Biochimica et Biophysica Acta, 468 (1977) 490—494

BBA Report

BBA 71299

COOPERATIVE UNIT SIZE IN THE GEL-LIQUID CRYSTALLINE PHASE TRANSITION OF DIPALMITOYL PHOSPHATIDYLCHOLINE-WATER MULTILAYERS: AN ESTIMATE FROM RAMAN SPECTROSCOPY

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(Received April 4th, 1977)

Summary

The temperature dependence of the Raman spectral transitions assigned to the acyl chain C-C stretching modes of dipalmitoyl phosphatidylcholine was determined for the gel, phase transition and liquid crystalline states of the lipid multilayers. The van't Hoff enthalpy differences ΔH_{VH} between *trans* and *gauche* rotational isomers were obtained from the Raman spectral data for the temperature region characteristic of each bilayer state. An average size for the cooperative unit undergoing the chain melting process during the phase transition was estimated from the ratio of the appropriate van't Hoff enthalpy to an adjusted calorimetric enthalpy.

For phospholipid bilayer systems undergoing gel to liquid crystalline phase transitions, values for the average size of the cooperative units participating in the hydrocarbon chain disordering process are important parameters both for indicating the extent of lipid-lipid interactions in membrane related assemblies and for the further theoretical modeling of transition behavior. In a recent series of studies we determined the Raman spectral temperature dependence of acyl chain carbon-carbon (C-C) stretching modes and applied these data toward probing the intramolecular disorder, or flexibility, of the hydrocarbon chains of a variety of bilayer dispersions (ref. 1; Levin, I.W. and Spiker, Jr., R.C., unpublished data). Specifically, van't Hoff enthalpy differences ΔH_{VH} between *trans* and *gauche* forms of the acyl chains were obtained for systems both in the relatively ordered gel form and in the fluid, liquid crystalline state. In the present report we emphasize an extension of these

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ideas first by determining the van't Hoff enthalpy ΔH_{VH} for the thermal phase transition of dipalmitoyl phosphatidylcholine-water multilayers from Raman spectroscopy and then by comparing ΔH_{VH} to an adjusted calorimetric enthalpy in order to estimate the cluster size of a cooperative unit undergoing the chain melting phenomenon.

High purity samples of 1,2-dipalmitoyl-D,L-phosphatidylcholine were commerically obtained from Sigma Chemical Company. Since these samples either gave a single spot by thin-layer chromatography or produced no major spectral contaminants, the samples were used without further purification. The procedures used to prepare the lecithin multilayers have been previously described [1—3]. Vibrational Raman spectral data were collected on two different spectrometers utilizing a variety of sampling techniques [2—4]. In general, the spectral resolution varied between 2 and 5 cm^{-1}. Absolute spectral frequencies were monitored by either argon or neon lines. Although the data from the separate spectrometers are in overall accord, only one set [3] will be discussed in detail since the narrow temperature intervals between these data points for this experiment allow the lower phase transition to be more faithfully reproduced.

Effects of *trans-gauche* isomerization, the intramolecular disordering mechanism for acyl chains, are conveniently monitored by following the temperature dependence of the C-C skeletal stretching modes in the 1100 cm^{-1} region of the Raman spectrum. For dipalmitoyl lecithin-water multilayers in the gel form, vibrational features at 1130, 1101 and 1062 cm^{-1} are assigned at liquid nitrogen temperatures to all-*trans* C-C stretching modes. (The phosphate symmetric and C-O stretching modes lie under the 1090 and 1050 cm^{-1} regions, but are quite weak in intensity.) As the temperature of the bilayer increases, these modes exhibit moderate frequency shifts and intensity changes as gauche rotamers are introduced into the hydrocarbon chains. Spectral features in the 1090—1085 cm^{-1} region are specifically assigned to C-C modes characteristic of gauche conformations. The most dramatic spectral effects occur as the multilayers pass through the gel-liquid crystalline phase transition, the narrow temperature range during which the chains cooperatively melt. Above the phase transition, the changes in the Raman spectrum are again more gradual as further gauche conformers are formed and populated. The general spectral appearance of dipalmitoyl lecithin in these three characteristic temperature intervals appears, for example, in ref. 2. In the liquid crystalline form of the multilayer dispersion, the intensity of the mode at about 1130 cm^{-1} in the gel decreases significantly and is replaced by a vibrational transition at approximately 1124 cm^{-1}. This feature is probably now best assigned as being representative of C-C skeletal stretching modes of an entire acyl chain consisting of perhaps various *trans* segments appropriately separated by gauche bond rotations [1]. The important point to note is that for the molecular state defined either by the melting of the hydrocarbon chains during the phase transition or by the liquid crystalline form of the multilayer, the decrease in intensity of the $\sim$1124 cm^{-1} feature represents a decrease in chain order, while the increase in intensity of the 1090—1085 cm^{-1} region represents an increase in chain disorder.

Once specific spectral features are assigned to either *gauche* or *trans* chain configurations, for the gel state, for example, enthalpy differences ΔH between acyl chain rotamers may be conveniently estimated from the temperature dependent Raman intensities [1]. For the phase transition and liquid crystalline regions, the vibrational transitions are assigned either to the relatively ordered or disordered chain forms. Thus, for the gel the enthalpy ΔH is related to the Raman peak intensities I by the integrated form of the van't Hoff equation $\ln(I_{\text{gauche}}/I_{\text{trans}}) = -\Delta H_{\text{vH}}/RT + C$, where I_{gauche} and I_{trans} are proportional to the concentration of *gauche* and *trans* rotamers, respectively. R, T and C represent the gas constant, temperature and a constant of integration, respectively. For the phase transition and liquid crystalline regions in which the ~1024 and 1090 cm^{-1} features represent the relatively ordered and the relatively disordered states, the left hand side of the equation for the enthalpy change becomes $\ln(I_{\text{disordered}}/I_{\text{ordered}})$. Although integrated Raman intensities should be used in the van't Hoff expression, peak height values appear to be sufficient in the absence of an accurate decomposition of the contours in this spectrally congested region [1].

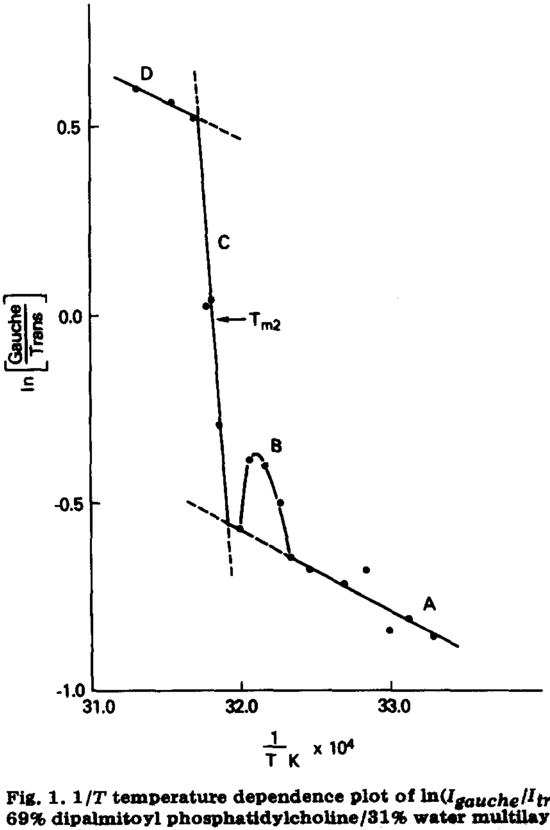

Fig. 1. $1/T$ temperature dependence plot of $\ln(I_{gauche}/I_{trans})$ for hydrocarbon chain isomerization for 69% dipalmitoyl phosphatidylcholine/31% water multilayers in the gel, phase transition and liquid crystalline states. I_{gauche} and I_{trans} represent peak height intensities of the Raman transitions in the 1090 and 1130 cm^{-1} regions, respectively. Note that for both the phase transition and liquid crystalline regions, the ~1090—1085 and ~1024 cm^{-1} features represent the relatively disordered and the relatively ordered acyl chain forms, respectively. Thus, the temperature dependence plots for the phase transition and liquid crystalline regions become $\ln(I_{\text{disordered}}/I_{\text{ordered}})$ vs. $1/T$. T_{m_2} defines the midpoint of the phase transition curve, which, for these data, occurs at 41°C.

A plot of $\ln(I_{1088}/I_{\sim 1130})$ as a function of $1/T$ defines four distinct temperature regions, A, B and C and D, as shown in Fig. 1, which correspond to the gel, lower phase transition, main phase transition and liquid crystalline states. The slopes for the lines in each region yield a value for the van't Hoff enthalpy ΔH_{vH} representing the change between the respective ordered or disordered chain states. (The band intensities for the 1090 cm^{-1} gauche region were corrected in the gel state for the low temperature all-trans background [1].)

In region B of the van't Hoff plot we note particularly the appearance of the lower phase transition ($1/T \times 10^4 \approx 37°\text{C}$). After the completion of the lower phase transition, $\ln(I_{\text{gauche}}/I_{\text{trans}})$ reverts back to a value characteristic of the gel state prior to the onset of the lower transition. Values for ΔH determined from least-squares fits for the gel and liquid crystalline regions, A and D are 4.4 ± 1.4 and 3.5 ± 1.7 kcal/mol, respectively, where dispersions in the enthalpies are estimated by accepting values of twice the least-squares standard deviations*.

Region C in the figure depicts the narrow temperature range of the phase transition for the lecithin multilayers. The van't Hoff enthalpy ΔH_{vH} between the relatively ordered and disordered molecular states for the chain melting region is 107 kcal/mol. For a lower limit we obtain a ΔH_{vH} value of 71 kcal/mol by requiring the van't Hoff plot to pass through the end-points of regions A and D.

The size of a cooperative unit of the bilayer undergoing chain melting behavior is given by the ratio of the van't Hoff enthalpy for the phase transition region to the calorimetric enthalpy [5—7]. In identifying ΔH_{vH} with the enthalpy for the phase transition of a cooperative unit, one assumes that the mechanism involves a two step process; that is, a conversion from the relatively ordered, gel state to the final relatively disordered, liquid crystalline state. Since the van't Hoff enthalpy derived from the temperature dependent Raman spectral data explicitly reflects acyl chain trans-gauche isomerization, it would appear reasonable to correct the observed calorimetric enthalpy [6] in order to express only the energy change entailing the rotational isomeric contribution. Nagle [8] has estimated the rotational contribution by subtracting a calculated van der Waals chain interaction energy of 5.7 kcal/mol and an estimate of the head group interaction term of 0.5 kcal/mol [9] from the observed calorimetric 9.69 kcal/mol [6] phase transition enthalpy. Thus, from $\Delta H_{\text{vH}}^{\text{order-disorder}}/\Delta H_{\text{calorimetric (corrected)}}^{\text{order-disorder}}$ we estimate the cluster size of a cooperative unit of dipalmitoyl lecithin to be ≈31 molecules (107 (kcal/mol of coop. units)/3.5 (kcal/mol)). Clearly, a lower limit in the unit size would then be given by the van't Hoff enthalpy divided directly by the observed calorimetric enthalpy; that is, 107 (kcal/mol of coop. units)/9.69 (kcal/mol) ≈11 molecules.

We may compare the size of a cooperative unit of dipalmitoyl lecithin determined from Raman data with values obtained from both fluorescence [10] and calorimetric measurements [6]. Since the widths of the phase transi-

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*Ref. (1) obtained a value of 3.4 ± 0.4 kcal/mol for ΔH_{vH} in the gel region. Although this value differs from the present one, they are within their respective range of errors. The 3.4 ± 0.4 kcal/mol is probably the more reliable value as it is representative of a van't Hoff plot covering a greater temperature span.

tion vary somewhat as a function of sample preparation [6], comparisons between physical techniques should perhaps be considered only illustrative at the present time. Thus, a cluster size estimate for dipalmitoyl lecithin determined from the fluorescence properties of an imbeded probe molecule is about 19 molecules [10]*, while that from scanning calorimetry [6] is about 70 ± 10 molecules. In view both of the uncertainties involved in sampling and of the different molecular properties reflected by each experimental method, the range in values between the various physical techniques for determining the size of a cooperative unit may perhaps be considered satisfactory at the present.

Note added in proof: (Received May 31st, 1977)

Recently, a calorimetric value of 8.2 kcal/mol was determined for ΔH of the high temperature phase transition of dipalmitoyl phosphatidylcholine multilayers [11]. The rotational isomeric contribution to the calorimetric value would be about 2.2 kcal/mol from Nagle's estimates of headgroup and chain-chain interactions [8,9]. For this value, however, the size of the cooperative unit then becomes ≈ 49 molecules.

Acknowledgment

One of us (N.Y.) acknowledges the support provided by a National Institutes of Health Visiting Fellow appointment. A portion of this work was carried out in the Chemistry Department of Hunter College of the City University of New York. In this respect, we wish to express our thanks to Dr. B.J. Bulkin for his kind cooperation.

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*This value was obtained by the ratio of the van't Hoff enthalpy determined from fluorescence measurements (185 kcal/mol) [10] to the current calorimetric enthalpy of 9.69 kcal/mol [6].