DMPC MLVs with or without 25 mol percent cholesterol, are shown in Fig. 3. In DMPC MLVs, the broad amide I absorption centered at 1638 cm^{-1} in the gel state consists of two components centered near 1628 and 1646 cm^{-1}, while in the liquid-crystalline state, the overall center

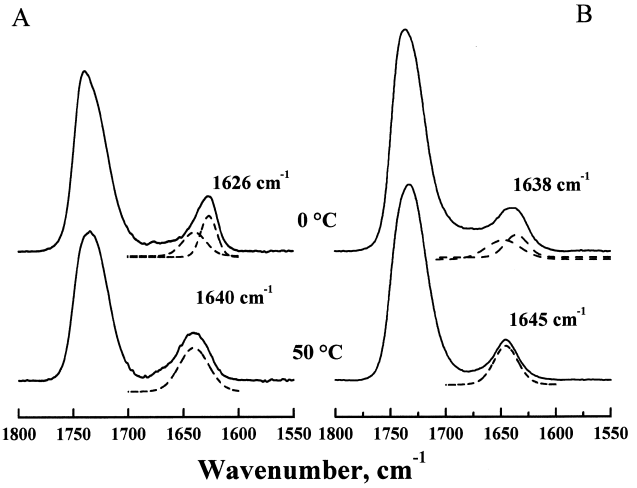

Fig. 3. FTIR spectra showing the C=O stretching and amide I bands of cholesterol-free (A) and cholesterol-containing (B) DMPC liposomes. The upper traces show spectra acquired at temperatures below the onset of the lipid hydrocarbon chain-melting phase transition (0°C) whereas the lower traces show spectra acquired at temperatures above the completion of the lipid hydrocarbon chain-melting phase transition (50°C). GS-containing samples were prepared at lipid:peptide ratios of 25:1 and the cholesterol-rich lipid samples contained 25 mol percent cholesterol. The dotted lines represent the single components that could be resolved in the peaks.

of gravity of the now sharper amide I band shifts to a frequency near 1645 cm^{-1} and is not obviously multicomponent. We have shown previously that the upward shift in amide I band frequency with temperature is due to a decrease in environmental polarity resulting from the increased penetration of GS molecules into the interfacial region of DMPC bilayers in the liquid-crystalline state, and that the amide I band component at 1628 cm^{-1} observed in the gel state arises from GS molecules in a relatively polar environment, either in the aqueous phase or at the bilayer surface [11]. Although a generally similar behavior is noted in the DMPC/cholesterol MLVs, at both low and high temperatures the frequency of the GS amide I band is lower than that observed in the cholesterol-free DMPC MLVs. In fact, at 0°C, the GS amide I band frequency of 1626 cm^{-1} in the DMPC/cholesterol MLVs is similar to that observed for GS dissolved in D_2O-based buffer [11]. These results indicate that in the gel state, cholesterol promotes the exclusion of the GS molecules from the DMPC bilayer and that in the liquid-crystalline state, the GS molecules on average reside in an environment of lower polarity, indicating less extensive penetration of the GS molecules into DMPC bilayers in the presence of cholesterol.

The ^{31}P-NMR spectra shown in Fig. 4 illustrate the effect of GS on the thermotropic phase behavior of cholesterol-free and cholesterol-containing dipalmitelaidoylphosphatidylethanolamine (DPEPE) membranes. In the absence of GS, cholesterol-free DPEPE preparations exhibit ^{31}P-NMR powder patterns consistent with their phosphate moieties undergoing fast axially symmetric motion in liquid-crystalline bilayers at all temperatures between 25 and 75°C. This result is consistent with the known phase behavior of DPEPE, which exhibits a L_β/L_α phase transition at temperatures near 21°C and a L_α/H_{II} phase transition at temperatures near 92°C [12]. The incorporation of GS into these cholesterol-free membranes has two major effects on the ^{31}P-NMR spectra observed. First, like the peptide-free sample, the ^{31}P-NMR powder patterns observed at temperatures of 40°C and below are indicative of the DPEPE forming predominantly a lamellar liquid-crystalline phase. However, in these spectra the intensity of all downfield components are markedly reduced relative to what is observed with DPEPE

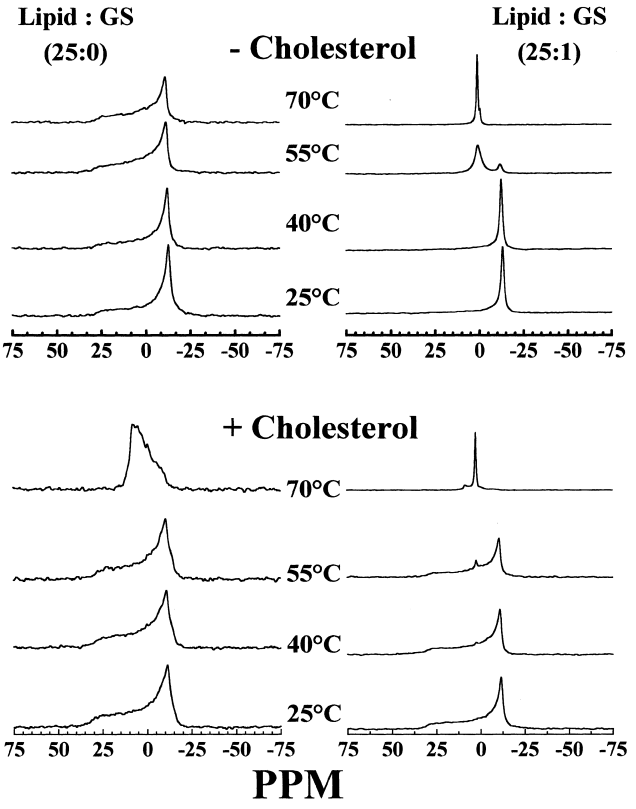

Fig. 4. Proton decoupled ^{31}P-NMR spectra showing the effect of GS on cholesterol-free (top panel) and cholesterol-containing (bottom panel) DPEPE liposomes. The data presented were acquired in the heating mode at the temperatures indicated. The data acquired with the peptide-free samples are located on the left and those observed with the peptide-containing samples (lipid:peptide ratio = 25:1) are located on the right.

alone. This pattern of behavior was observed in previous ^{31}P-NMR studies of GS-phospholipid interactions [9] and is consistent with the idea that GS makes phospholipid vesicles more susceptible to magnetically induced sample orientation under our conditions (for more details, see [9] and references therein). Second, the incorporation of GS into this DPEPE dispersion causes a marked reduction in the high-temperature stability of the lamellar liquid-crystalline phase, since at temperatures above 40°C the bilayer powder patterns are progressively replaced by an isotropic peak at higher temperatures. This pattern of behavior has been ascribed to the GS-induced formation of inverted cubic phases in these DPEPE dispersions [9] and in dispersions of the total membrane lipids from *Escherichia coli* [13]. Available data

suggest that the capacity of GS to induce cubic phase formation in lipid bilayers is a function of the intrinsic nonlamellar-forming tendencies of the host lipid and may also be a key to the hemolytic and antimicrobial activities of this peptide [9,13].

The results presented in Fig. 4 also show that the incorporation of cholesterol itself into DPEPE membranes markedly enhances the propensity of those membranes to form inverted nonlamellar structures. As noted above, lipid bilayers composed of pure DPEPE exhibit a L_α/H_{II} phase transition at temperatures near 92°C [12]. However, DPEPE dispersions containing 25 mol percent cholesterol exhibit ^{31}P-NMR powder patterns consistent with the formation of lamellar phases at temperatures only up to 55°C and powder patterns consistent with the formation of inverted hexagonal phases at temperatures of 70°C. A cholesterol-induced increase in inverted phase-forming propensity has been observed previously with other PE bilayers [14–17]. Fig. 4 shows that GS also affects the phase preferences of the cholesterol-containing DPEPE dispersion, as shown by the appearance of a small isotropic peak beginning at temperatures near 55°C, which grows in intensity and predominates at temperatures above 70°C. As noted above, the appearance of an isotropic peak in the ^{31}P-NMR spectra of GS-containing phospholipid dispersions has been ascribed to the GS-induced formation of an inverted cubic phase. Thus, cholesterol-free DPEPE membranes are more susceptible to GS-induced cubic phase formation than are the corresponding cholesterol-containing preparations. Moreover, GS-containing cholesterol-free preparations are more susceptible to magnetically induced sample orientation than are the corresponding cholesterol-containing samples (see spectra acquired at $T \leq 40$°C in Fig. 4). In particular, the loss of intensity in the downfield components of the axially symmetric powder patterns is more pronounced with the cholesterol-free GS-containing than with cholesterol-containing DPEPE dispersions. Together, these differences between the effects of GS on the cholesterol-free and cholesterol-containing DPEPE membranes provide strong evidence for a cholesterol-mediated attenuation of GS effects on the inverted nonlamellar phase-forming propensity of these phospholipid bilayers.

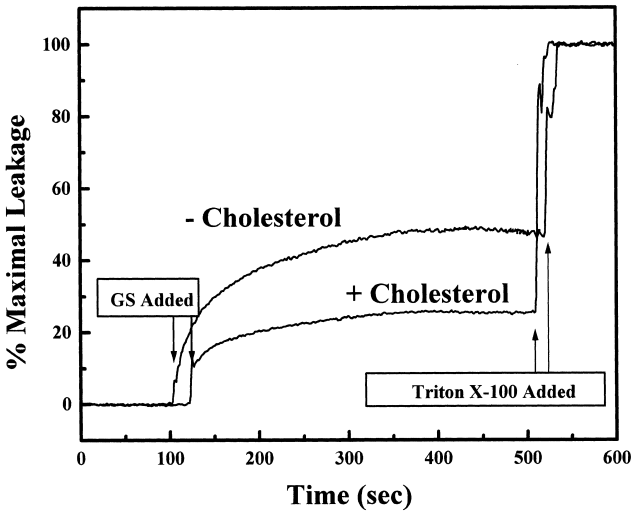

Fig. 5. GS-induced calcein leakage from POPC LUVs (25 μM lipid) in the absence and presence of cholesterol (25 mol percent) and a lipid:peptide ratio of 25:1 (mol/mol) is shown as a function of time. Maximum dye release was induced by adding 100 μl of Triton X-100 (1%) to completely lyse the LUVs.

After studying the membrane interaction and potential structural changes in the lipid bilayers induced by GS, we wished to investigate if any of the spectroscopically detectable differences in the effects of GS on cholesterol-containing and cholesterol-free model membranes are relevant to the actual membrane-disruptive potential of this peptide. We thus performed dye release experiments and induced calcein leakage from LUVs of cholesterol-free and cholesterol-containing 1-palmitoyl,2-oleoyl-phosphatidylcholine (POPC) at lipid-to-peptide ratios of 25:1. As shown in Fig. 5, the cholesterol-free and cholesterol-containing samples are both susceptible to GS-induced calcein leakage under those conditions, but the relative amount of calcein released from the cholesterol-free vesicles significantly exceeds that released from the corresponding cholesterol-containing sample. Indeed, we generally find cholesterol-containing POPC vesicles are consistently more resistant to GS-induced leakage than are the corresponding cholesterol-free vesicles under all conditions where measurable amounts of GS-induced leakage occurs. This indicates that the susceptibility of lipid membranes to GS-induced disruption is also attenuated by the presence of significant amounts of cholesterol.

4. Discussion

The CD spectrum of GS dissolved in a relatively less polar but hydrogen-bond forming organic solvent such as TFE results in an overall enhancement of molar ellipticity, an increase in the ratio of the molar ellipticity of the lower frequency minimum relative to the higher frequency minimum, and a shift of the ellipticity minima to lower frequencies as compared to H_2O, while the values in egg PC and egg PC/cholesterol SUV's are intermediate. However, the overall shapes of the CD spectra exhibited by GS in aqueous buffer, in egg PC or egg PC/cholesterol SUV's, or in TFE/H_2O (1:1, v/v) are generally similar, indicating that this molecule retains its overall β-sheet/β-turn conformation in all four systems, as expected from previous work confirming the relative conformational invariance of this peptide in a variety of environments (see [1–3]). However, the increase in the ratio of the molar ellipticity of the lower frequency minima to the higher frequency minima and the overall increase in the negative ellipticity values as one proceeds from aqueous buffer to TFE may reflect a minor conformational change, possibly a slight alteration in the twist of the antiparallel β-sheet backbone [18]. Alternatively, these spectral changes may result from an increase in the conformational rigidity of the GS molecule, due to an enhancement of intramolecular hydrogen bonding in the molecule in the less polar, less strongly hydrogen bonding environment of TFE. Alcohols of relatively lower polarity such as TFE are believed to be less effective than H_2O in their ability to form hydrogen bonds with the carbonyl and amide groups of the peptide backbone, thus increasing the strength of intramolecular hydrogen bonding and the stability and rigidity of secondary structures in general [19,20]. Moreover, the blue shift in the two minima in the CD spectra observed in going from water to TFE is expected due to a decrease in the polarity of the environment of the GS molecule [18]. Interestingly, the blue shifts in the CD spectra of GS in egg PC or egg PC/cholesterol SUV's are intermediate between those of water and TFA. This result is compatible with our previous FTIR study, which indicated that GS resides in an environment of intermediate polarity in liquid-crystalline PC bilayers, such as occurs at the glycerol backbone region of the phospholipid molecule [11].

Our present FTIR study of the effect of cholesterol on the interactions of GS with DMPC MLVs indicate that the polarity of the environment of the peptide molecule in cholesterol-containing DMPC bilayers in the biologically relevant liquid-crystalline state is higher than that of cholesterol-free DMPC bilayers, as shown by the lower frequency of the GS amide I absorption band (1640 cm^{-1} versus 1645 cm^{-1}, respectively). Since cholesterol is known to condense and order liquid-crystalline phospholipid bilayers (see [21]), this finding is consistent with the results of our previous calorimetric [22] and FTIR [11] spectroscopic studies, which indicate that GS interacts more strongly with, and penetrates more deeply into, more fluid bilayers. However, the present FTIR spectroscopic data suggest that the presence of cholesterol actually results in a greater exclusion of GS molecules in gel state DMPC bilayers as well, which is not expected, since cholesterol is well known to expand and disorder phospholipid bilayers in the gel state (see [7,21]). It is possible that the rigid fused ring system of the cholesterol molecule, and the more hydrophobic and less hydrated interface between the polar headgroups and the hydrophobic fatty acyl chains in the phospholipid bilayer produced by this sterol, may hinder the insertion of GS molecules, even though cholesterol has an overall net disordering effect on gel-phase DMPC bilayer. Clearly additional experimental work, possibly employing various cholesterol analogs, will be required to resolve this issue.

Our ^{31}P-NMR spectroscopic data show that GS-induced cubic phase formation is initiated at lower temperatures in cholesterol-free DPEPE dispersions than in the corresponding cholesterol-containing system, indicating that the presence of cholesterol markedly reduces the susceptibility of the DPEPE bilayer to the potentially disruptive effects of GS interaction. This observation is particularly notable because the cholesterol-induced attenuation of GS-mediated inverted cubic phase formation occurs despite the fact that cholesterol itself markedly promotes H_{II} phase formation in DPEPE dispersions in the absence of peptide. Since the ability of GS to induce inverted nonlamellar phases in model membranes composed of the total membrane lipids from bacteria

has been demonstrated previously and may be an important aspect of the membrane lytic ability of this peptide [9,13], this result is a potentially important one.

The results of our present spectroscopic studies indicate that the presence of cholesterol attenuates the interaction of GS with PC bilayers and the propensity of this peptide to induce inverted nonlamellar phases in PE bilayers. Moreover, the results of our calcein leakage experiments indicate that these effects are also manifest in the ability of this peptide to induce lipid bilayer membrane permeabilization or lysis, since cholesterol-containing POPC LUVs are more resistant to GS-induced permeabilization than are cholesterol-free POPC LUVs. The present results support an earlier report which indicated that the addition of cholesterol (amount unspecified) to egg PC LUVs decreased the rate of K^+ leakage by about 25% when GS was added at a phospholipid/peptide ratio of 125:1 [23]. Note, however, that although the presence of 25 mol percent cholesterol decreases the rate of calcein leakage by about half in POPC LUVs, these model membranes are nevertheless still permeabilized by GS at a phospholipid/peptide ratio of 25:1 and can be completely lysed at higher peptide concentrations. This finding is compatible with the action of GS on natural membranes, where previous studies have shown that this peptide will disrupt both cholesterol-containing eukaryotic plasma membranes and cholesterol-free bacterial inner membranes but is more effective against the latter (see [1,2]). In this regard it is interesting to note that in general, the actions of the antibacterial peptides synthesized by higher organisms are inhibited by the presence of cholesterol [24–28], in keeping with a minimization of cell membrane damage to the host animal by these agents. In contrast, the actions of the membrane lytic protein toxins or macrolide antifungal agents secreted by bacteria are generally not only not inhibited by the presence of cholesterol, but this sterol may even be a specific target for the disruption of the membrane lipid permeability barrier [29–32]. The fact that GS is relatively more potent against cholesterol-free prokaryotic than against eukaryotic cholesterol-containing membrane suggests that the primary target of this bacterially synthesized antimicrobial peptide is competing bacterial species in its natural environment.

The decreased sensitivity of cholesterol-containing model and biological membranes to the action of GS should aid us in our search for GS analogs with increased antimicrobial activity and decreased hemolytic activity, since we can, in principle, design GS analogs whose interactions with cholesterol-containing phospholipid model membranes are diminished with respect to the parent compound. However, our previous calorimetric [22] and FTIR spectroscopic [11] studies indicate that the strength of GS interactions with various phospholipid bilayers increases with the degree of negative charge and with the fluidity of these model membranes, and our previous ^{31}P-NMR spectroscopic [9] and X-ray diffraction studies [13] also suggest that GS destabilizes lipid bilayers by increasing the negative monolayer curvature of such structures through interaction with inverted nonlamellar phase-forming phospho- and glycolipids. The question of whether or not cholesterol exerts its attenuating effects on GS action through its effects on one or more of these three factors, or through another mechanism, awaits further study. However, the rational design of GS analogs with a higher therapeutic index must take into account the fact that GS appears to interact with model and biological membranes in complex, multifaceted ways.

Acknowledgements

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