EFFECTS OF CYCLOSPORINE A ON BIOMEMBRANES

Vibrational Spectroscopic, Calorimetric and Hemolysis Studies

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ABSTRACT Cyclosporine A (CSA)-dipalmitoylphosphatidylcholine (DPPC) interactions were investigated using scanning calorimetry, infrared spectroscopy, and Raman spectroscopy. CSA reduced both the temperature and the maximum heat capacity of the lipid bilayer gel-to-liquid crystalline phase transition; the relationship between the shift in transition temperature and CSA concentration indicates that the peptide does not partition ideally between DPPC gel and liquid crystalline phases. This nonideality can be accounted for by excluded volume interactions between peptide molecules. CSA exhibited a similar but much more pronounced effect on the pretransition; at concentrations of 1 mol % CSA the amplitude of the pretransition was <20% of its value in the pure lipid. Raman spectroscopy confirmed that the effects of CSA on the phase transitions are not accompanied by major structural alterations in either the lipid headgroup or acyl chain regions at temperatures away from the phase changes. Both infrared and Raman spectroscopic results demonstrated that CSA in the lipid bilayer exists largely in a β-turn conformation, as expected from single crystal x-ray data; the lipid phase transition does not induce structural alterations in CSA. Although the polypeptide significantly affects DPPC model membrane bilayers, CSA neither inhibited hypotonic hemolysis nor caused erythrocyte hemolysis, in contrast to many chemical agents that are believed to act through membrane-mediated pathways. Thus, agents, such as CSA, that perturb phospholipid phase transitions do not necessarily cause functional changes in cell membranes.

INTRODUCTION

Protein-lipid interactions are generally considered to be important mediators of biomembrane structure and function. For the most part, experimental studies have focused on the protein-induced perturbations of membrane phase transitions (for example, Epand and Sturtevant, 1984), on lipid conformation (see, for example, Susi et al., 1979), or on lipid mobility (see, for example, Yeagle, 1982). While these studies have contributed greatly to characterizing lipid-peptide interactions, they have not provided the necessary structural information required for a detailed understanding of the properties and behavior of these lipid-peptide aggregates. For example, calorimetric studies of peptide hormone-lipid bilayer interactions do not usually consider the possible effects of peptide conformational changes associated with the gel-to-liquid crystalline phase transition (see, for example, Epand and Sturtevant, 1984). Indeed, even when peptide crystal structures are known, the relevance of the conformational studies of isolated systems to that of bilayer associated peptides is generally not known.

Since theories of lipid-protein interactions generally model the effects of peptides on acyl chain conformation, a detailed test of such theories requires that the perturbation by peptide incorporation of lipid acyl chain *trans-gauche* isomerization be determined. Raman spectroscopy is ideally suited to this task since information on both chain isomerization and chain packing is obtained (Levin, 1984). Quantitative application of lipid-peptide interaction theories (Abney and Owicki, 1985) requires, in addition, that the peptide geometry be known both below and above the gel-to-liquid crystalline phase transition temperature. If detailed conformational information for isolated peptides from x-ray diffraction or nuclear magnetic resonance experiments is available, infrared spectroscopy provides a convenient method both for determining the relevance of this information to the bilayer-peptide system and for assessing temperature dependent changes in the peptide structure. Together, infrared and Raman spectroscopy can provide a structural basis for peptide-induced changes in the themodynamic properties of lipid melting transitions measured by scanning calorimetry.

To understand the biological relevance of both experimental and theoretical studies of lipid-peptide interactions, information on the effects of peptide incorporation on intact cellular membranes is required in addition to data acquired from model systems. Studies on general anesthetics, drugs that are considered to function through membrane-mediated mechanisms, have demonstrated that

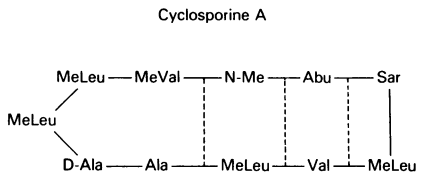

FIGURE 1 Primary structure of cyclosporine A, with locations of β-structures (from Petcher et al. 1976). N-Me denotes 2S-methylamino-3R-hydroxy-4R-methyl-oct-6-en-1-oic acid. Abu denotes α-aminobutyric acid. Sar denotes sarcosine (N-methyl glycine).

these agents generally perturb both the pretransition and the main phase transition of single-component phospholipid dispersions (Seeman, 1972; Mountcastle et al., 1978; Hill, 1978; O'Leary, et al., 1984), as well as inhibit osmotic hemolysis (Seeman, 1972). By analogy, we might expect membrane active peptides also to perturb bilayer phase transitions and, in addition, perhaps to inhibit hypotonic hemolysis. Cyclosporine A (CSA) (Fig. 1) is an ideal peptide with which to study the relationship between peptide effects in model and intact membrane systems, both since it is poorly soluble in water (0.04 mg/ml at 25°C; McEvoy, 1985), insuring that it will remain in the lipid phase, and since its secondary structure is known and can be monitored by vibrational spectroscopic techniques. This cyclic undecapeptide, produced by *Trichoderma polysporum Rifai* (Petchers et al., 1976), is a potent suppressor of T-lymphocyte function that has found widespread use in the prevention of graft rejection (Merion et al., 1984). Although specific CSA receptors have been reported to be present on lymphocytes (Ryffel et al., 1982), the CSA binding affinities of B-lymphocytes, T-lymphocytes, and phospholipids are approximately the same (LeGrue et al., 1983). This has led to the suggestion that CSA may exert its immunosuppressive effect through a nonspecific membrane-mediated mechanism (LeGrue et al., 1983), although interaction with a cytosolic receptor seems more likely (Handschumacher et al., 1984).

In the present study we examine first the interactions of CSA with dipalmitoylphosphatidylcholine (DPPC) multilamellar dispersions using scanning calorimetric and vibrational spectroscopic techniques, and then the effect of CSA on erythrocyte membranes using osmotic hemolysis. These physical methods allow us to monitor conveniently the changes induced by CSA in the two phase transitions of the lipid bilayer and to probe the structural alterations that characterize the interactions between CSA and the bilayer lipid. The results are interpreted in terms of the thermodynamics of the membrane phase transition and of the scaled particle theory of solutions.

EXPERIMENTAL

DL-dipalmitoylphosphatidylcholine was purchased from Sigma Chemical Co. (St. Louis, MO); cyclosporine A was a gift of Sandoz Ltd. DPPC-CSA mixtures were prepared by dissolving both DPPC and CSA in chloroform, drying to a gelatinous consistency under flowing nitrogen gas and then lyophilizing for 14–16 h at 10^{-4} torr. Samples were then dispersed in distilled water at 1:2 (wt/wt) ratios for recording their infrared and Raman spectra and at 1:10 (wt/wt) ratios for obtaining calorimetric data.

The computer-controlled Raman (Huang et al., 1982) and dispersive infrared (Vincent et al., 1984) spectrometers and the respective thermostatically controlled sampling accessories have been described previously. Effects of CSA on the phospholipid headgroup region were examined by monitoring the 717 cm^{-1} choline symmetric methyl C—N stretching mode region at 2° and 47°C. Effects of CSA on the lipid acyl chain lateral order and *trans-gauche* chain isomerization were monitored by constructing temperature profiles from the peak-height intensity ratios $I_{2,850}/I_{2,880}$, and $I_{2,935}/I_{2,880}$, derived from spectra recorded in the 2,800–3,100 cm^{-1} CH stretching mode region. These measurements were determined for 10 mol % CSA/DPPC multilayers, using signal-averaged scans recorded at a 5 cm^{-1} spectral slit width.

Changes in conformation within the phospholipid bilayer interface region and in CSA resulting from CSA-lipid interactions were monitored using infrared data recorded in the lipid 1,700–1,800 cm^{-1} carbonyl C=O stretching mode region and in the 1,600–1,700 cm^{-1} polypeptide amide I spectral interval. Phospholipid dispersions were placed in a temperature-controlled variable path length cell with CaF_2 windows. Bubbles were eliminated by careful cyclic variation of the path length, which was ultimately set at approximately four microns. Data were collected using a spectrophotometer (model 580B; Perkin-Elmer Corp., Norwalk, CT), which was constantly purged with nitrogen gas. Acquisition was controlled using a LSI 11/23-based laboratory microcomputer. Spectra were acquired for 10 mol % CSA dispersions at 28° and 43°, and for 30 mol % CSA dispersions at 45°. In addition, a spectrum of CSA deposited from chloroform on a CaF_2 plate was obtained. All spectra were obtained over the 2,200–1,200 cm^{-1} range, with a spectral resolution of 1.8 cm^{-1}. The water background was subtracted using the 2,130 cm^{-1} water association band as a normalizing spectral feature, as described previously (Vincent et al., 1984). Briefly, a library of water spectra for each sample temperature was constructed by slightly varying the cell path length. Subtraction of the water reference spectrum from the lipid dispersion spectrum was based upon the contour of the 2,130 cm^{-1} water association band. The reference spectrum, which best reproduced that of the sample in this region, was scaled as necessary (by 1–2%) to achieve a level baseline in the 2,200–1,850 cm^{-1} region.

Calorimetric measurements were carried out using a previously described apparatus (Ross and Goldberg, 1974). In a typical experiment, ~60 mg of the phospholipid dispersion were heated from 25° to 50°C at a scanning rate of 15.1 Kh^{-1}; data were reduced and analyzed using the procedures described by O'Leary et al. (1984). Transition temperatures obtained from these measurements were reproducible to within ±0.03°C. The main transition enthalpy values were obtained in a uniform manner by integrating the excess heat capacity curve over a linear baseline spanning the 38.06°–47.62°C temperature range.

To determine whether CSA alters the passive permeability of a membrane, hemolysis studies were performed using human erythrocytes. In these experiments 1 vol of washed packed cells was suspended in 10 to 1,000 vol of either 2.5 mM aqueous solutions of NaH_2PO_4, 2.5 mM Na_2HPO_4 (pH 7.2), and 150 mM NaCl, or in aqueous solutions of 2.5 mM NaH_2PO_4, 2.5 mM Na_2HPO_4 (pH 7.2), and 300 mM mannitol, sucrose, or raffinose. CSA concentrations were varied from 10^{-8} M to saturation (~10^{-4} M). After incubation for 20 min, the suspension was centrifuged for 15 s. at 10,000 g in a microfuge (Beckman Instruments, Inc., Fullerton, CA). Hemoglobin release was measured by determining the 415-nm absorbance of the supernatant.

To examine possible stabilization of the erythrocyte membrane by CSA, hemolysis studies were determined by suspending 1 vol of packed eythrocytes in 100 vol of 5mM Na_2HPO_4 containing separately 0, 30, 50, 60, 70, 75, 80, 90, 100, and 150 mM NaCl. Hemolysis was measured as described above both in the presence of CSA at 10^{-7} to 10^{-4} M concentrations and in the absence of CSA.

RESULTS AND DISCUSSION

Raman spectra of polycrystalline cyclosporine A in the amide I region show major features at 1,672, 1,665, 1,652, 1,640, and 1,620 cm^{-1} (Fig. 2). These features are consistent with the presence of the β-sheet, β-turn, and disordered structures determined by x-ray diffraction studies of single crystals (Petcher et al. 1976); α-helical conformations (which are often associated with vibrational features at ~1,650 cm^{-1}) cannot form in this short cyclic peptide. Raman spectra of anhydrous 30 mol % CSA/DPPC bilayers (Fig. 2 *B*) similarly show intense, broadened, features at 1,669 and 1,650 cm^{-1} (encompassing the 1,650, 1,640, and 1,620 cm^{-1} features of the crystal spectrum), indicating that CSA exists in essentially the same conformation both in the crystal and in the bilayer systems. Infrared spectra of the anhydrous polypeptide in Fig. 3 display a major feature at ~1,632 cm^{-1}, which is consistent with the presence of a β-sheet conformation. The somewhat less intense features at ~1,680 cm^{-1} could result from either the antiparallel β-sheet or the β-turn structure (Bandekar and Krimm, 1980).

Amide I region spectra of 30 mol % CSA/DPPC bilayers are almost identical to those of the crystal, consistent with the conclusion that bilayer-associated CSA and polycrystalline CSA are structurally very similar. In contrast, the amide I region spectra of 10 mol % CSA/DPPC bilayers are symmetrically narrowed. The interpretation of the differences is difficult, since changes in normal coordinates specifying the vibrational frequencies, reaction fields, and dielectric constant may all contribute to the observed spectral changes (Lavialle et al., 1982). In the case of CSA, however, the cyclic primary structure and the necessary presence of β-sheet sufficiently restrict the possible changes in secondary structure; thus, we interpret the reduction in linewidth to result from the change from an environment where the CSA molecule is surrounded by other CSA molecules to an environment, in which it is primarily surrounded by lipid. The frequency and lineshape of the amide I feature change little with temperature (ν_{max} = 1,634 cm^{-1} at 28°C, 1,636 cm^{-1} at 43°C), demonstrating that the lipid bilayer phase transition induces no major perturbation of the polypeptide structure. At the higher temperature the amide I halfwidth decreases

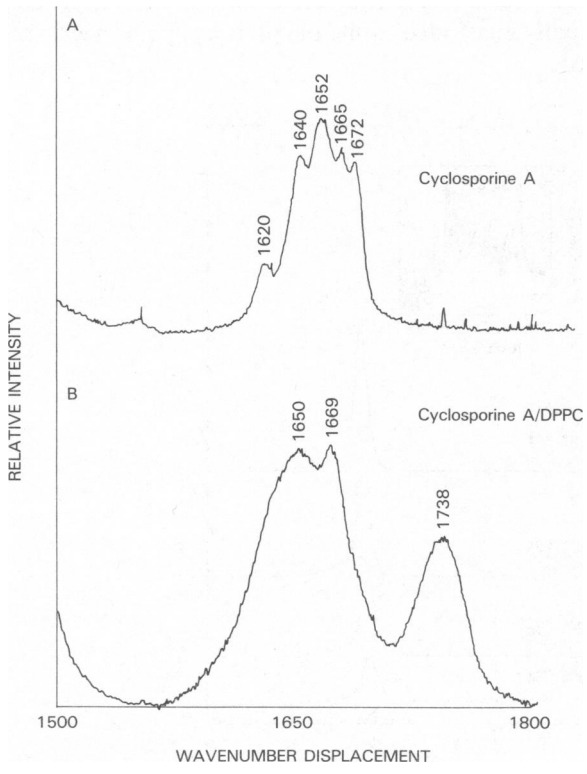

FIGURE 2 Raman spectra of polycrystalline (*A*) and lipid bilayer (*B*) CSA in the Amide I spectral region. Similarity in spectra indicates that CSA has a bilayer conformation very similar to that of the crystal.

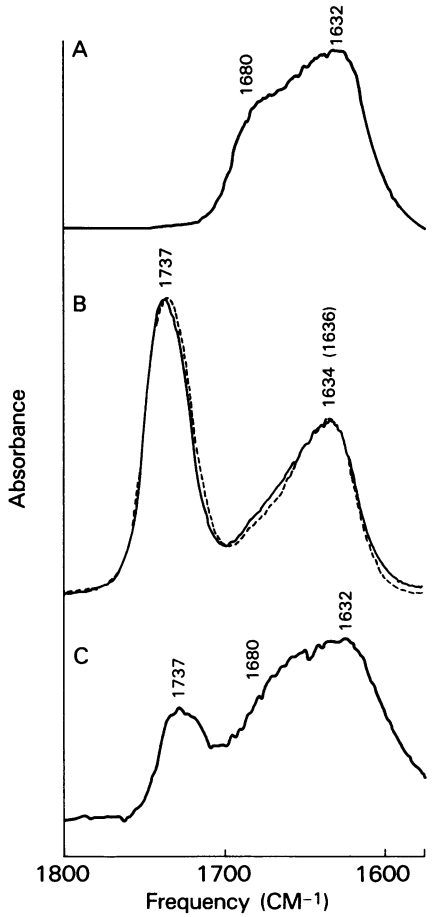

FIGURE 3 Infrared spectra of CSA and of CSA-DPPC bilayers in the 1,700–1,800 cm^{-1} lipid carbonyl, and 1,600–1,700 cm^{-1} polypeptide Amide I stretching mode regions (*A*) Anhydrous CSA, (*B*) 10% CSA-DPPC bilayers below (—28°C) and above [----43°C] the bilayer phase transitions. The amide I peak frequency is typical of antiparallel β-pleated sheets (Krimm and Bandekar, 1980). The similarity in these spectra reflects the absence of conformational changes of the polypeptide in the liposome over this temperature range. (*C*) 30% CSA-DPPC bilayers above (43°C) the bilayer phase transition.

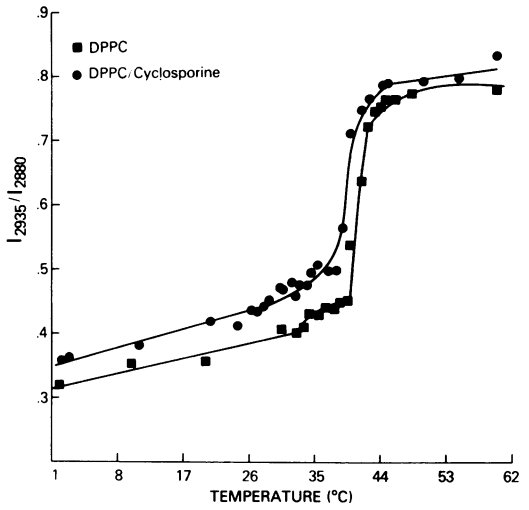

FIGURE 4 Raman intensity peak height intensity ratios $I_{2,935}/I_{2,880}$ as a function of T for pure DPPC (■) and 10% CSA (●) bilayers. The increase in this ratio in the gel state for CSA-containing bilayers reflects an increased fraction of *gauche* conformers along the lipid acyl chains, as well as a small contribution from the CSA spectrum.

slightly from 53.4 cm^{-1} to 50.8 cm^{-1}. This results from a decrease in the intensity in the 1,650–1,660 cm^{-1} region and may indicate either a more heterogeneous arrangement of CSA molecules in the gel phase or very slight changes in average conformation.

Temperature profiles derived from Raman spectral peak height intensity ratios determined from the C—H stretching mode region, (Fig. 4) demonstrate that 10 mol % CSA induces a small increase in the *gauche/trans* ratio in both the low and high temperature sides of the phase transition. This results from a very slight disordering of the bilayer both above and below the gel-to-liquid crystalline phase transition in addition to CSA signal in this spectral region. Thermodynamic parameters obtained from the amplitude of the sigmoidal phase transition curve (Huang et al., 1982) agree with our calorimetric results (vide infra) that CSA causes little change in the enthalpy of the main transition. CSA containing bilayers exhibit only slight narrowing ($\Delta\nu_{1/2} < 1$ cm^{-1}) of the Raman spectral 717 cm^{-1} choline C—N stretching mode, suggesting that CSA exerts no significant effect on the lipid headgroup conformation. Infrared and Raman spectral frequencies and contours in the phospholipid 1,700–1,800 cm^{-1} C=O stretching mode region are the same for both pure DPPC liposomes and those containing 10% CSA, indicating that no conformational differences exist between the two systems within the lipid interface region.

Calorimetric results presented in Figs. 5 and 6 demonstrate that CSA lowers the temperature and maximum heat capacity of both the bilayer gel-to-liquid crystalline phase transition and the pretransition. The thermodynamic data presented in Table I, which suggest a small decrease in the enthalpy of transition on addition of CSA, are consistent with the Raman results showing a perturbation of the acyl chain region by CSA. Marked broadening of both phase transitions also occurs. The lipid bilayer pretransition is much more sensitive than the main transition to the effects of CSA; at 1% CSA the main transition is virtually unaffected, while the pretransition is nearly abolished.

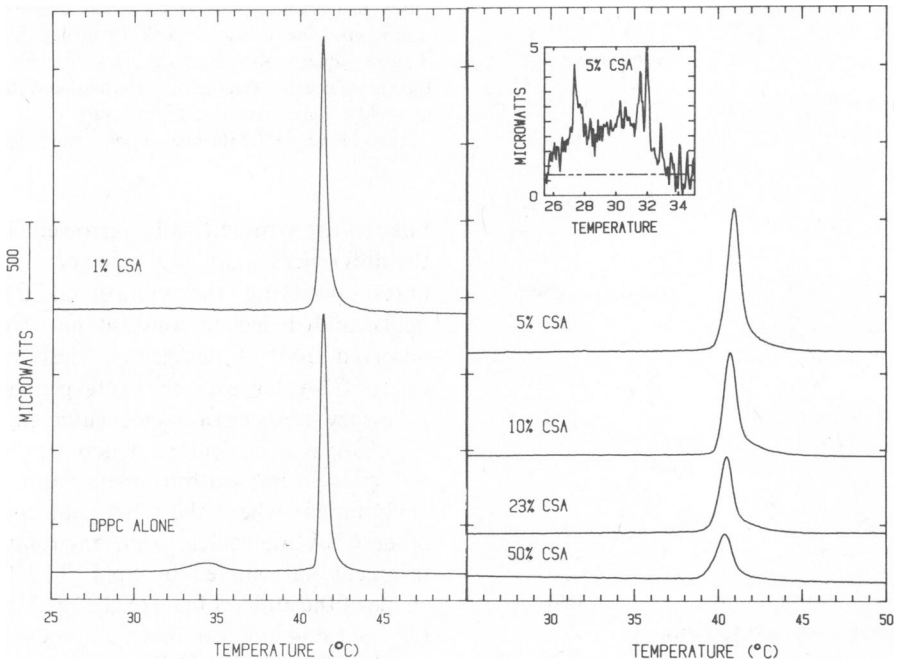

FIGURE 5 Calorimetric traces for CSA-DPPC dispersions. CSA dramatically lowers both the temperature and the height of both pre- and main-transitions. Evidence of the pretransition persists up to 5 mol % CSA (*inset*).

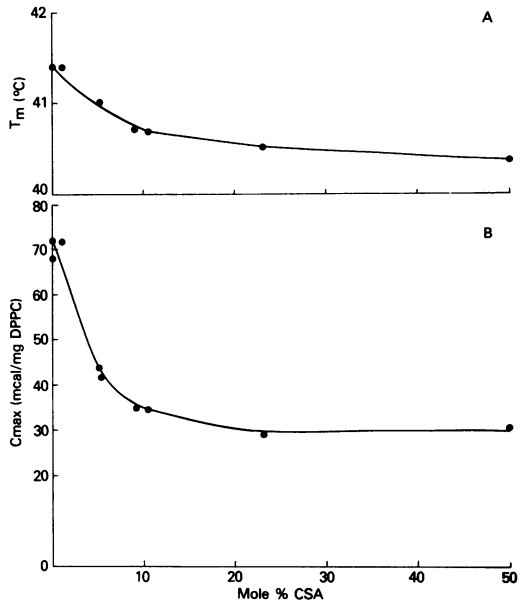

FIGURE 6 (*A*) T_m vs. mole fraction CSA for the main bilayer phase transition. (*B*) Maximum heat capacity in the transition region (C_{max}) vs. mole fraction CSA for the main phase transition.

Potential sources of the observed broadening and decreased amplitudes of the pretransition and gel-to-liquid crystalline transition would include loss of cooperativity and partitioning of CSA between solutions formed in both the gel and liquid crystalline phases. Sturtevant (1982) has formulated theoretical expressions for the heat capacity in the transition region for a system forming such solutions. This theory associates changes in the heat capacity with the calorimetric and van't-Hoff enthalpies of the pure lipid, the mole fraction of impurity, and the equilibrium constant K relating the gel phase impurity concentration to that in the liquid crystalline phase.The relationship between impurity concentration and the excess heat capacity is given (Sturtevant, 1980) by

$$T_0/T = 1 + RT_0\left[\frac{1}{\Delta H_{vH}}\ln\left(\frac{1-\alpha}{\alpha}\right) - \frac{\ln X_1}{\Delta H_{cal}}\frac{1}{\left(\frac{K}{1-K}\right)+\alpha}\right] \quad (1)$$

$$C_{ex} = \Delta H_{cal}\frac{d\alpha}{dT}, \quad (2)$$

where ΔH_{cal} is the calorimetric enthalpy of the phase transition; ΔH_{vH} is the van't Hoff enthalpy of the phase transition; α is the extent of transition; X_1 is the mole fraction lipid; T_m is the temperature at which the maximum heat capacity C_{max} occurs; C_{ex} is the excess heat capacity; T_0 is the midpoint temperature of the pure lipid phase transition ($\alpha = 0.5$); and K is the gel/liquid crystalline partition constant for CSA.

If we assume applicability of these equations, ΔH_{vH} and K may be determined from T_m and C_{max}. For a constant calorimetric enthalpy ΔH_{cal}, the gel/liquid crystalline partition constant K is uniquely determined by T_m for any given peptide concentration. The van't Hoff enthalpy ΔH_{vH} can then be determined from the value of C_{max}. Thus, for each sample we first determined K by adjusting the value of this parameter such that the T_m obtained from Eq. 1 agreed with the experimental value to within 0.04°. Then, we determined ΔH_{vH} by adjusting its value such that the ratio of C_{max} to that of pure lipid given by Eq. 1 agreed with the experimental value. Values for K and ΔH_{vH} determined using Sturtevant's equations for various CSA concentrations are shown in Table II. These values demonstrate that the broadening and decrease in amplitude of the main transition results from both partitioning of CSA between the gel and liquid crystalline phases and a decreased cooperativity of melting. The change in K with concentration of CSA reflects nonideal partitioning of CSA between the gel and liquid crystalline phases. Extrapolation of these results to 0 mol % CSA yields a partition constant K of ~0.5, indicating that CSA has a strong preference for insertion into the liquid crystalline phase.

TABLE I
CALORIMETRIC DATA FOR PURE DPPC AND FOR VARIOUS CSA-DPPC DISPERSIONS

Mole fraction CSA	T_m	ΔT_m^*	C_{max}	ΔH_{vH}‡	ΔH cal§
	°C	°C	*Kcal/mole-K*	*Kcal/mole*	*Kcal/mole*
0	41.39	0.52	12.86	1277	8.03
1	41.39	0.43	12.85	1262	8.12
5.2	40.99	0.94	7.44	663	8.92
5.2	40.99	0.89	7.19	694	8.24
9.1	40.73	0.96	6.32	639	7.85
10.6	40.73	0.93	6.22	651	7.51
23.0	40.53	0.96	5.17	633	6.47
50.0	40.38	1.14	5.42	568	7.56

*Full width at half maximum.
‡Calculated from the relationship $\Delta H_{vH} = 4RT_0^2\, C_{ex}/\Delta H_{cal}$ where T_0 = temperature at which the transition is half completed.
§Integrated from 38.06°C – 47.62°C.

TABLE II
THERMODYNAMIC PARAMETERS DETERMINED FOR THE DPPC-CSA SYSTEM USING STURTEVANT'S THEORY*

Mole fraction CSA	T_m (fit)	C_{max}‡ (fit)	ΔH_{vH}	K
	°C		*kcal/mole lipid*	
0.00	314.54	1.00	1.25×10^3	—
0.05	314.15	0.57	8.0×10^2	0.73
0.10	313.86	0.51	7.4×10^2	0.77
0.23	313.64	0.41	5.5×10^2	0.87
0.50	313.52	0.41	5.3×10^2	0.94

*Sturtevant, J. M. 1982. *Proc. Natl. Acad. Sci. USA.* 79:3963–3967.
‡Normalized to 1 for pure lipid.

To understand further this nonideal behavior, we have constructed a simple theoretical model of the lipid-peptide system using scaled particle theory (Lebowitz et al., 1965), which enables calculation of the free energy change of a system upon introduction of a hard particle of known geometry. Such a particle is conformationally inflexible, and interacts with other particles by repulsion arising from physical contact. Since this free energy change is related to the thermodynamic activity of the introduced particle, the theory allows us to examine the effect of excluded volume interactions on the partitioning of CSA between the gel and liquid crystal phases. We assume that membrane lipids may be treated as a two-dimensional array of hard disks, with an area A_L, which are packed at densities of ρ_G and ρ_L in the gel and liquid crystalline phases, respectively. CSA is treated as a disk of area A_C.

We calculate the partitioning of CSA between phases using the relationships:

$$\mu_{\text{gel}}^{\text{CSA}} = \mu_{\text{lc}}^{\text{CSA}} \tag{3}$$

at equilibrium, and (Scott and Cheng, 1979)

$$\beta_{\mu_i} = kT \ln\left[\rho_i \frac{h^2}{2\pi m_i kT}\right] + \left[-\ln(1 - \pi\Sigma\rho_i R_i^2) + \left(\frac{2\pi(\Sigma\rho_i R_i)R_i}{1 - \pi\Sigma\rho_i R_i^2}\right) + \beta\pi\rho R_i^2\right], \tag{4}$$

where μ_i is the chemical potential of species i; β is $1/kT$; k is the Boltzmann's constant; h is the Planck's constant; T is the temperature (Kelvin); ρ_i is the number density of species i; R_i is the radius of a particle of species i; and m_i is the mass of a particle of species i.

In the scaled particle calculation perturbation of the transition by the peptide is not considered. Instead, we calculated the partition constant for several CSA mole fractions between 0.0 and 0.5, and for several combinations of A_C/A_L, ρ_G and ρ_L, assuming in all cases that $A_C > A_L$, as evident from x-ray diffraction data, and that $\rho_G > \rho_L$. With these general assumptions, K always approaches 1 in the same nonlinear manner shown in Table II as the CSA concentration is increased, regardless of specific assumptions about A_C, A_L, ρ_G, and ρ_L. This result is physically reasonable, since at high CSA concentrations the environment of a CSA molecule largely reflects other CSA molecules, rather than either the gel phase or liquid crystalline phase lipids themselves. The calculation thus indicates that the nonideal partitioning behavior inferred from the use of Sturtevant's theory may arise from excluded volume interactions alone.

The greater perturbation by CSA of the pretransition compared to the gel-to-liquid crystal phase transition has also been seen with other agents, including cholesterol (Mabrey et al., 1978) and steroids (O'Leary et al., 1984). Sturtevant's theory may also be useful in explaining this greater sensitivity of the pretransition over the main transition to bilayer perturbants. Our DPPC samples have typically shown main transition calorimetric enthalpies ΔH_{cal} of ~7.8 Kcal/mol, with van't-Hoff enthalpies ΔH_{vH} of ~1.25 × 10^3 Kcal/mol. The pretransition, with calorimetric and van't Hoff enthalpies of 1.1 Kcal/mol and ~300 Kcal/mol, respectively, is more cooperative than the primary phase transition. Sturtevant's theory predicts that the more highly cooperative transition would exhibit a greater sensitivity to solid solution broadening and amplitude effects. Although the temperature of the perturbed transition is independent of transition cooperativity, the amplitude of the more cooperative transition is reduced to a greater extent by small concentrations of perturbants.

An important feature of our analysis is that lipid phase separation in the transition region is accompanied by significant differences in gel and liquid crystalline phase peptide concentrations, particularly at low mole fractions of peptide. In addition, the results suggest that at high peptide concentrations, peptide-peptide excluded volume interactions have a significant role in determining the thermodynamic properties of the gel to liquid crystalline phase transition. Most theories of lipid-peptide interactions (Marcelja, 1976; Owicki et al., 1978; Owicki et al., 1979; Jahnig, 1981*a*, *b*; Schroeder, 1977; Pink and Chapman, 1979; Scott and Coe, 1982; O'Leary, 1983; Lookman et al., 1982; and Pearson et al., 1984) ignore one or both of these factors. Because our analysis is model dependent, we cannot state unequivocally that both factors are necessary to explain our experimental data. None of the theories referred to above, however, appears capable of giving a reasonable quantitative fit to our results. For this reason, we believe that much progress remains to be made in the theoretical understanding of lipid-peptide interactions.

Experiments on model membrane systems, such as those we have examined here, may not necessarily indicate the presence or absence of effects on intact biological membranes. Since the most important function of most membranes is to separate intra- and extracellular contents, we assessed the effects of CSA on this function by means of hemolysis experiments on erythrocytes. Our experiments showed that CSA neither induces hemolysis at concentrations ranging from 10^{-8} to 10^{-4} M, nor protects erythrocytes from hypotonic hemolysis in this concentration range. Thus, CSA had no effect on red cell membrane integrity. These results do not support the hypothesis that the observed immunologic effects of CSA are mediated through nonspecific membrane interactions. The absence of an effect on hemolysis also demonstrates that even strong perturbation of model membrane phase transitions by impurities does not necessarily imply membrane-mediated effects in cells, in contrast to the behavior of general anesthetics.

In summary, we have established that membrane-associated CSA has a conformation similar to that of the polycrystalline peptide and that CSA undergoes no significant conformational change at the DPPC gel-to-liquid

crystalline phase transition. CSA perturbs both the gel-to-liquid crystalline phase transition of DPPC and the pretransition. Raman spectra show that this perturbation is accompanied by a small increase in membrane disorder in both gel and liquid crystalline phases, but that the difference in the change in lipid order at the phase transition upon addition of CSA is small. For these reasons, the two-state solid solution model (Sturtevant, 1982) for perturbation of membrane phase transitions appears to provide a reasonable description of this system. Application of this theory to calorimetric data characterizing the phase transition suggests that there is a loss of transition cooperativity in the presence of peptide in addition to solution broadening. As a result of excluded volume interactions between CSA molecules, partitioning of CSA between the gel and liquid crystalline phase lipids depends on CSA concentration. In spite of dramatic effects of CSA on model membrane phase transitions, no effects on osmotic hemolysis are observed, demonstrating that perturbations of model membrane phase transitions by CSA do not necessarily indicate corresponding effects on intact biological membranes.

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