Vol. 168, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 16, 1990 Pages 85-90

A QUANTITATIVE INFRARED DETERMINATION OF ACYL CHAIN CONFORMATION IN GRAMICIDIN/DIPALMITOYLPHOSPHATIDYLCHOLINE MIXTURES

Mark A. Davies, Joseph W. Brauner, Herbert F. Schuster and Richard Mendelsohn

Department of Chemistry,Newark College of Arts and Science, Rutgers University, 73 Warren Street Newark, N.J. 07102

Received February 14, 1990

A quantitative infrared characterization of phospholipid acyl chain disordering in 6,6,6'6'-d4 dipalmitoylphosphatidylcholine/ Gramicidin D bilayers has been made. Three CD2 rocking modes, at 622 cm-1, 646-649 cm-1, and 651-653 cm-1,assigned to particular conformers,were used to determine disorder in the presence of peptide,as well as percentages of particular classes of conformer within the total gauche population. At 44C, the gauche percentages in 10:1 and 30:1 lipid/peptide mixtures were 15% and 17%, respectively. At 34C, the corresponding values were 9.8% and 2.6%. The percentage of (single gauche bend + kink) conformers, relative to multiple gauche forms, decreases dramatically from 78% in the 30:1 mixture to 15% in the 10:1 mixture at 44C. These data provide the first quantitative measure of the extent to which a membrane-spanning peptide disorders phospholipid gel phases and orders liquid crystal phases.

Model membrane systems containing Gramicidin, a channel-forming pentadecapeptide specific for monovalent cations, have been the subject of many biophysical investigations (1, and references therein). Though the function of this peptide is well understood from a physiological point of view, a detailed picture of its interaction with lipids, particularly with regard to lipid acyl chain conformational states, has not emerged. Snyder and co-workers (2-4), in their seminal infrared studies of alkane structure in condensed phases, introduced a method of structure determination making use of the sensitivity of CD_2 rocking frequencies and intensities to the trans-gauche isomerization process. In this study, we take advantage of the high sensitivity of FT-IR technology for studies of phospholipid acyl chain structure in aqueous media and put forth, for the first time, a quantitative description of phospholipid acyl chain conformational disorder in a peptide-containing model membrane system.

MATERIALS AND METHODS

The experimental protocols were described in detail previously (5,6); a brief summary is given here. Samples were prepared by dissolving appropriate proportional amounts of 6-d4 DPPC and Gramicidin D (Dubos) (Sigma, St. Louis, MO) in chloroform in a culture tube. Excess solvent

Abbreviations used : 6-d_4 DPPC : 6,6,6',6'-d_4 dipalmitoylphosphatidylcholine; 12-d_4 DPPC : 12,12, 12',12'-d_4 dipalmitoylphosphatidylcholine; FT-IR : Fourier Transform Infrared; T_m : main gel>liquid crystal phase transition temperature; t : trans; g : gauche.

0006-291X/90 $1.50

was evaporated under a stream of nitrogen. Samples were dried under vacuum overnight. Following addition of D2O (2:1 water/lipid, mol/mol), the culture tube was sealed, incubated at 55C for 1.5 hours with frequent vortex mixing. The sample was placed between two AgCl windows and contained with a 6 μ spacer. This assembly was thermostatted and 2000 interferograms were collected at a spectral resolution of 4 cm-1 on a Mattson Sirius 100 FT-IR intrument (Mattson Instruments, Inc., Madison, WI) equipped with a HgCdTe dectector. The interferograms were apodized with a triangular function and Fourier transformed. Data were encoded every 1.0 cm-1. Spectral band positions had an uncertainty of less than 0.1 cm-1. For subtraction of the D2O librational background, spectra of mixtures of proteated DPPC and Gramicidin D, otherwise identical to those of deuterated samples, were collected under the conditions described above.

RESULTS

FT-IR spectra of the CD_2 rocking mode region of 6-d_4 DPPC/Gramicidin D mixtures taken at temperatures above and below the gel>liquid crystal phase transition are shown in Figure 1. Spectra of 10:1 mixtures are shown in Panel A; those of 30:1 mixtures are shown in Panel B. Three spectral features, arising from CD_2 groups in particular conformations, are observed. The most intense of these in all spectra, occurring at 622 cm^{-1}, is due to a CD_2 rocking mode from a group adjoining a pair of trans (t) bonds; that is, a ttt acyl chain segment in which underlined bonds are adjacent to the CD_2 group. Spectral features in the 10 :1 sample arising from gauche (g) conformers of the type g'tgt (kink) and ttgt (single gauche bend) occur at 653 cm^{-1}, while those of the type ttgg or g'tgg (multiple gauche) are observed at 649 cm^{-1}. The corresponding bands in the 30:1 sample (Panel B) are decreased by 1-2 cm^{-1}. A fourth band, observed at 643 cm^{-1} in Panel A, is due to incomplete removal of Gramicidin D bands by the spectral subtraction process and does not interfere with any of the trans-gauche conformational markers. The weak band occurring at 657 cm^{-1} in Panel B is the CHD rock, arising from small amounts of lipid incompletely deuterated at the 6 position. Several characteristics of the spectra in Figure 1 are notable. First, the intensity of the gauche markers (649 cm^{-1} and 653 cm^{-1}) increase with

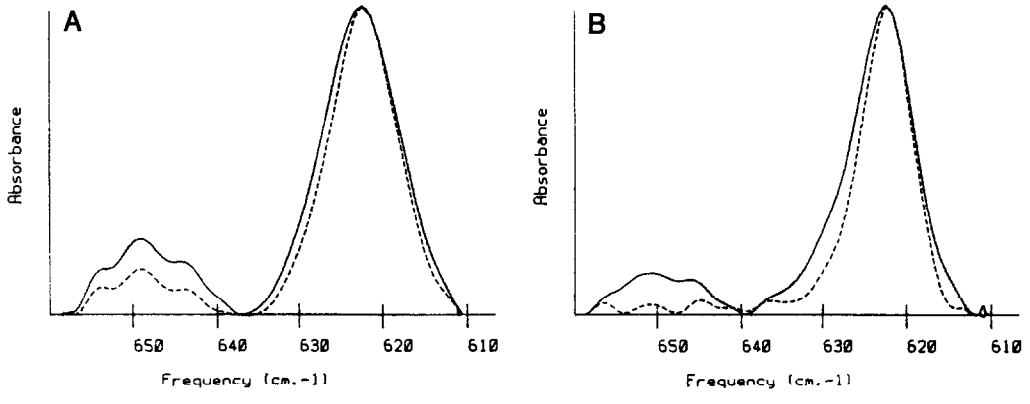

Figure 1A : FT-IR spectrum of the CD_2 rocking mode region of 10:1 6-d_4 DPPC / Gramicidin D. (——) : 44°C; (----) : 34°C.

Figure 1B : FT-IR spectrum of the CD_2 rocking mode region of 30:1 6-d_4 DPPC / Gramicidin D. (——) : 44°C; (----) : 34°C.

Table 1 : Percent of Total Gauche Conformers for 6-d_4 DPPC Alone , and in Binary Mixtures with Gramcidin D or Cholesterol

	Temperature °C					
Mixture	5	25	34	38	44	48
6-d_4 DPPC	-	-	1.7±0.3	-	2.3±1.1	32.3±2.3
10:1[a]	5.8±3.0	6.3±3.2	9.8±3.3	-	15.0±3.0	-
30:1	1.0±0.5	1.1±0.6	2.6±1.3	-	17.7±3.4	-
12-d_4/Chol[b]	4.5±3.0	7.6±3.0	6.7±3.0	8.4±2.1	10.1±2.5	10.6±2.7

[a] mole ratios of 6-d_4 DPPC/Gramicidin D
[b] mole ratio 12-d_4 DPPC/Cholesterol = 2:1

increasing temperature, particularly in the range between 34 and 44°C. Second, the fraction of gauche conformers increases with increasing Gramicidin D content. Finally, there is a reversal in the relative intensity of the 649 cm^{-1} and 653 cm^{-1} markers on going from 30:1 lipid/protein ratio to 10:1. The total percentage of gauche conformers for each mixture and temperature are given in Table 1. The gauche percentage is calculated by dividing the sum of the areas of the gauche markers by the gauche area sum plus the area of the 622 cm^{-1} trans marker. Extinction coefficients for the three marker bands are equal (3,4). Percentages of the gauche rotamer population due to particular gauche classes, calculated by dividing the area of an individual band by the sum of the areas of both gauche markers, are given in Table 2. Also included in the Tables are data for DPPC/cholesterol mixtures (2:1 mol/mol) and for pure 6-d_4 DPPC.

DISCUSSION

Despite many investigations regarding the nature of Gramicidin / phospholipid interactions in model membrane systems, a quantitative description of these in terms of particular phospholipid motions has proven an elusive goal, and is the motivation for the current work. It has been recently demonstrated (5,6) that acyl chain CD_2 rocking modes in phospholipids deuterated at specific positions are sensitive, non-perturbing probes of lipid conformational disorder. The direct sensitivity of these modes to acyl chain trans-gauche isomerization has been used to address the following issues :

Table 2 : Percent of tgt and tgg Gauche Conformer Classes

Mixture	tgt Class (%)		tgg Class (%)	
	34°C	44°C	34°C	44°C
6-d_4 DPPC	37±10	74±18[a]	62±10	25±18
10:1[b]	20±10	15±10	79±10	84±10
30:1[b]	53±10	78±16	46±10	21±16
12-d_4 /Chol[c]	33±10	54±15	66±10	45±15

[a] 48°C
[b] mole ratios of 6-d_4 DPPC/Gramicidin D
[c] mole ratio 12-d_4 DPPC/cholesterol = 2:1
tgg class determined by difference

1. What are the fractions of trans and gauche conformers at a specific acyl chain position (in this instance, the 6 and 6'positions) at various temperatures and levels of Gramicidin?

2. What is the detailed nature of lipid acyl chain conformational disorder in the presence of Gramicidin; i.e., what are the relative amounts of gauche conformers arising from the sum of kink + single gauche bends compared with multiple gauche bends ?

3. How do these results compare with those in aqueous preparations of 6-d_4 DPPC and mixtures of 6-d_4 DPPC and cholesterol?

As in our previous reports for phospholipid bilayers (5) and for DPPC / cholesterol mixtures (6), two assumptions have been made in the data analysis :

1. The relative trans/gauche absorptivities of the CD_2 rocking modes calculated by Maroncelli, et al. (3,4), are transferable from alkanes to phospholipid acyl chains. In addition, it is assumed that the relative extinction coefficients of these modes are not sensitive to the presence of Gramicidin in the bilayer.

2. CD_2 rocking frequencies are sensitive primarily to trans /gauche isomerization because the rapid time scale of molecular vibrations uncouples these from slower motions detected in NMR and other studies.

Infrared spectroscopy was used by Chapman and co-workers (7,8) in studies of the effects of insertion of Gramicidin into acyl-chain perdeuterated dimyristoylphosphatidylcholine (DMPC) bilayers. C-D stretching frequencies and bandwidths were studied as a function of temperature and Gramicidin concentration. The following qualitative features of lipid/peptide interaction were deduced. First, peptide insertion caused an overall increase in gauche conformers in DMPC below T_m. At temperatures above T_m, a decrease in gauche conformers was observed for lipid/Gramicidin ratios greater than 10. No further decrease in order was observed with additional Gramicidin insertion. Short et al. (9) monitored CH_2 twisting, C-H stretching, and carbon skeletal stretching modes of DMPC in a Raman spectroscopic investigation and also observed an increase in lipid acyl chain conformational disorder below T_m of the pure lipid. The current results place the above conclusions of acyl chain disorder on a more quantitative foundation. Below T_m for 6-d_4 DPPC, Gramicidin D insertion causes the overall percentage of gauche rotamers to increase (Table 1). At 34°C, the total gauche rotamer percentage in 10:1 6-d_4 DPPC/Gramicidin D is 9.8 %, a factor of ~6 increased compared with 1.7 % in pure 6-d_4 DPPC. Similar trends are evident at lower temperatures. Above T_m for pure 6-d_4 DPPC, gauche rotamer percentages are 32.3, 17.7 and 15.0%, for pure DPPC at 48.5°C, and 30:1 and 10:1 DPPC/Gramicidin mixtures at 44°C, respectively. Thus, at the temperatures and lipid / protein ratios studied, Gramicidin D induces conformational disorder in the lipid gel phase and orders the liquid crystalline phase.

A unique advantage of the current experimental approach is the possibility of delineating the nature of the peptide-induced disordering process in the lipid gel phase. The areas of the 646 cm^{-1} and 652 cm^{-1} conformational markers can, taken together, be used to calculate fractions of conformers arising from either multiple gauche forms (646 cm^{-1} marker) or the sum of kinks and

single gauche bends (652 cm^{-1} marker) within the total gauche conformer population. At 44°C for a 30:1 lipid/Gramicidin D sample, 78±16% of the gauche conformers arise from kinks of the type tg'tgt and single gauche bends of the type ttgtt and is, within stated errors, the same as that found in pure 6-d_4 DPPC (74±18%) (Table 2). In the 10:1 mixture at 44°C, the percentage of (kinks + gauche) drops dramatically to 15±10%; the disordering process thus arises from multiple gauche forms such as tg'tggt. It is speculated that these forms are best suited to complement the shape of the exterior peptide surface, and that they therefore dominate at high peptide levels. Increasing the temperature from 34 to 44°C does not alter the relative intensities of the gauche marker bands in the 10:1 mixture, whereas in the 30:1 sample the 652 cm^{-1}/622 cm^{-1} ratio increases much more rapidly than the 646 cm^{-1}/622 cm^{-1} and is clearly the main source of disordering induced in the acyl chains. Results similar to the latter were observed in cholesterol mixed with (2:1 lipid/sterol) DPPC deuterated at the 12 position, in which the relative intensity of the 646 cm^{-1} marker was constant over the temperature range 34-44°C while that of the 652 cm^{-1} marker increased from 1.7% to 5.8%. Therefore, we conclude that Gramicidin D, in addition to modulating gauche conformer populations, exerts a strong influence on the type of acyl chain geometry induced upon its insertion into bilayers. The relative contributions of particular gauche forms to acyl chain disordering as a function of temperature and protein content can thus begin to be distinguished.

It is interesting to compare the current quantitative estimates of disordering with those available from other experimental approaches. Morrow and Davis (10) have used ^2H NMR and DSC to map out Gramicidin/DPPC phase behavior and order and concluded from the temperature dependence of the first moment of the DPPC-d_{62} signal that the peptide has a strong disordering effect just below the transition and a weaker ordering effect just above the transition, which itself was abolished at about 2-3 % peptide. Our quantitative determinations of ordering,although carried out at higher peptide levels, are in agreement with their general observations, although we can make no comment on the cooperativity of the observed temperature dependence of the disordering, due to insufficient temperature resolution. Rice and Oldfield (11) studied the interaction of specifically deuterated DMPC with Gramicidin at 30°C at concentrations up to 67 wt % of peptide. Up to 6 mol%, they observed partial ordering of the acyl chains by the peptide. At higher levels, a disordering was noted. These results are in partial accord with the current data, although the different acyl chain lengths and deuteron position used in the analysis may vitiate attempted detailed comparisons.

The CD_2 probe method is a powerful tool in the investigation of membrane conformational disorder, uncovering subtle details of the acyl chain trans-gauche isomerization process. Work is currently underway to apply this techique to systems containing phospholipids specifically deuterated at various positions in order to probe the depth dependence of membrane disorder. Of interest in this regard is the extensive work of de Kruijff and his collaborators (1, and references therein), on the Gramicidin-induced H_{II} phase.

Acknowledgment : This work was supported by the Public Health Service through NIH grant GM29864 to RM.

REFERENCES

1. de Kruijff, B., Cullis, P.R., Verkleij, A.J., Hope, M.J., van Echteld, C.J.A., Taraschi, T.F., van Hoogevest, P., Killian, J.A., Rietveld, A., van der Steen, A.T.M. (1985) in Progress in Protein-Lipid Interactions (A. Watts and J.J.H.H.M. DePont, Eds.), Vol. 1, pp. 89-142
2. Snyder, R.G., Poore, M.W. (1973) Macromolecules, **6**, 708-715
3. Maroncelli, M., Strauss, H.L., Snyder, R.G. (1985) J. Phys. Chem. **89**, 4390-4395
4. Maroncelli, M., Strauss, H.L., Snyder, R.G. (1985) J. Chem. Phys. **82**, 2811-2824
5. Mendelsohn, R., Davies, M.A., Brauner, J.W., Schuster, H.F., Dluhy, R.A. (1989) Biochemistry **28**, 8934-8939
6. Davies, M.A., Schuster, H.F., Brauner, J.W., Mendelsohn, R. (1990) Biochemistry, in press
7. Cortijo, M., Alonso, A., Gomez-Fernandez, J.C., Chapman, D. (1982) J. Mol. Biol. **157**,597-618
8. Lee, D.C., Durrani, A.A., Chapman, D. (1984) Biochim. Biophys. Acta **769**, 49-56
9. Short, K.W., Wallace, B.A., Myers, R.A., Fodor, S.P.A., Dunker, A.K. (1987) Biochemistry **26**, 557-562
10. Morrow, M.R., Davis, J.H. (1988) Biochemistry **27**, 2024-2032
11. Rice, D., Oldfield, E. (1979) Biochemistry **18**, 3272-3279