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Partitioning of gramicidin A′ between coexisting fluid and gel phospholipid phases

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The partitioning behavior of gramicidin A′ was investigated in four binary phospholipid mixtures with coexisting fluid and gel phases. The ratio of the equilibrium peptide concentration in the fluid phase to that in the gel phase (i.e., the partition coefficient, K_P) was determined by analysis of the quenching of gramicidin A′ tryptophanyl fluorescence by a spin-labeled phosphatidylcholine. The partition coefficient was used as a measure of the relative solubility of gramicidin A′ in the four types of gel phases analyzed. The composition of the gel phase was entirely Ca(dioleoylphosphatidylserine)$_2$ (Ca(di18:1-PS)$_2$), or was rich in either distearoylphosphatidylcholine (di18:0-PC), dipalmitoylphosphatidylcholine (di16:0-PC), or dimyristoylphosphatidylcholine (di14:0-PC). Except in the last case, the gel phase was depleted of gramicidin A′: $K_P \sim 30$ when the gel phase was Ca(di18:1-PS)$_2$ or di18:0-PC-rich, $K_P \sim 10$ when the gel phase was di16:0-PC-rich, and $K_P \sim 1$ when the gel phase was di14:0-PC-rich. The hydrophobic mismatch between the length of gramicidin A′ and the length of the phospholipid acyl chains in the bulk gel phase is greatest with di18:1-PS and di18:0-PC, intermediate with di16:0-PC, and least with di14:0-PC. The K_P measurements presented here are consistent with increasing solubility of gramicidin A′ in the gel phase with decreasing hydrophobic mismatch.

Introduction

The fact that thermotropic gel and fluid phases can coexist in biological membranes has been known since the scanning calorimetry experiments of Steim et al. [1] and the X-ray diffraction experiments of Engelman [2] which proved that the lipids of *Acholeplasma laidlawii* are in a bilayer. The gel-to-fluid phase transition was later observed in living *A. laidlawii* cells [3,4]. Wolf and coworkers have reported that the mammalian sperm plasma membrane has coexisting fluid and gel phases at physiologically relevant temperatures [5]. Electron microscopy [6–8], transport protein activity [9] and fluorescence quenching [10] experiments have shown that proteins can be partially cleared from the gel phase when gel and fluid phases coexist. It is possible that such protein clearing from a Ca^{2+}-induced gel phase is involved at a stage of membrane fusion [11]. However, it has not been firmly established that biological membranes ordinarily can undergo phase transitions under physiological conditions.

We are interested in a quantitative description of the partitioning behavior of membrane proteins when fluid and gel lipid phases coexist. Such information would make it possible to compare the partitioning behavior not only of different proteins, but also of a given protein in the presence of different kinds of gel phases. This study is of the latter type, and compares the partitioning of gramicidin A′ in four different phospholipid systems in which gel-fluid phase coexistence can be induced. Gramicidin A′ is a linear pentade-

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Abbreviations: K_P, the partition coefficient, i.e., the ratio of gramicidin A′ concentration in the fluid phase to that in the gel phase at equilibrium; di14:0-PC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; di16:0-PC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; di18:0-PC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; di18:1-PS, 1,2-dioleoyl-*sn*-glycero-3-phosphoserine; Ca(di18:1-PS)$_2$, Ca^{2+}-induced di18:1-PS gel phase; (7,6)PC, 1-acyl-2-[2-(6-carboxyhexyl)-2-octyl-4,4-dimethyloxazolidinyl-3-oxy]-*sn*-glycero-3-phosphocholine; [(7,6)PC], the overall mole fraction of (7,6)PC; [(7,6)PC]$_L$, the mole fraction of (7,6)PC at the liquidus boundary of the phase diagram; [(7,6)PC]$_S$, the mole fraction of (7,6)PC at the solidus boundary of the phase diagram; F, observed or calculated fluorescence intensity; F_L, observed fluorescence intensity at the liquidus boundary of the phase diagram; F_S, observed fluorescence intensity at the solidus boundary of the phase diagram; F_0, observed fluorescence intensity at 0 mole fraction (7,6)PC; F_{min}, observed fluorescence intensity at 1 mole fraction (7,6)PC; DPH, 1,6-diphenyl-1,3,5-hexatriene; Pipes, piperazine-1,4-bis(2-ethanesulfonic acid).

capeptide, two monomers of which span the bilayer in the $\beta^{6,3}$-helical conformation [12,13]. This peptide is a convenient model for the membrane-spanning domain of transmembrane proteins.

The partitioning behavior of a wide variety of membrane-bound fluorescent probes can be quantitatively analyzed by a fluorescence quenching technique developed several years ago in this laboratory [10]. Early studies with gramicidin A′ showed that the peptide strongly favors the fluid phase when both a fluid and a di16:0-PC-rich gel phase coexist [10]. However, attempts to quantitate this partitioning behavior were hindered because an accurate phase diagram for the binary phospholipid mixture used was not available. Later experiments showed that the membrane-bound coat protein from bacteriophage M13 partitions strongly out of a Ca^{2+}-induced di18:1-PS gel phase [14]. This gel phase has the stoichiometry $Ca(PS)_2$ [15], and has been characterized by differential scanning calorimetry and X-ray diffraction [16], as well as by electron paramagnetic resonance and fluorescence techniques [17]. Because of the known phase behavior of the lipid system used in the M13 coat protein experiments, it was possible to determine that the equilibrium ratio of protein concentration in the fluid phase to that in the $Ca(di18:1\text{-}PS)_2$ gel phase (K_P) is about 25 [14].

This study examines the partitioning behavior of gramicidin A′ in four binary phospholipid mixtures whose phase behavior is known. When two-phase coexistence was induced, the gel phase was composed predominantly of di14:0-PC, di16:0-PC, or di18:0-PC, or entirely of $Ca(di18:1\text{-}PS)_2$. The partition coefficient is taken to be a measure of the relative solubility of gramicidin A′ in these gel phases (see Discussion). The results support the view that the ability of a gel phase to accommodate gramicidin A′ is largely governed by the degree of hydrophobic mismatch between the length of the peptide and the length of the phospholipid acyl chains in the bulk gel phase.

A preliminary account of this work was presented at a symposium on Cell and Model Membrane Interactions (155th American Chemical Society Meeting, 1990, Boston, MA), and later included in a proceedings volume for this symposium [18].

Materials and Methods

Materials

Phospholipids were from Avanti Polar Lipids (Birmingham, AL). The spin-labeled phosphatidylcholine, (7,6)PC, was prepared as previously described [19]. All phospholipids used in these experiments were judged to be >99% pure by thin-layer chromatography of 0.1 mg of each lipid on Adsorbosil-Plus 1 P* plates (Alltech Associates, Deerfield, IL), using chloroform/methanol/concentrated ammonium hydroxide (65:25:5, v/v). The natural mixture of gramicidins A, B and C from *Bacillus brevis* was purchased from Sigma Chemical Co. (St. Louis, MO) and Serva Biochemicals (Westbury, NY). The mixture of gramicidins A, B, and C is referred to as gramicidin A′. Water was purified with a Milli-Q system (Millipore Corp., Bedford, MA). Chloroform and methanol (HPLC grade) were from Mallinckrodt (Paris, KY). Pipes buffer and DPH were BioChemika MicroSelect grade and puriss grade, respectively, from Fluka (Ronkonkoma, NY). All other chemicals were reagent grade.

Stock solutions

Phospholipids in chloroform were stored under an argon atmosphere at −20°C. The phospholipid concentration of these solutions was periodically determined by phosphorus analysis [20]. Gramicidin A′ in methanol was stored under an argon atmosphere at −20°C. The concentration of this solution was periodically determined spectrophotometrically, using $\epsilon_{280} = 20.7\ cm^{-1}\,mM^{-1}$ [21].

Preparation of multilamellar vesicles

Aliquots of stock solutions of lipid (6–10 mM) in chloroform were delivered to borosilicate culture tubes to produce binary lipid mixtures of (7,6)PC together with di14:0-PC, di16:0-PC, di18:0-PC, or di18:1-PS, at mole fractions of (7,6)PC from 0 to 1. An aliquot of a stock solution of gramicidin A′ (50–100 mM) in methanol or DPH (22 mM) in chloroform was added so that the gramicidin A′/lipid mole ratio was 1/200 or the DPH/lipid mole ratio was 1/1000. Blanks containing lipid without gramicidin A′ or DPH were also prepared in order to determine the background fluorescence. Samples were dried to a thin film under a stream of nitrogen, then lyophilized from benzene/methanol (19/1, v/v) for a minimum of 12 h. Each freeze-dried sample was hydrated with buffer (20 mM Pipes, 100 mM KCl, pH 7.0) for 1 h under argon. All samples were in the fluid phase throughout the hydration period. Hydration was at 30°C and 1 mM lipid for di14:0-PC/(7,6)PC dispersions, 48°C and 1 mM lipid for di16:0-PC/(7,6)PC dispersions, 61°C and 1 mM lipid for di18:0-PC/(7,6)PC dispersions, or room temperature and 2 mM lipid for di18:1-PS/(7,6)PC dispersions. Midway through the hydration period, samples containing di14:0-PC, di16:0-PC, or di18:0-PC were briefly vortexed. At the end of the hydration period, samples containing di14:0-PC, di16:0-PC, or di18:0-PC were cooled at a rate not exceeding 0.5 C°/min to the temperature at which the fluorescence measurements were to be made. At the end of the hydration period, samples containing di18:1-PS were briefly vortexed and brought to 1 mM lipid by a 2-fold dilution with buffer containing 40 mM $CaCl_2$. The

di18:1-PS/(7,6)PC multilayer dispersions were equilibrated with Ca^{2+} by 15 cycles of freezing at −10°C and thawing in a water bath at ambient temperature, as previously described [14].

Fluorescence quenching assay

Fluorescence measurements for all samples except those containing di14:0-PC were made with a home-built spectrofluorometer [22] utilizing conventional 90° optics and equipped with double monochromators in both excitation and emission optics. The excitation and emission wavelengths were 285 and 345 nm, respectively. Nominal excitation and emission bandwidths were 2 and 8 nm, respectively. For samples containing di14:0-PC, fluorescence measurements were made with a Hitachi F-3010 fluorescence spectrophotometer with a 290 nm interference filter in the excitation beam when the fluorophor was gramicidin A′ and a 365 nm interference filter in the excitation beam when the fluorophor was DPH. The emission wavelength was 345 nm for gramicidin A′ and 425 nm for DPH. Nominal excitation and emission bandwidths were 10 and 20 nm, respectively.

Immediately before the measurement of fluorescence intensity, each sample was diluted ten-fold with buffer to a final lipid concentration of 0.1 mM. At this lipid concentration, light scattering did not contribute significantly to the fluorescence signal. The diluting buffer was identical to the buffer of the sample in composition and temperature. Background fluorescence intensity from the phospholipids was determined and used to correct the observed fluorescence intensity of the samples. Within the two-phase region of the phase diagram, this background ranged from 10 to 50% of the total signal when the fluorophor was gramicidin A′ and from 3 to 9% of the total signal when the fluorophor was DPH. Samples containing di14:0-PC, di16:0-PC or di18:0-PC were placed into a quartz microcuvette in a temperature-controlled cuvette holder. Each sample containing di18:1-PS was thawed (completing its last freeze-thaw cycle) immediately before dilution and determination of fluorescence intensity at ambient temperature (~24°C). Thawing was delayed in order to avoid complications arising from the aggregation of PS vesicles treated with excess Ca^{2+}. This aggregation becomes significant after 0.5 to 1 h at room temperature [17]. Also, PS vesicles treated with excess Ca^{2+} stick to glass, resulting in variable transfer efficiencies of the di18:1-PS/(7,6)PC dispersions [17]. Thus, after each fluorescence measurement (using a disposable acrylic cuvette; Sarstedt, Inc., Princeton, NJ) a 0.100 ml aliquot was removed from the cuvette for determination of phospholipid concentration by phosphorus analysis [20]. This determination of transfer efficiencies was used to correct the fluorescence measurements.

Determination of the partition coefficient, K_P

Quenching of the tryptophanyl fluorescence of gramicidin A′ by (7,6)PC occurs when the spin-labeled phospholipid is in contact with the peptide [19]. For this reason, the local lipid environment of gramicidin A′ can be ascertained by measuring the fluorescence intensity at various mole fractions of (7,6)PC in a binary lipid mixture whose phase behavior is known. The temperature-composition phase diagrams have been determined for the di16:0-PC/(7,6)PC and di18:0-PC/(7,6)PC dispersions by differential scanning calorimetry [23]. In addition, the phase boundaries have been established for di18:1-PS/(7,6)PC dispersions equilibrated with 20 mM Ca^{2+} at 23°C by EPR spectroscopy, X-ray diffraction, and fluorescence measurements [17]. The phase boundaries for di14:0-PC/(7,6)PC dispersions at 15°C were determined here by an analysis of the fluorescence quenching of DPH in this system. At the solidus phase boundaries, at all temperatures used in this study, the gel phase is predominantly di14:0-PC, di16:0-PC or di18:0-PC, or is entirely $Ca(di18:1\text{-}PS)_2$. Thus, the gel phase is either greatly or completely depleted of (7,6)PC. The coexisting fluid phase is enriched with the spin-labeled lipid. The partition coefficient, K_P, was determined directly from the fluorescence quenching data within the two-phase region of the phase diagram, by using the relation

$$F = F_L + \frac{[G](F_S - F_L)}{K_P(1-[G])+[G]} \quad (1)$$

where F is the observed or calculated fluorescence intensity, $F_{L(S)}$ is the observed fluorescence intensity at the liquidus (solidus) boundary of the phase diagram, and [G] is the mole fraction of total lipids in the gel phase [10]. The value of [G] is calculated from the lever rule for this two-phase system,

$$[G] = \frac{[(7,6)PC]_L - [(7,6)PC]}{[(7,6)PC]_L - [(7,6)PC]_S} \quad (2)$$

where $[(7,6)PC]_{L(S)}$ is the mole fraction of (7,6)PC at the liquidus (solidus) boundary of the phase diagram. The partition coefficient, the only unknown in Eqn. 1, was determined by fitting the experimental data to theoretical curves of F vs. [(7,6)PC] within the two-phase region of the phase diagram.

Results

Self-quenching of gramicidin A′

In order to minimize self-quenching of gramicidin A′ fluorescence during the quenching experiments, the dependence of fluorescence intensity on the peptide/lipid mole ratio was investigated at ~24°C with multi-

lamellar vesicles composed of Ca(di18:1-PS)$_2$, di16:0-PC, or di18:1-PS/(7,6)PC (0.44/0.56, mol/mol; data not shown). Self-quenching of gramicidin A′, as detected by a decrease in slope from an initially linear dependence of fluorescence on gramicidin A′ concentration, was not seen in the gel phases until a peptide/lipid mole ratio of about 1/50. In the fluid di18:1-PS/(7,6)PC phase, fluorescence increased linearly with gramicidin A′ concentration until a peptide/lipid mole ratio of about 1/35; at higher peptide/lipid mole ratios, complex self-quenching behavior was observed. This self-quenching may be due to the appearance of a gramicidin-rich H_{II} phase when the overall peptide/lipid mole ratio is greater than 1:35. For the fluorescence quenching experiments, a gramicidin A′/lipid mole ratio of 1/200 was chosen in order to obtain a sufficient fluorescence signal without significant self-quenching at any mole fraction of (7,6)PC. Note that when $K_P \gg 1$ the peptide/lipid mole ratio in the fluid phase can be quite high near the solidus boundary of the phase diagram. Thus, it is possible for self-quenching, or other phenomena dependent upon peptide concentration, to occur at some mole fractions of (7,6)PC while not at others.

Phase behavior of di14:0-PC/(7,6)PC dispersions at 15°C

The hydrophobic fluorescent probe DPH has been shown to partition about equally between coexisting fluid and gel phospholipid phases when the latter is di16:0-PC-rich or di18:0-PC-rich [23]. Because the partition coefficient of DPH is close to unity in these systems, the solidus and liquidus phase boundaries of di16:0-PC/(7,6)PC and di18:0-PC/(7,6)PC dispersions are easily observed as discontinuities in the slope of the DPH fluorescence quenching curves. In the present study, we made use of this phenomenon to determine the phase boundaries for di14:0-PC/(7,6)PC dispersions at 15°C, a temperature which is optimal for partitioning analyses with this binary mixture. At 15°C, discontinuities in the slope of the DPH fluorescence quenching curve are readily apparent at [(7,6)PC] ~ 0.06 and [(7,6)PC] ~ 0.30 (Fig. 1). When [(7,6)PC] ≤ 0.06 and when [(7,6)PC] ≥ 0.30, the data conform to the equation

$$\frac{(F - F_{min})}{(F_0 - F_{min})} = \{1 - [(7,6)PC]\}^n \quad (3)$$

where F_0 and F_{min} are the observed fluorescence intensities when [(7,6)PC] = 0 and 1, respectively. This equation describes the fluorescence quenching of a probe throughout compositions where only one phospholipid phase exists [19] and where quenching is efficient [24]. However, when 0.06 ≤ [(7,6)PC] ≤ 0.30, the data do not conform to this equation; rather, the data

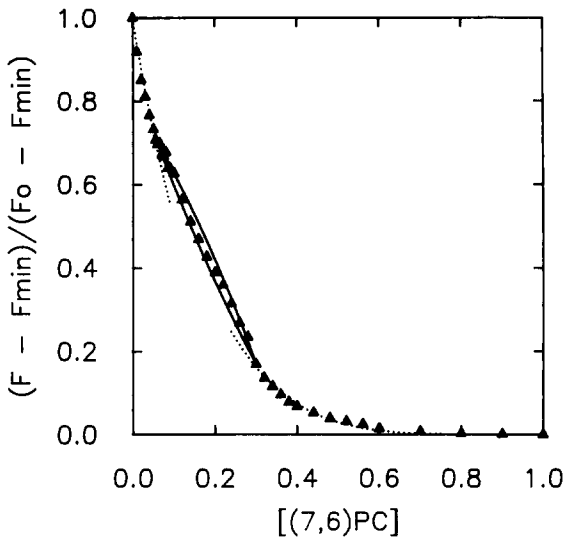


Fig. 1. Fluorescence quenching of DPH in multilamellar vesicles composed of di14:0-PC/(7,6)PC at 15°C. From [(7,6)PC] = 0 to [(7,6)PC] = 0.06, the normalized data were fit to a theoretical fluorescence quenching curve which was calculated by use of Eqn. 3 (text) setting $n = 6.2$ (upper dotted curve). Similarly, from [(7,6)PC] = 0.30 to [(7,6)PC] = 1, the normalized data were fit by Eqn. 3 (text) setting $n = 5.1$ (lower dotted curve). Throughout the compositions of gel-fluid phase coexistence, judged to be 0.06 < [(7,6)PC] < 0.30, the normalized data were fit to theoretical curves which were calculated by use of Eqn. 1 (text) setting $[(7,6)PC]_S = 0.06$, $[(7,6)PC]_L = 0.30$, $(F_S - F_{min})/(F_0 - F_{min}) = 0.70$, $(F_L - F_{min})/(F_0 - F_{min}) = 0.17$ and $K_P = 0.8$ (upper solid curve) or 1.2 (lower solid curve).

are well described by Eqn. 1 with $K_P = 1$. Thus, we infer that the solidus and liquidus phase boundaries of di14:0-PC/(7,6)PC dispersions at 15°C are $[(7,6)PC]_S$

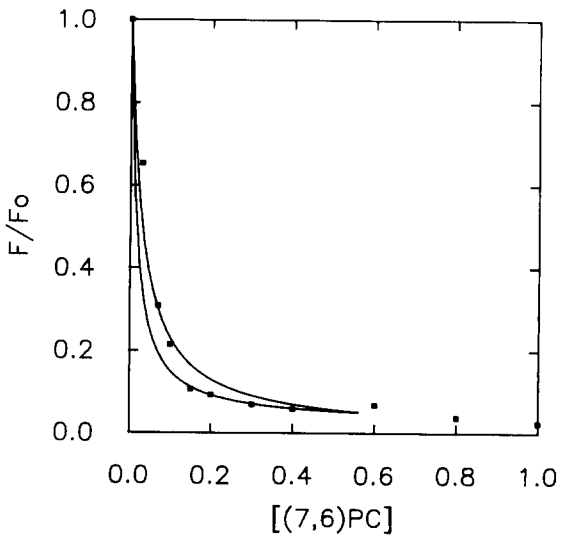
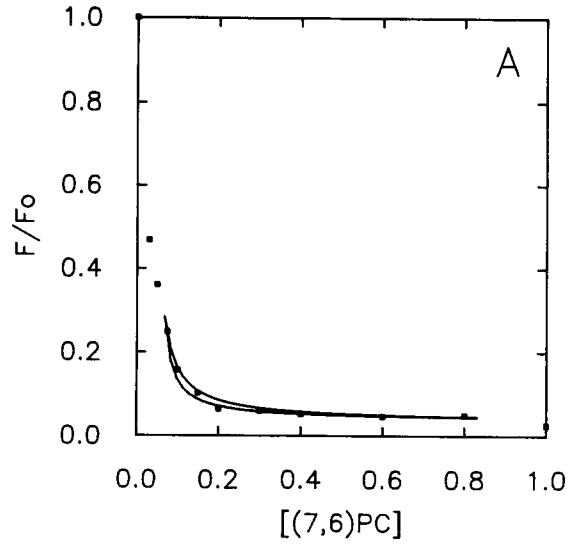
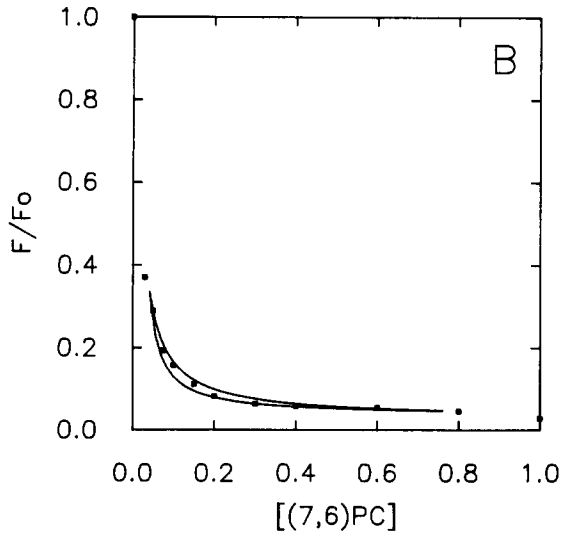


Fig. 2. Fluorescence quenching of gramicidin A′ in multilamellar vesicles composed of di18:1-PS/(7,6)PC equilibrated with 20 mM Ca^{2+} at ambient temperature (~ 24°C). All data points are averages of duplicate samples, except the [(7,6)PC] = 0 point, which is the average of quadruplicate samples. The normalized data were fit to theoretical curves which were calculated by use of Eqn. 1 (text) setting $[(7,6)PC]_S = 0.00$, $[(7,6)PC]_L = 0.56$, $F_S/F_0 = 1.00$, $F_L/F_0 = 0.051$ and $K_P = 20$ (upper curve) or 40 (lower curve).





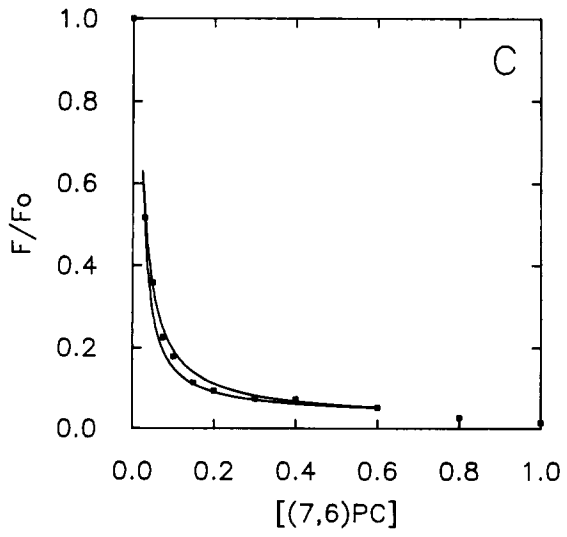


Fig. 3. Fluorescence quenching of gramicidin A′ in multilamellar vesicles composed of di18:0-PC/(7,6)PC at 35°C (A), 40°C (B) and 46°C (C). All data points are averages of duplicate samples. The normalized data were fit to theoretical curves which were calculated by use of Eqn. 1 (text) setting (A) $[(7,6)PC]_S = 0.067$, $[(7,6)PC]_L = 0.83$, $F_S/F_0 = 0.285$, $F_L/F_0 = 0.046$, and $K_P = 24$ (upper curve) or 40 (lower curve); (B) $[(7,6)PC]_S = 0.040$, $[(7,6)PC]_L = 0.76$, $F_S/F_0 = 0.33$, $F_L/F_0 = 0.047$, and $K_P = 16$ (upper curve) or 28 (lower curve); and (C) $[(7,6)PC]_S = 0.023$, $[(7,6)PC]_L = 0.59$, $F_S/F_0 = 0.63$, $F_L/F_0 = 0.053$, and $K_P = 20$ (upper curve) or 32 (lower curve).

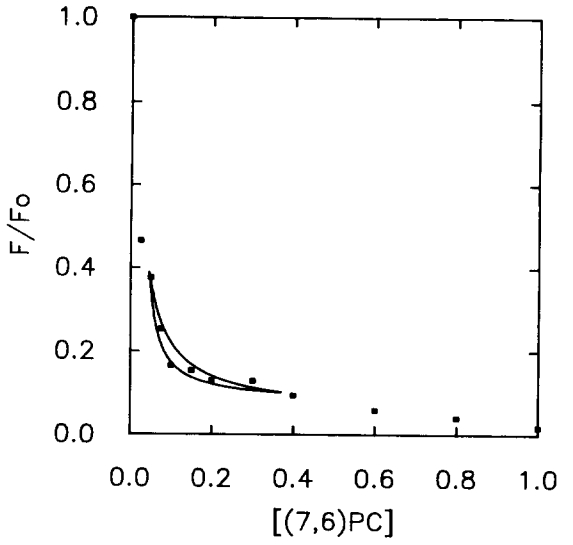


Fig. 4. Fluorescence quenching of gramicidin A′ in multilamellar vesicles composed of di16:0-PC/(7,6)PC at 34°C. All data points are averages of duplicate samples. The normalized data were fit to theoretical curves which were calculated by use of Eqn. 1 (text) setting $[(7,6)PC]_S = 0.045$, $[(7,6)PC]_L = 0.37$, $F_S/F_0 = 0.39$, $F_L/F_0 = 0.102$ and $K_P = 7$ (upper curve) or 15 (lower curve).

$= 0.06$ and $[(7,6)PC]_L = 0.30$. In other words, when $[(7,6)PC] \leq 0.06$, only a gel phase exists; when $[(7,6)PC] \geq 0.30$, only a fluid phase exists; and when $0.06 < [(7,6)PC] < 0.30$, a gel phase of composition $[(7,6)PC] = 0.06$ coexists with a fluid phase of composition $[(7,6)PC] = 0.30$, and the relative amounts of these two phases is given by the lever rule.

Partitioning behavior of gramicidin A′

It is clear from Eqn. 1 that accurate estimates of K_P require that the probe fluorescence at the solidus phase boundary, F_S, be sufficiently different than the probe fluorescence at the liquidus phase boundary, F_L. Thus, useful fluorescence quenching measurements can be made only over a limited temperature range with any given binary lipid system. For example, with gramicidin A′ in di18:0-PC/(7,6)PC dispersions at ~ 24°C, F/F_0 at the solidus phase boundary was found to be only 0.10, much too low for a reasonable determination of K_P. In order to obtain higher fluorescence at the solidus phase boundary, $[(7,6)PC]_S$ was lowered by rais-

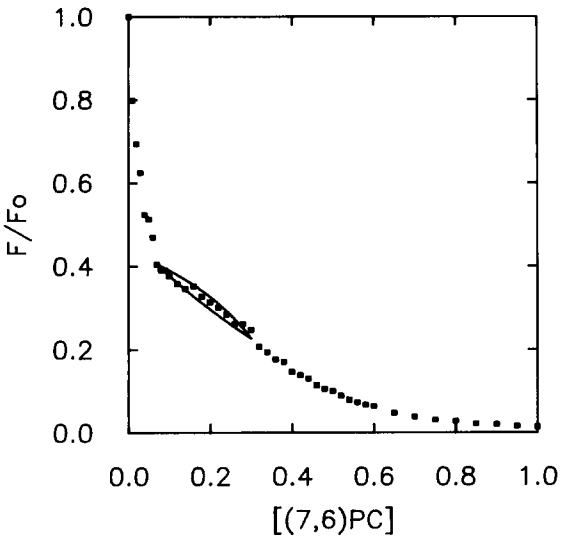


Fig. 5. Fluorescence quenching of gramicidin A' in multilamellar vesicles composed of di14:0-PC/(7,6)PC at 15°C. The normalized data were fit to theoretical curves which were calculated by use of Eqn. 1 (text) setting $[(7,6)PC]_S = 0.07$, $[(7,6)PC]_L = 0.30$, $F_S/F_0 = 0.40$, $F_L/F_0 = 0.23$ and $K_P = 0.6$ (upper curve) or 1.2 (lower curve).

ing the temperature at which the fluorescence quenching data were collected. We found that the best determinations of K_P could be made between about 40 and 46°C for di18:0-PC/(7,6)PC dispersions, at approx. 34°C for di16:0-PC/(7,6)PC dispersions, and at approx. 15°C for di14:0-PC/(7,6)PC dispersions. Data were also collected with di18:0-PC/(7,6)PC dispersions at 35°C in an attempt to detect any temperature dependence of K_P.

Figs. 2–5 show fluorescence quenching measurements for gramicidin A', with the data in the two-phase region fit to Eqn. 1. The two solid curves in each figure represent the range of K_P values which were judged to reasonably fit the data. Except when the gel phase was enriched in di14:0-PC, gramicidin A' was found to partition strongly into the fluid phase, with $K_P = 30 \pm 10$ for the peptide in di18:1-PS/(7,6)PC equilibrated with 20 mM Ca^{2+} at ~24°C (Fig. 2); $K_P = 32 \pm 8$, 22 ± 6 and 26 ± 6 for the peptide in di18:0-PC/(7,6)PC at 35, 40 and 46°C, respectively (Fig. 3); $K_P = 11 \pm 4$ for gramicidin A' in di16:0-PC/(7,6)PC at 34°C (Fig. 4); and $K_P = 0.9 \pm 0.3$ for gramicidin A' in di14:0-PC/(7,6)PC at 15°C (Fig. 5).

Discussion

In order to quantitate the partitioning of gramicidin A' between two coexisting phases by the fluorescence quenching technique used in this study, it is necessary to know the lipid compositions of both phases. We have determined the phase behavior of the four binary phospholipid mixtures used in these experiments [17,23]. However, it is important to know whether gramicidin A' significantly alters the phase behavior of these lipid systems at the peptide/lipid mole ratio used (1/200). The effect of gramicidin A' on the solidus boundaries of the di14:0-PC/(7,6)PC, di16:0-PC/(7,6)PC and di18:0-PC/(7,6)PC phase diagrams can be estimated by calculating the freezing point depression of the thermotropic gel phases when the peptide is present. Given that the enthalpy change of fusion is approx. 10 kcal/mol [25], and making the first-order approximations that the peptide mixes ideally in the fluid phase but is insoluble in the gel phase, the freezing point depression is only about 0.1°C. It follows that the liquidus phase boundaries also are not significantly affected by the presence of gramicidin A', when the peptide/lipid mole ratio is 1/200. Furthermore, the solidus phase boundary of di14:0-PC/(7,6)PC dispersions at 15°C, as determined by observing discontinuities in the slopes of the gramicidin A' and DPH fluorescence quenching curves, was essentially the same whether the fluorescent probe was gramicidin A' at a probe/lipid mole ratio of 1/200 or DPH at a probe/lipid mole ratio of only 1/1000.

It was generally observed that (7,6)PC was extremely efficient at quenching gramicidin A' tryptophanyl fluorescence in all of the thermotropic gel phases analyzed. We attribute this phenomenon to a nonrandom distribution of lipids and gramicidin A' in the thermotropic gel phases, with (7,6)PC preferentially in contact with the peptide. However, this nonrandomness does not affect our data analysis because Eqn. 1 requires only the *values* of fluorescence at the phase boundaries, without regard to a model of the mixing behavior within each phase.

While the best determinations of K_P could be made between about 40 and 46°C for di18:0-PC/(7,6)PC dispersions, data were also collected with this system at 35°C in an attempt to detect any temperature dependence of K_P. However, given the range of reasonable fits of Eqn. 1 to our data, no clear temperature dependence of K_P was found from 35 to 46°C. Thus, within the error limits of our measurements, it is reasonable to compare all of the partition coefficients determined in this study, even though they were obtained at different temperatures.

We assume that the solubility of gramicidin A' in the fluid phase is similar with all four binary phospholipid mixtures analyzed because the fluid phase compositions are similar; each liquidus boundary has a large fraction of (7,6)PC. This assumption allows K_P to be used as a measure of the relative solubility of gramicidin A' in the four types of gel phase analyzed.

In this study, four binary phospholipid mixtures were compared. The partition coefficient of gramicidin A' was not the same in the four systems. This implies that the solubility of gramicidin A' in a gel phase is sensitive to the nature of the gel phase. One way that

these gel phases differ is in the degree of hydrophobic mismatch between the length of gramicidin A′ and the length of the phospholipid acyl chains in the bulk gel phase. In these experiments, with the exception of the di14:0-PC-rich gel phase, the length of gramicidin A′ [26] was significantly less than the length of the acyl chains in the bulk gel phase [27]. We estimate from space-filling models that the di18:0-PC-rich gel phases and the highly ordered Ca(di18:1-PS)$_2$ gel phase have hydrophobic thicknesses which are about equal, and that the di16:0-PC-rich gel phase is about 5 Å thinner than these phases, and that the di14:0-PC-rich gel phase is about 5 Å thinner yet. Our K_P measurements suggest that hydrophobic mismatch may be a key factor in determining the solubility of gramicidin A′ in a gel phase, with peptide solubility increasing with decreasing hydrophobic mismatch. Similar findings have been reported for indocarbocyanine dyes partitioning between coexisting fluid and gel phases; dye solubility in the gel phase is maximized when there is an approximate match between the dye alkyl chain length and the phospholipid acyl chain length in one leaflet of the gel phase bilayer [28,29].

Of course, the solubility of gramicidin A′ in a fluid phase could also be affected by the degree of hydrophobic mismatch. However, we cannot address this issue here because the large mole fraction of (7,6)PC at each of the liquidus phase boundaries minimizes the differences in hydrophobic thickness among the fluid phases.

The mattress model of Mouritsen and Bloom [30] provides a plausible explanation for a strong dependence of the solubility of gramicidin A′ in a bilayer phase on hydrophobic mismatch. According to the mattress model, the distortion energy of a bilayer due to the incorporation of a transmembrane protein is minimized when the hydrophobic mismatch is minimized. In our study, the length of the gramicidin A′ dimer matches the bulk phase hydrophobic thickness best with the fluid phases and with the di14:0-PC-rich gel phase, less well with the di16:0-PC-rich gel phase, and least well with the di18:0-PC-rich gel phases and the Ca(di18:1-PS)$_2$ gel phase. We propose that the accommodation of gramicidin A′ in a relatively thick gel phase is accomplished by a dimpling of the bilayer, with a fluidized lipid annulus surrounding the peptide (Fig. 6); and as the distortion of the bilayer required to accomplish this increases, the ability of the gel phase to accommodate gramicidin A′ decreases. Note that the fluidization of a few layers of lipid around a single peptide molecule does not represent the appearance of a new phase. Rather, it represents the mechanism by which the peptide is accommodated within one phase.

When $K_P \gg 1$ the peptide/lipid mole ratio in the gel phase can be quite low, especially near the liquidus phase boundary. Due to the effect of mass action, the lower peptide concentration in the gel phase shifts the gramicidin A′ monomer-dimer equilibrium toward the monomer. Indeed, a significant proportion of the gramicidin A′ in the di16:0-PC-rich, di18:0-PC-rich and Ca(di18:1-PS)$_2$ gel phases could be monomeric. However, this does not affect our conclusion that the solubility of gramicidin A′ in a gel phase is largely dependent on the degree of hydrophobic mismatch. In the case of monomeric gramicidin A′, the mismatch would occur only in one leaflet of the bilayer (Fig. 6).

Properties of the gel phase other than hydrophobic thickness might also influence the solubility of a membrane-bound peptide. For instance, the degree of order of the phospholipid acyl chains in the bulk gel phase might have an effect. The Ca^{2+}-induced di18:1-PS gel phase is more highly ordered than the temperature-induced gel phases used in this study, as shown by X-ray diffraction [16] and EPR spectroscopic measurements [17]. Despite these differences in order, we found that $K_P \sim 30$ for gramicidin A′ in di18:0-PC/(7,6)PC dispersions and in di18:1-PS/(7,6)PC dispersions equilibrated with 20 mM Ca^{2+}. This suggests that the order

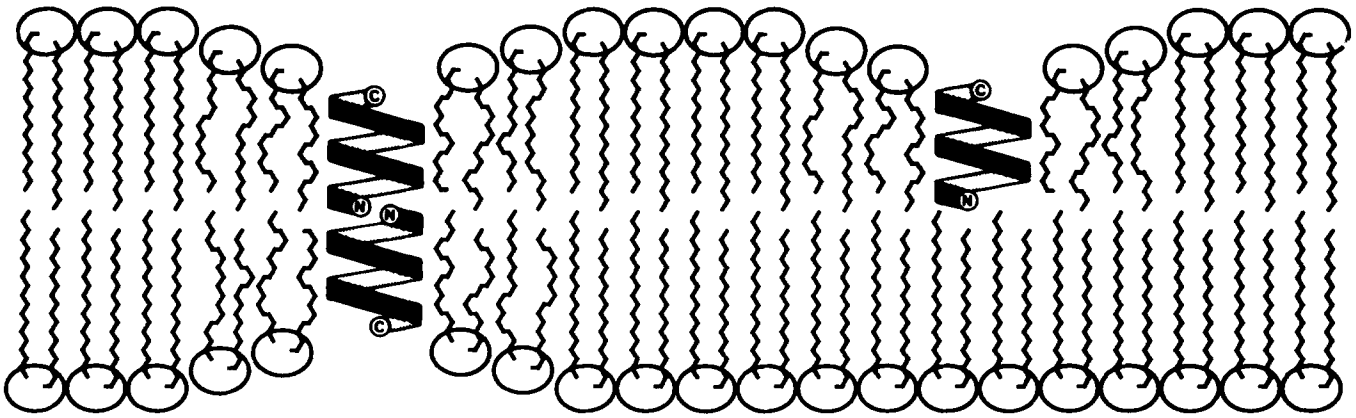


Fig. 6. Schematic drawing of gramicidin A′ in a gel phase where there is hydrophobic mismatch between the length of the peptide and the length of the phospholipid acyl chains in the bulk gel phase, with the peptide being shorter.

of a gel phase may not be a dominant factor in determining the solubility of gramicidin A′ within that phase.

An earlier study showed that the partition coefficient of M13 bacteriophage coat protein in di18:1-PS/(7,6)PC equilibrated with 20 mM Ca^{2+} is 25 [14]. This is quite similar to the partition coefficient measured here for gramicidin A′ in the same binary phospholipid system. The similarity of the partitioning behavior of the coat protein and gramicidin A′ implies that neither the chemical nature of the amino acid residues nor their secondary structure dominates the partitioning behavior. Membrane-bound gramicidin A′ is a $\beta^{6.3}$-helix [12,13], while there is strong evidence that the 50-residue M13 coat protein traverses the bilayer by means of a stretch of nineteen predominantly hydrophobic amino acids in an α-helical conformation [31]. It is possible that the similar partitioning behavior of gramicidin A′ and M13 coat protein is due to both molecules having comparable transbilayer hydrophobic distances. The length of the gramicidin A′ dimer is 26–30 Å [26]. The proposed membrane-spanning 19-residue α-helix of M13 coat protein has a length which falls within this range.

In conclusion, our data support the view that hydrophobic mismatch between the length of gramicidin A′ and the length of the phospholipid acyl chains in the bulk gel phase is a key factor in determining the solubility of the peptide in the gel phase. The ability of the gel phase to accommodate gramicidin A′ increases as hydrophobic mismatch decreases.

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