Gel-liquid crystalline transition of some multilamellar lipid bilayers follows classical kinetics with a fractional dimensionality of approximately two

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ABSTRACT The relaxation kinetics of the gel-liquid crystalline transition of phosphatidylcholine ($DC_{14}PC$, $DC_{16}PC$, and $DC_{18}PC$) multilamellar vesicles have been examined using volume-perturbation calorimetry. The time-dependent temperature and pressure changes associated with a periodic volume perturbation are monitored in real time. Data collected in the time domain are transformed to the frequency domain using Fourier series representations of the perturbation and response functions. Because a very small perturbation is imposed during the experiment, linear response theory is suitable for analysis of the relaxation process. The Laplace transform of the classical Kolmogorov-Avrami relation of transition kinetics is used to describe the dynamic response in the frequency domain. For $DC_{14}PC$ and $DC_{16}PC$, the relaxation process is better fit with an effective dimensionality of $n = 2$ rather than $n = 1$. For $DC_{18}PC$, we estimate that an effective dimensionality of ~ 1.5 will best fit the data. These results indicate that the gel-liquid crystalline transition of these lipid bilayers follows the classical Kolmogorov-Avrami kinetic model with an effective dimensionality > 1 and the assumption of simple exponential decay ($n = 1$) commonly used in data analysis may not always be valid for lipid transitions. Insofar as the dimensionality of the relaxation reflects the geometry of fluctuating lipid clusters, this parameter may be useful in connecting experimental thermodynamic and kinetic results with those obtained from Monte Carlo simulations.

INTRODUCTION

Various techniques have been developed to study the kinetics of the gel-liquid crystalline transition of lipid bilayer systems. Reviews of such studies can be found in van Osdol et al. (1989) and Caffrey (1989). A wide range of relaxation times, from tens of nanoseconds to seconds, has been reported. A general qualitative agreement among these different results has been found in that both relaxation times and amplitudes are maximal at or near the transition temperature, T_m. We recently described the relaxation kinetics of the gel-liquid crystalline transition of five different phosphatidylcholine multilamellar vesicles obtained using volume-perturbation calorimetry (van Osdol et al., 1991). The data reported in that earlier publication were analyzed in terms of a single, major relaxation process exponential in time. For all these lipids, the relaxation time was maximal near the T_m and equal to 2–4 s.

A major question is what theoretical model is quantitatively suitable to describe the kinetic process of the bilayer main transition. The assumption of simple exponential decay kinetics has been universally adopted in the data analysis without any critical justification. However, Yang and Nagle (1988) reported the results of a careful dilatometric and differential scanning calorimetric studies of the kinetics of the sub- and pretransition of dipalmitoylphosphatidylcholine multilamellar vesicles, and concluded that these transitions follow the classical Kolmogorov-Avrami relation (Kolmogorov, 1937; Avrami, 1939, 1940, 1941) with a fractional dimensionality slightly > 1. The main transition is too fast to have been studied with their instrumentation, but they suggested Kolmogorov-Avrami theory with an effective dimensionality of two may apply to the kinetics of the main transition. In this communication, we apply this cluster growth model to describe the relaxation kinetics of the main transition of three different phosphatidylcholine multilamellar bilayers and conclude that indeed $n > 1$ for these systems.

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THEORY

The classical Kolmogorov-Avrami kinetic theory in the time domain has been reviewed and summarized by Yang and Nagle (1988). Briefly, the fractional completion, f, of sample transformed to the new phase after a step perturbation is given by,

$$df/dt = N(1 - f)dV/dt, \quad (1)$$

where N is a fixed number of randomly distributed nuclei

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per unit vol, each of which will grow to a vol V at time t if it does not meet another domain. For reasonably smooth domains,

$$V = gr^n = gu^n t^n, \tag{2}$$

where u is the radial growth rate, g is a geometrical factor which is $4\pi/3$ for spheres and π for circles, and n is the effective dimensionality of the growing domain. Eq. 1 can be solved to yield

$$f = 1 - \exp(-Ngu^n t^n) = -\exp[-(t/\tau)^n], \tag{3}$$

where

$$\tau = 1/[u(Ng)^{1/n}].$$

The corresponding impulse response function is then,

$$I(t) = df/dt = [nt^{(n-1)}/\tau^n]\exp[-(t/\tau)^n]. \tag{4}$$

For a linear response system, the frequency spectrum can be obtained by the Laplace transform, $L(S)$, of the impulse response function. The Laplace transform of Eq. 4 is,

$$\text{for } n = 0.5: L(s) = 0.5\,(\pi/\tau s)^{1/2}\exp(1/4\tau s) \cdot \{1 - erf\,[1/2(\tau s)^{1/2}]\} \tag{5a}$$

$$n = 1: L(s) = (1 + \tau s)^{-1} \tag{5b}$$

$$n = 2: L(s) = 1 - \tau s\,\pi^{1/2}/2\exp[(\tau s)^2/4] \cdot \{1 - \mathrm{erf}(\tau s/2)\}, \tag{5c}$$

where the error function: $\mathrm{erf}(t) = 2/\pi^{1/2}\int_0^t \exp(-u^2)du$. The frequency spectrum of the kinetic process is obtained by taking $s = \sqrt{-1}\omega$, where ω is the perturbation frequency.

MATERIALS AND METHODS

A home-built, volume-perturbation calorimeter (Johnson et al., 1983; van Osdol et al., 1989) was used for these kinetic studies. The design, calibration, and operation of the instrument and the method of data analysis are described in detail in the review article by van Osdol et al. (1989). Phosphatidylcholines were purchased from Avanti Polar Lipids, Inc. (Birmingham, AL) and stored frozen until used. The purity of each lipid was checked by thin layer chromatography. Lipid dispersions were prepared and characterized as described previously (van Osdol et al., 1991).

RESULTS

Fig. 1 shows the calculated frequency spectra for linear response systems following the classical Kolmogorov-Avrami kinetics with a time constant of 2 s and fractional dimensionality, n, of 0.5, 1, and 2, respectively. It can be seen that the relaxation amplitude decreases more sharply for larger n as the perturbation frequency increases. This behavior is independent of the relaxation time constant.

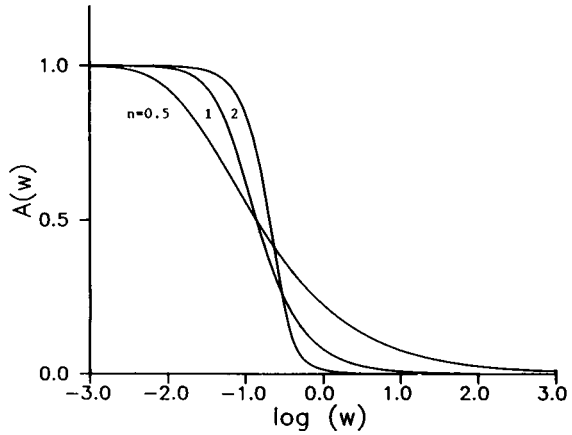

FIGURE 1 The normalized relaxation amplitude $A(\omega)$ vs. $\log_{10}(\omega)$, where ω is the perturbation frequency, calculated according to the Kolmogorov-Avrami theory, assuming a relaxation time of two seconds and dimensionalities of 0.5, 1, and 2, respectively.

In Fig. 2, the frequency dependent relaxation amplitudes of $DC_{14}PC$, $DC_{16}PC$, and $DC_{18}PC$ multilamellar vesicles (MLV) at three temperatures from just below to just above their respective transition temperatures are shown. Due to the computational complexity of fitting the data with noninteger n, we have only analyzed data with $n = 1$ and 2 to demonstrate the validity of employing Kolmogorov-Avrami theory to describe the relaxation kinetics of lipid bilayers. The data fitting for $n = 1$ was done by a nonlinear, least square analysis program (van Osdol et al., 1989) and for $n = 2$ was done manually using calculated relaxation curves such as shown in Fig. 1. Because a perturbation frequency of >30 Hz is approaching the dynamic response limit of the thermistor, the data obtained at frequencies >30 Hz were not used in the curve fitting. Correction for the thermistor time response indicated that the high frequency data represent normal Joule-Thompson heating of the lipid dispersion and are not associated with relaxation modes of the transition (van Osdol et al., 1989, 1991). None of the data can be well fit with $n = 0.5$. The $DC_{14}PC$ and $DC_{16}PC$ data are better fit with $n = 2$ rather than $n = 1$, with the best fit using a value of n slightly <2. A typical residual distribution in Fig. 3 clearly shows that the fitting with $n = 1$ exhibits greater systematic deviation than that with $n = 2$. In contrast, the $DC_{18}PC$ data appear to be equally well fit with $n = 1$ and $n = 2$. However, the systematic deviations of the two

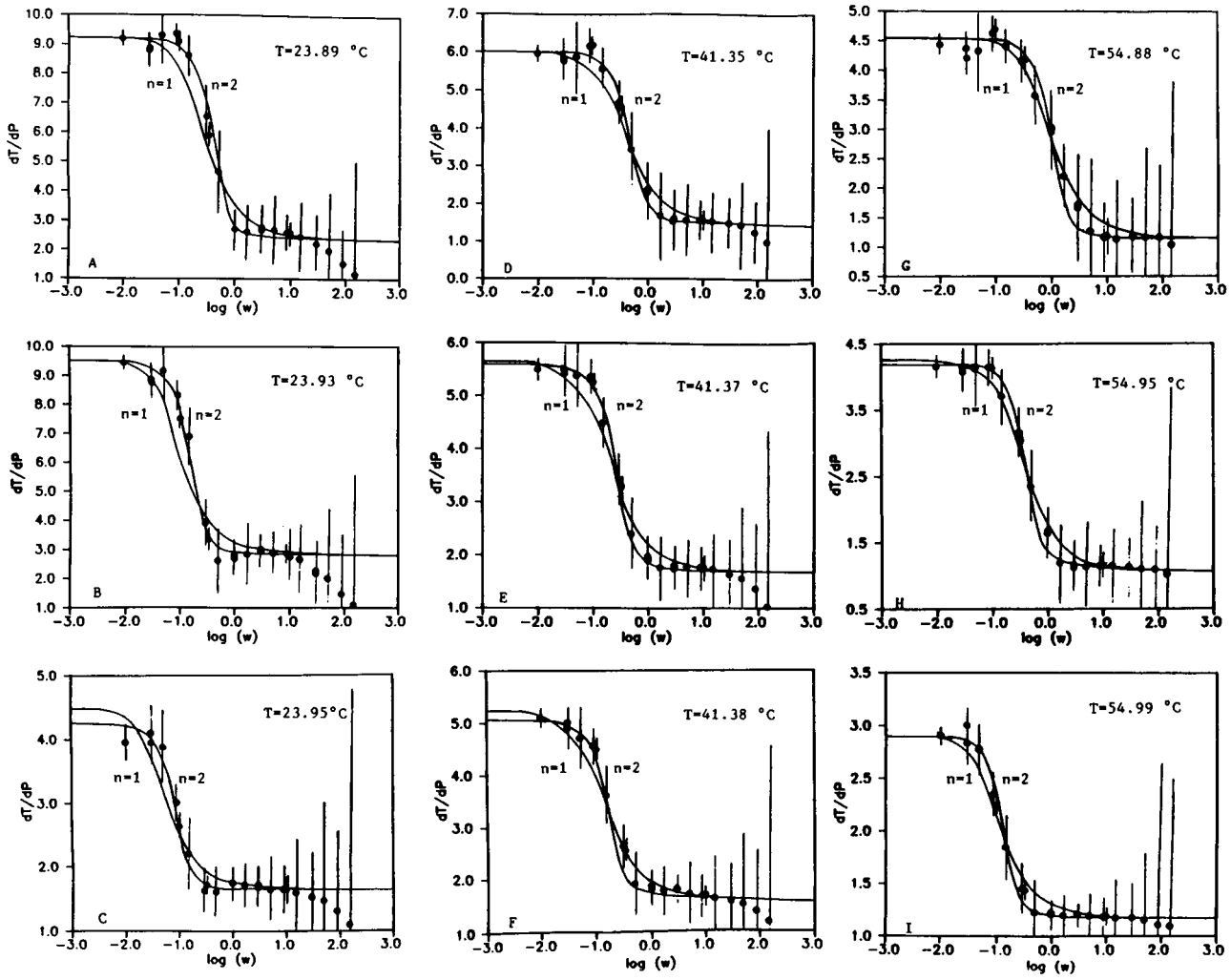

FIGURE 2 Relaxation amplitudes vs. $\log_{10}$ of the perturbation frequency for $DC_{14}PC$ (*A–C*), $DC_{16}PC$ (*D–F*) and $DC_{18}PC$ (*G–I*) at three temperatures near their respective T_m. The lipid concentrations were 150–180 mM. The ordinate is in units of the response due to water at the respective temperature as previously described (van Osdol et al., 1991). The standard errors of the data are indicated by the vertical lines. The two solid lines in each graph represent the best fit of the data to the Kolmogorov-Avrami model with $n = 1$ and $n = 2$, respectively. The data obtained at frequencies higher than 30 Hz were assumed to reflect simple Joule-Thompson relaxation of the sample and therefore, not used in the curve fitting (see text).

fits indicate that a best fit would be obtained with n at ~ 1.5. The statistics of fittings with $n = 1$ and $n = 2$ are summarized in Table 1.

DISCUSSION

This is the first application of the classical Kolmogorov-Avrami kinetic model to describe the main transition of lipid bilayer systems. It was found that the gel-liquid crystalline transition of $DC_{14}PC$ MLV follows the classical Kolmogorov-Avrami kinetics with an effective fractional dimensionality of ~ 2. This apparent dimensionality decreases as the acyl chainlength increases for saturated di-acyl phosphatidylcholines. This model is a mechanistic description of domain growth with time when physical conditions are changed. Because of the complexity of lipid phase transitions, there is no well-established theory to describe the main transition kinetics quantitatively. Most interpretations of kinetic experiments in the literature have been qualitative in spirit. It now appears that the classical Kolmogorov-Avrami theory will allow a more mechanistic interpretation of the relaxation behavior of bilayer systems in the gel to liquid crystalline transition region.

Within the scope of classical kinetic theory, the phase

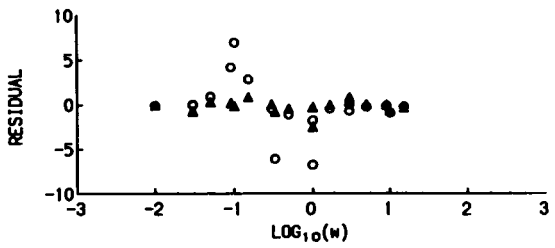

FIGURE 3 A typical residual distribution for fittings for $n = 1$ (0) and $n = 2$ (▲), as obtained for $DC_{14}PC$ MLV relaxation at T = 23.93°C (graph *B* in Fig. 2). The residual is defined as the ratio of the deviation of the data point from the fitting curve to the standard error of that data point. Because three harmonics have been used for each foundamental perturbation frequency, more than one datum has been collected at some frequencies (van Osdol et al., 1989).

transition begins with nucleation and then nuclei proceed to grow. Nuclei are formed due to thermodynamic fluctuations which are greatly enhanced in the transition region. The critical size which dictates whether a nucleus will grow or dissipate is determined by the physical conditions. The rate of nucleation and growth is an increasing function of the extent of supercooling and the detailed kinetics of nucleation and growth could be quite different, depending on the magnitude of perturbation (Dunning, 1969; Landau and Lifshitz, 1980).

Most kinetic experiments, such as temperature jump (Tsong and Kanehisa, 1977; Gruenewald, 1982; Laggner et al., 1987), pressure jump (Gruenewald et al., 1980; Yager and Peticolas, 1982), pH-jump (Strehlow and Jahnig, 1981), et cetera, are done with a "large perturbation" resulting in the bilayer changing completely from one phase to another. This is because the nearly first order transition in phosphatidylcholine multilamellar vesicles has a transition half width of only ~0.07°C for $DC_{14}PC$ and $DC_{16}PC$ MLV. A unique feature of the volume-perturbation calorimeter is its "small perturbation" amplitude which only forces a small fraction of the bilayer to change state. An isothermal pressure perturbation of ±2 atm is equivalent to an isobaric temperature perturbation of ~ ±0.05 °C. In our experiments using 150 mM lipid, the fractional change in the degree of melting was only 3–4%. This was because the attendant temperature and pressure changes counteracted the effect of the initial pressure perturbation on the system. It should be noted that a previous study by van Osdol (1988) found that the dynamic response of lipid bilayers due to a small bidirectional perturbation was linear with respect to the magnitude of the perturbation and that the response to the perturbation was symmetric.

TABLE 1 Summary of statistics for the analysis of data shown in Fig. 2

	T_m (°C)	T (°C)	f	rms ($n = 1$)	τ (s) ($n = 2$)	rms ($n = 1$)	τ (s) ($n = 2$)
		23.89	0.41	2.11	0.91	1.22	0.89
$DC_{14}PC$	23.92	23.93	0.65	2.76	2.71	1.37	2.59
		23.95	0.74	1.77	4.19	0.80	4.31
		41.35	0.57	1.41	0.61	0.78	0.96
$DC_{16}PC$	41.35	41.37	0.65	1.48	1.13	0.61	1.78
		41.38	0.73	1.13	1.69	0.59	1.95
		54.88	0.50	1.27	0.26	1.43	0.41
$DC_{18}PC$	54.92	54.95	0.75	1.09	0.69	0.74	0.82
		54.99	0.90	1.01	2.79	0.89	2.87

T_m is obtained from the heat capacity function at a scan rate of 0.1 °C/h and serves as the standard value for the calibration of temperature of the volume perturbation calorimeter. T is scaled by a pressure effect of 0.024 °C/atm (van Osdol et al., 1991). $T = T_o - (P - 1) \times (dT_m/dP)$ where T_o is the actual mean temperature of the experiment, P is the actual mean pressure of the experiment and dT_m/dP, the pressure dependence of the melting temperature (Mountcastle, et al., 1978; Nagle and Wilkinson, 1978). This scaling procedure reduced the equilibrium melting profile to a common temperature scale at all pressures (van Osdol et al., 1991). f is the fractional degree of melting. τ is the estimated relaxation time, defined in Eq. 3. rms is the root mean square of the deviation of the fit.

The fluctuation-dissipation theorem (Callen and Welton, 1951) indicates that the dynamic response of a system to a small external perturbation is the same as the decay of spontaneous fluctuations about equilibrium. This implies that to study equilibrium thermal fluctuations, as small an external perturbation as possible must be used. The small perturbation used in our experiment offers the best possibility of separation of nucleation from growth by creating a situation where negligible new nucleation occurs and growth dominates the kinetics. Because the induced temperature change in our volume-perturbation experiment is only on the order of 0.05°C, the supercooling or superheating condition necessary for new nuclei to form is thus limited. The nuclei ready to grow upon external perturbation are primarily those which exist in the dynamic equilibrium state due to spontaneous thermal fluctuations (Landau and Lifshitz, 1980).

In experiments with large perturbations, the response function of the bilayer system is outside the linear response range. In these cases, the relaxation process may be very complex due to the fact that it actually reflects a combination of several subprocesses overlapping in the time domain. Lipid domains will coalesce with one another as they grow and other structural reorganizations may occur. Thus, it is not surprising that the relaxation time for formation of the gel state in the bilayer starting from a temperature in the transition region may be different from that obtained from melting of the bilayer following a perturbation at the same temperature as was found in the pressure-jump experi-

ments of Yager and Peticolas (1982). If nucleation continuously takes place at a constant rate while growth of the existing nuclei proceed, the fractional dimensionality n should then be $n + 1$ (Kolmogorov, 1937; Avrami, 1939, 1940, 1941). This is less likely to occur in a small perturbation experiment such as the ones described in this report.

Several conclusions can be drawn from this study. First, water diffusion does not appear to be the rate-limiting step of the phase transition under our experimental conditions. The permeability of water has been found to undergo a sharp increase as the bilayer changes phase from gel to liquid crystalline state (Blume, 1986). If water diffusion is the rate-limiting step, the formation of gel domains during the freezing process would be progressively hindered as more lipid becomes gel. This decrease of the growth rate of gel domains would be consistent with a decrease of n. On the other hand, the melting process would result in a progressive increase of the growth rate due to the formation of more liquid crystalline lipid, consistent with an increase of n. However, such behavior is inconsistent with the fact that a symmetrical relaxation is observed in the time domain (van Osdol, 1988) and the fact that the relaxation time reaches a maximum at the transition point as the temperature is increased, instead of a monotonic decrease as would be expected for a water-diffusion limited process.

Second, heat diffusion across the multibilayers is not the rate-limiting step during the phase transition process because the transient process of heat diffusion from the bilayers to the aqueous medium should be characterized by $n = 1$ (i.e., $\exp[-t/\tau]$). Due to the fact that the lipid main transition proceeds much faster than the sub- and pretransition, Yang and Nagle (1988) suggested that heat diffusion might become the rate-limiting step. However, the results of our dimensionality analysis indicates that this is not true under our experimental conditions.

Third, the fractional dimensionality of ~ 2 for $DC_{14}PC$ and $DC_{16}PC$ MLV suggests that the growing domains are very compact and nearly circular in shape in the bilayer plane. This compactness of domains indicates a highly unfavorable Gibb's free energy of interaction between unlike nearest neighbors and therefore a high degree of cooperativity in the transition process (Biltonen, 1990). The fact that n is close to two supports the prediction made by Yang and Nagle (1988) for the lipid main transition. The apparently lower fractional dimensionality of $DC_{18}PC$ MLV in contrast to those of $DC_{14}PC$ and $DC_{16}PC$ MLV indicates a smaller unfavorable Gibb's free energy of interaction between unlike nearest neighbors and therefore a lower cooperativity for the transition. This conclusion is consistent with the temperature-dependent heat capacity results of Mabrey and Sturtevant (1976) who found that the cooperativity of saturated diacylphosphatidylcholine bilayers decreases as the acyl chains become longer. Thus, this dimensionality analysis appears to have the potential of connecting experimental thermodynamic and kinetic results with these obtained from Monte Carlo simulations (Mouritsen, 1990) to achieve a comprehensive understanding of the main transition. It should be noted that a low fractional dimensionality may be an indication of domains growing in a fractal mode (Yang and Nagle, 1988) as has been suggested to occur in lipid monolayers containing dye impurities when rapid supercooling is imposed (Miller et al., 1986).

The common assumption of a sum of one dimensional exponential decays ($n = 1$) generally used in data analysis may not be valid for lipid transitions. Due to the lack of a well established kinetic theory for the main transition of lipid bilayers, this assumption has been adopted without sufficient justification. Whereas use of an exponential decay to estimate the time scale in a relaxation phenomenon is justified, one should be cautious of assigning each nominal exponential decay to a specific step of the overall physical process, such as nucleation, growing, annealing, et cetera, because the fractional dimensionality for each step has been artificially fixed to unity. Any relaxation process of apparent dimensionality n can be fit by a summation of several relaxations, each with a dimensionality larger than n. Even if the apparent dimensionality is equal to one, the overall process may involve several steps of higher dimensionality. On the other hand, no relaxation process of apparent dimensionality n can be described quantitatively by a summation of relaxation steps each of dimensionality less than n.

There is one further point we wish to address. If the MLV preparations were heterogeneous in such a way as being a distribution of vesicles with different relaxation times at a given temperature, then the apparent dimensionality n would be less than the true dimensionality of the relaxing system. Because we find $n \approx 2$, such heterogeneity must be negligible if the ideal dimensionality is 2 as suggested by Yang and Nagle (1988). If this statement of negligible kinetic or geometric heterogeneity applies to the subgel and pretransitions examined by Yang and Nagle (1988), then the lower values of $n \approx 1.08$ they obtained are consistent with fractal growth occuring under their experimental conditions.

Finally, we would like to note that the more detailed analysis of the relaxation behavior of lipid bilayers presented in this report does not alter any of the conclusions based on similar data, which were analyzed assuming the kinetic processes were exponential in time (van Osdol et al., 1991).

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