Effects of the anesthetic dibucaine on the kinetics of the gel-liquid crystalline transition of dipalmitoylphosphatidylcholine multilamellar vesicles

William W. van Osdol, Qiang Ye, Michael L. Johnson, and Rodney L. Biltonen
Departments of Biochemistry and Pharmacology and The Biophysics Program, University of Virginia, Charlottesville, Virginia 22908

ABSTRACT The effects of the anesthetic dibucaine on the relaxation kinetics of the gel-liquid crystalline transition of dipalmitoylphosphatidylcholine ($DC_{16}PC$) multilamellar vesicles have been investigated using volume-perturbation calorimetry. The temperature and pressure responses to a periodic volume perturbation were measured in real time. Data collected in the time domain were subsequently converted into and analyzed in the frequency domain using Fourier series representations of the perturbation and response functions. The Laplace transform of the classical Kolmogorov-Avrami kinetic relation was employed to describe the relaxation dynamics in the frequency domain. The relaxation time of anesthetic-lipid mixtures, as a function of the fractional degree of melting, appears to be qualitatively similar to that of pure lipid systems, with a pronounced maximum, τ_{max}, observed at a temperature corresponding to greater than 75% melting. The τ_{max} decreases by a factor of ~2 as the nominal anesthetic/lipid mole ratio increases from 0 to 0.013 and exhibits no further change as the nominal anesthetic/lipid mole ratio is increased. However, the fractional dimensionality of the relaxation process decreases monotonically from slightly less than two to approximately one as the anesthetic/lipid mole ratio increases from 0 to 0.027. At higher ratios, the dimensionality appears to be less than one. These results are interpreted in terms of the classical kinetic theory and related to those obtained from Monte Carlo simulations. Specifically, low concentrations of dibucaine appear to reduce the average cluster size and cause the fluctuating lipid clusters to become more ramified. At the highest concentration of dibucaine, where $n < 1$, the system must be kinetically heterogeneous.

INTRODUCTION

In previous articles we have discussed the theory and implementation of volume perturbation calorimetry and presented the results of our investigations of the kinetics of the gel-liquid crystalline (or main) transition in multilamellar bilayer vesicles of phosphatidylcholines (van Osdol et al., 1989, 1991). These systems have essentially two components: the aqueous medium and the lipid. In the present article we report the results of studies in which a third component, an anesthetic that partitions between the aqueous and lipid phases, is introduced.

The effects of both general and local anesthetics on the phase transition properties of phospholipid bilayers have been experimentally investigated with a variety of techniques (Trudell et al., 1975; Papahadjopoulos et al., 1975; Mountcastle et al., 1978). The alteration of the equilibrium thermodynamics of the bilayer phase transition by an anesthetic depends on the details of the interactions between the lipid, the aqueous medium, and the anesthetic (Jorgensen et al., 1991). In general, these perturbants lower the gel-liquid crystalline transition temperature, T_m, broaden the equilibrium heat capacity curve, and reduce the maximal excess heat capacity in a dose-dependent fashion, with little alteration in the molar enthalpy change of the transition. Since volatile anesthetics do not change the pressure dependence of T_m, the volume change for the transition does not appear to be altered by the foreign molecule and moderate pressures (0–340 atm) do not displace the anesthetic from the lipid (Mountcastle et al., 1978; MacDonald, 1978; Kamaya et al., 1979). The presence of local anesthetics in the lipid bilayer has also been found to increase the rate of molecular transport across the lipid membrane (Tsong et al., 1977). These findings are consistent with the idea that anesthetics induce more phase boundaries while reducing the average size of the lipid clusters (Tsong et al., 1977; Freire and Biltonen, 1978).

The partitioning properties of a variety of small amphipathic molecules and anesthetics between biological membranes and their environment have been previously examined (Papahadjopoulos et al., 1975; Janoff and Miller, 1982; Eftink et al., 1985). The ability of the drug molecules to partition into and perturb the lipid membranes has attracted considerable attention in both experimental and theoretical research (Freire and Biltonen, 1978; Jorgensen et al., 1991). Many efforts have been focused on investigating the orientation and degree of penetration of these drug molecules in the lipid bilayer. The idea of localized interactions of drug molecules with specific portions of the bilayer membrane has been proposed (Jain and Wu, 1977). A multistate lattice model for the main transition of lipid bilayers doped with foreign molecules (Jorgensen et al., 1991) has been used for a detailed Monte Carlo simulation study of anesthetic-lipid interactions at the molecular level. A principal result of those simulations is the finding that anesthetic molecules can have a dramatic effect on the bilayer fluctuations in the phase transition region, inducing regions of locally very high concentrations of anesthetics. This is

Address correspondence to Dr. Biltonen.
Dr. van Osdol's present address is Laboratory of Mathematical Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.

macroscopically reflected by a broadening of the heat capacity curve and a maximum in the membrane/water partition coefficient in the transition region.

In view of the striking effects that anesthetics of various kinds have on the equilibrium transition, we have examined the effects of dibucaine, a tertiary amine local anesthetic, on the kinetics of the gel-liquid crystalline transition in $DC_{16}PC$. The results we report below indicate that the anesthetic does not alter the qualitative features of the relaxation behavior of lipid in the transition region. The time scale of the relaxation is reduced by only a factor of two and a maximum in the relaxation time is still observed at a temperature slightly higher than the T_m. The major effect of the anesthetic is to alter the shape of the frequency-dependent response function. This effect is related to the dimensionality of the kinetic process interpreted within the scope of the classical Kolmogov-Avrami domain growth theory.

KOLMOGOROV-AVRAMI THEORY

In a previous article (Ye et al., 1991), we reported the use of the classical Kolmogorov-Avrami cluster growth theory (Yang and Nagle, 1988, and references cited therein) to analyze the phase transition kinetics of phosphatidylcholine multilamellar vesicles. This theory assumes that the phase transition begins with nucleation and proceeds by growth of the nuclei. The time development of the fractional completion, f, of the transition to the new phase following a step perturbation is given by

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

where N is the number of randomly distributed nuclei per unit volume, each of which grows to a volume V at time t, if it does not collide with another domain. For reasonably smooth domains,

$$V = g \cdot r^n = g \cdot u^n \cdot t^n, \quad (2)$$

where r is the radius of the domain, u the radial growth rate, g a geometrical factor which is $4\pi/3$ for spheres and π for circles, and n the effective dimensionality of the growing domain. Solving Eq. 1, one obtains:

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

where the relaxation time is $\tau = 1/[u(Ng)^{1/n}]$.

The frequency spectrum of such a process can be obtained from the Laplace transform of the impulse response function (df/dt), and used to analyze the frequency dependent heat capacity function measured by the volume perturbation calorimeter (Ye et al., 1991).

METHODS AND MATERIALS

Volume perturbation calorimetry

The theory and implementation of volume perturbation calorimetry have been described elsewhere (van Osdol et al., 1989). In brief, the technique involves using a small, externally induced volume change to perturb the equilibrium of a physico-chemical process. The temperature and pressure of the sample are measured as functions of time in order to follow the relaxation to the new equilibrium. In principle, any process accompanied by a sufficiently large volume change can be investigated by this technique.

The volume perturbations are produced by the voltage-dependent extension and retraction of a stack of piezoelectric crystals, permitting use of the instrument in both transient and steady-state modes. To date, we have used the latter mode, in which the relaxation properties of the system are recovered from the frequency dependence of the amplitude demodulation and phase shift of the system's temperature response to harmonic perturbation. In abstract terms, the instrument is designed to measure the power spectrum of enthalpy and density fluctuations. It does this by measuring two generalized linear susceptibilities (Landau and Lifshitz, 1980): the frequency-dependent heat capacity and isothermal compressibility.

Preparation of lipid vesicles

The dipalmitoylphosphatidylcholine ($DC_{16}PC$) used in these experiments was obtained as a chloroform solution from Avanti Polar Lipids, Inc. (Pelham, AL), the dibucaine from Sigma Chemical Company (St. Louis, MO), and the 1-dodecanol from Eastman Kodak Co. (Rochester, NY).

The aqueous medium and procedure for making the vesicles of $DC_{16}PC$ and anesthetic were identical to those used to make vesicles of pure PC, and are described elsewhere (van Osdol et al., 1991). If anesthetic is present in the aqueous medium in which vesicles are forming from dried lipid, it may not partition into the inner bilayers of the nascent vesicles (van Osdol, unpublished observations). To avoid this problem, the $DC_{16}PC$ and anesthetic were colyophilized according to the following procedure. First, $DC_{16}PC$ was lyophilized for at least 16 h. The dried lipid was weighed, an appropriate amount of anesthetic was dissolved in a small amount of chloroform and added to the lipid, and the resulting solution was vortexed vigorously for 30 s. The chloroform was evaporated under a stream of nitrogen, with occasional vortexing to assure mixing of the lipid and anesthetic during solidification. The mixture was then lyophilized again for at least 16 h.

The chemical structure of dibucaine is shown in the inset of Fig. 1. The tertiary amine group has a pK_a of 8.8. Our experiments were conducted at pH 7.4 (determined at 40°C), under which conditions the anesthetic was, on the average, 96% protonated. The partition coefficient of dibucaine defined in terms of weight concentrations of the aqueous and lipid phases was estimated to be 235 for multilamellar vesicles of $DC_{16}PC$ at pH 7.4 (Papahadjopoulos et al., 1975). This value corresponds to a dibucaine partition coefficient of 9.6×10^3 when defined in terms of mole-fraction concentrations in the aqueous and lipid phases. It should be noted that the method used by Papahadjopoulos et al. (1975) for vesicle preparation did not involve colyophilization of lipid and dibucaine. Anesthetic was present in the aqueous medium in which the lipid was dispersed, and it is possible that not all bilayers were accessible to the dibucaine in their experiments. Our experiments were conducted at the following series of nominal anesthetic/lipid mole ratios (a/L): 0.0033, 0.0067, 0.013, 0.027, and 0.053. Using the reported partition coefficient, the mole fractions in the vesicles were estimated to be 0.0031, 0.0062, 0.012, 0.025, and 0.050, respectively. All dispersions were ~70 mM in $DC_{16}PC$.

Lipid samples were characterized using a home-made high-sensitivity, heat-conduction differential scanning calorimeter (DSC) (Suurkuusk et al., 1976). Analysis of the DSC data provided the thermodynamic information necessary to plan the course of the kinetic experi-

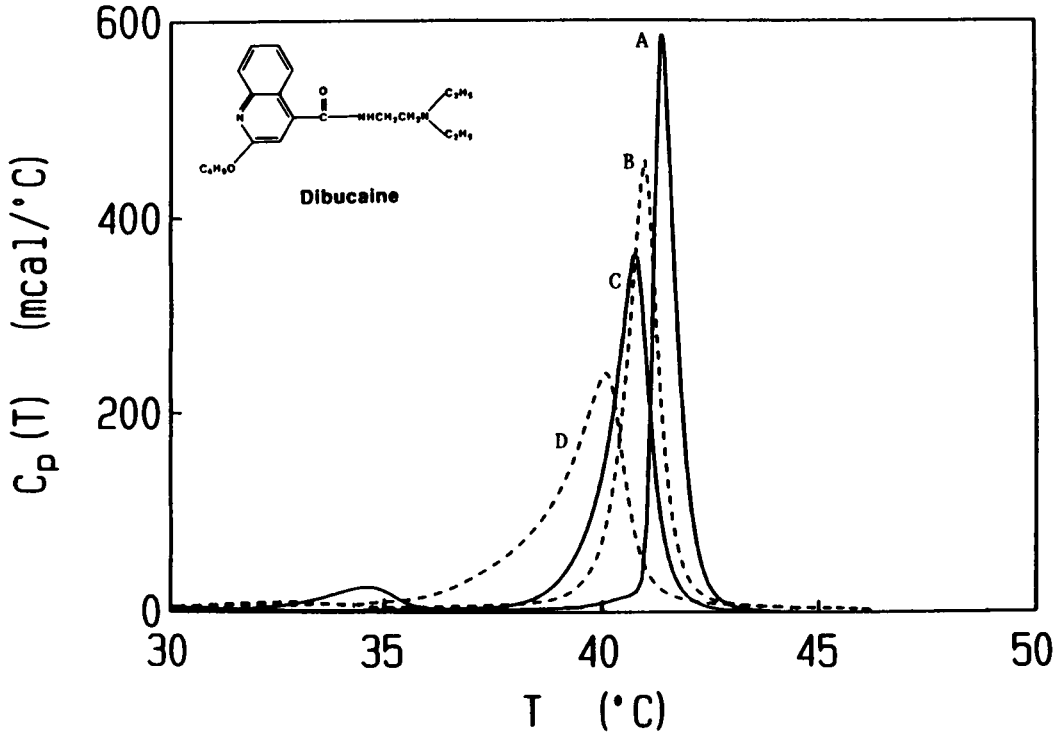

FIGURE 1 Apparent excess heat capacities of multilamellar vesicles of dibucaine-$DC_{16}PC$, as obtained by differential scanning calorimetry at a scan rate of 10°C/h, for nominal dibucaine/$DC_{16}PC$ mole ratios of (*A*) 0, (*B*) 0.013, (*C*) 0.027, and (*D*) 0.053. The results have not been corrected for the slow time response of the calorimeter. The inset shows the chemical structure of dibucaine.

ment: the temperature range of the transition and the heat capacity profile.

RESULTS

Differential scanning calorimetry (DSC)

The equilibrium characteristics of the pre- ($L_{\beta'} - P_{\beta'}$) and main ($P_{\beta'} - L_{\alpha}$) transitions of dibucaine-lipid dispersions were investigated by DSC prior to any kinetic experiments. The excess heat capacity curves are displayed in Fig. 1. All scans were performed at a scan rate of 10°C/h, which caused some broadening of the curves due to the finite response time of the calorimeter. However, this effect does not influence the interpretation of the data presented here. As a/L increases, the main transition temperature is lowered and broadened on the low temperature side, resulting in an increased transition half-width at half-height, $\Delta T_{1/2}$. Both the maximal excess heat capacity and the transition temperature are reduced, but the enthalpy change of the main transition is not altered by the presence of the anesthetic. The pretransition is not observed when the a/L ratio is greater than 0.027. These observations are in agreement with results obtained previously for $DC_{16}PC$ multilamellar vesicles in the presence of dibucaine (Papahadjopoulos et al., 1975).

Volume perturbation calorimetry (VPC)

Fig. 2, *a* and *b*, displays the relaxation amplitude of the hydrated lipid systems as a function of temperature and a/L ratio at a perturbation frequency of 0.1 Hz. This relaxation amplitude (dT/dP) given in units of the Joule-Thompson coefficient of water at the same temperature is proportional to the frequency-dependent heat capacity function $C_p(T, \omega)$. Clearly, the changes which dibucaine induced in the equilibrium excess heat capacity are mirrored in $C_p(T, \omega)$. For all values of a/L investigated, the scaled temperature at which the maximal relaxation amplitude was observed agreed closely with the T_m determined from differential scanning calorimetry.

The influence of dibucaine on the frequency dependence of the relaxation amplitude at T_m is shown in Fig. 3 *a* for several values of a/L. The displayed amplitudes have been corrected for the contributions of water and the dynamic characteristics of the instrument itself (van Osdol et al., 1989). We are unsure of the significance of the variation in the high frequency baselines as a/L is changed. In theory the baseline values for the corrected spectra should be unity, if all relevant relaxation processes are relatively slow. In any case, these relaxation spectra demonstrate that dibucaine causes a decrease in the low frequency amplitude and a small altering of the relaxation time.

$C_p(T, \omega)$ amplitude spectra from dibucaine/$DC_{16}PC$ dispersions were analyzed by a procedure described in

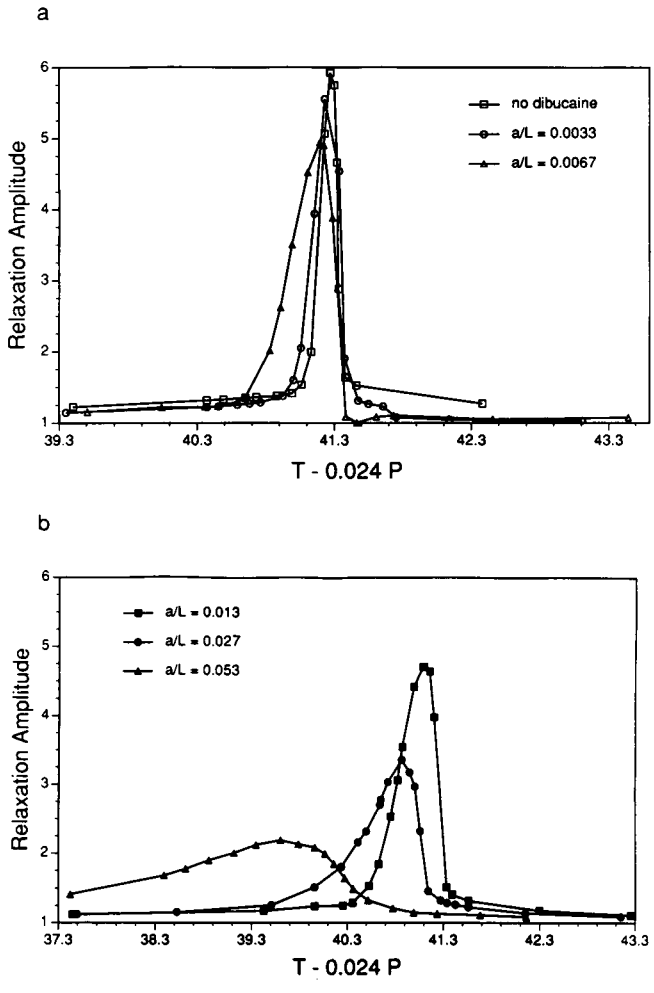

FIGURE 2 (*a* and *b*) The relaxation amplitude at a perturbation frequency of 0.1 Hz, as a function of the scaled temperature for samples of different dibucaine/$DC_{16}PC$ mole ratios (a/L). The abscissa is the average temperature of the lipid sample adjusted by a pressure effect of 0.024°C/atm to allow direct comparison to the DSC results obtained at P = 1 atm. The ordinate is the amplitude (dT/dP) of the sample's response to the pressure change relative to the amplitude of the response of water at the same average temperature (van Osdol et al., 1991). Error bars are omitted for clarity.

detail previously (van Osdol et al., 1989). The frequency spectra of $C_p(T, \omega)$ can be well-approximated by a single relaxation time, τ, and amplitude, and an apparent dimensionality, n, of the transition, as was found for multilamellar dispersions of pure $DC_{16}PC$ (Ye et al., 1991). The apparent dimensionality of the gel-liquid crystalline transition declined monotonically with increasing dibucaine mole fraction from slightly less than 2 for pure $DC_{16}PC$ vesicles to ~1 for dibucaine/$DC_{16}PC$ vesicles with a/L = 0.027. This reduction in dimensionality is depicted in Fig. 3 *a* where the relaxation spectra have been fit assuming n = 1. The systematic deviation of the experimental data from the fit curves (Fig. 3 *a*) are shown in Fig. 3 *b*. The derivation is large for pure DPPC but becomes less apparent as dibucaine is added. Finally, the deviation again becomes apparent at a/L = 0.053 but of opposite sign. The latter situation arises because the best n to describe these data is less than 1. The relaxation times (obtained assuming n = 1) and amplitudes resolved from the spectra of $C_p(T, \omega)$ are listed in Table 1, as a function of the fractional completion of the transition at which they were measured. Analyzing all data assuming n = 1 provided consistent meaning to τ. That is, $\tau = 1/(2\pi\omega_c)$ where ω_c is the perturbation frequency at which the response amplitude is equal to one-half of its maximum value. The fractional completion was estimated by integrating the $C_p(T)$ amplitude curves observed at 0.01 Hz perturbation frequency as described previously (van Osdol et al., 1991).

The relaxation times are shown in Fig. 4, *a* and *b*, plotted as a function of the fractional completion of the transition and a/L. For a/L = 0.0033, the relaxation times are indistinguishable from those observed in pure $DC_{16}PC$. At all higher mole ratios two changes are clear. The τ_{max} decreases, reaching a value of about 50% of the pure lipid at a/L = 0.053 and the fractional completion at τ_{max} increases as dibucaine is increased.

DISCUSSION

The effects of the tertiary amine local anesthetic dibucaine on the kinetics of the gel-liquid crystalline transition in multilamellar vesicles of $DC_{16}PC$ have been investigated. Although dibucaine has a dramatic influence on T_m and $\Delta T_{1/2}$, only a minor effect on the time scale of the relaxation kinetics of the gel-liquid crystalline transition was observed. The most striking effect of dibucaine on the transition kinetics is its alteration of the apparent dimensionality of the relaxation process.

We recently reported that the transition kinetics in multilamellar vesicles of $DC_{16}PC$ are best represented as a single relaxation process of dimensionality ~2 (Ye et al., 1991); that is, $f(t) = 1 - \exp\{-(t/\tau)^2\}$. This result was obtained by application of the classical Kolmogorov-Avrami theory, and suggests that domains of the minor phase (either gel or liquid crystalline) are highly compact. This implies that lipid states of many unlike nearest neighbors are highly unfavorable and that the Gibb's free energy of the planar bilayer is minimized by reducing the perimeter-to-area ratio of existing domains. The optimal way to minimize this ratio in two dimensions is by the formation of large, nearly circular domains, in which case $n \approx 2$. As shown in Fig. 3, the apparent dimensionality of the transition declined from ~2 to ~1 as the nominal mole ratio of dibucaine to $DC_{16}PC$ increased from 0 to 0.03. We interpret this to mean that in the presence of dibucaine, the liquid crystalline or gel phase domains become more ramified because the anesthetic tends to stabilize domain boundaries, where unlike nearest neighbors interact. This interpretation is consistent with the observation that the equilibrium excess heat capacity curve (and other measures of the transition) is greatly broadened in the presence of anesthetics. This interpre-

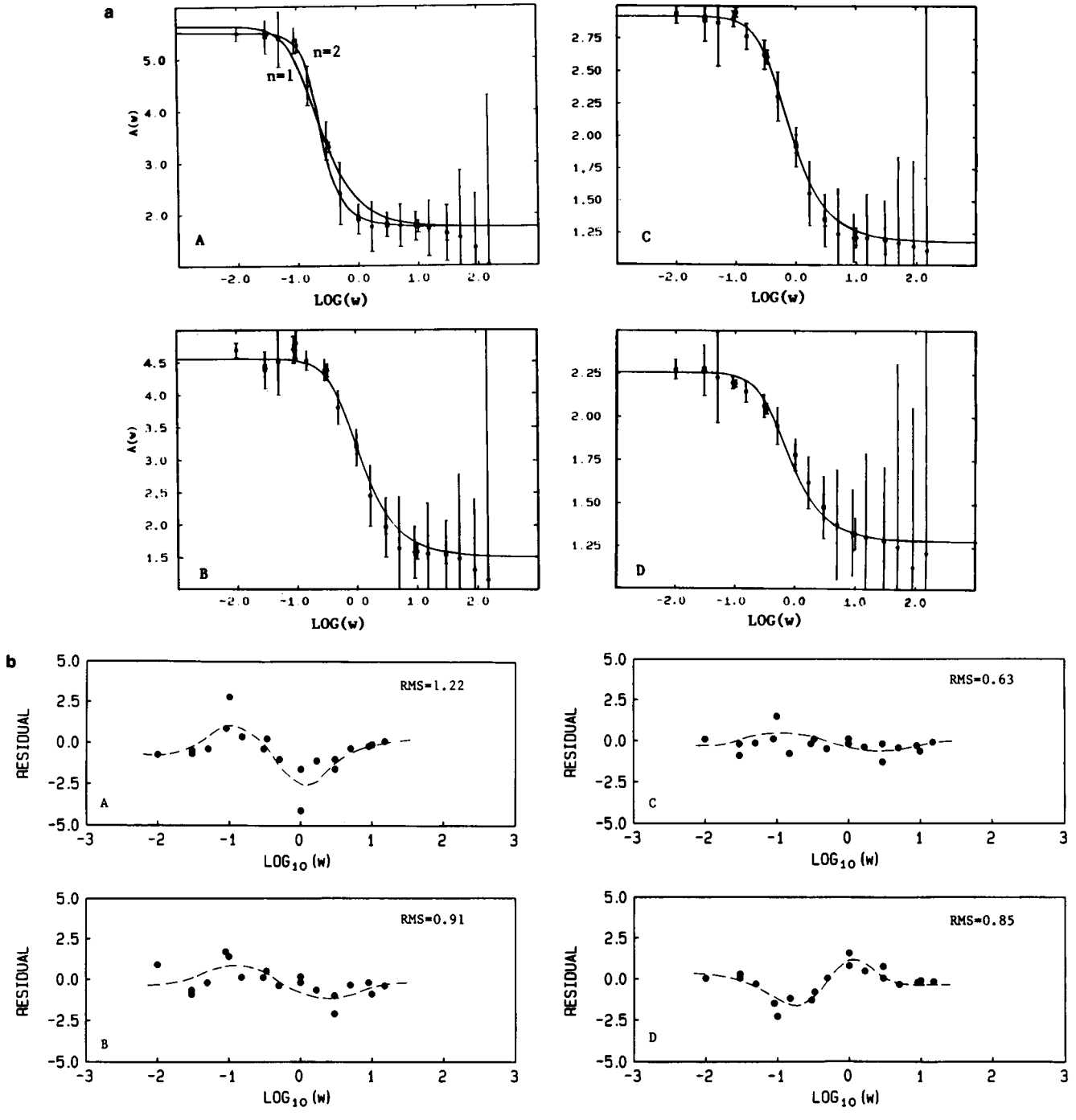

FIGURE 3 (*a*) Relaxation amplitudes vs $\log_{10}$ of the perturbation frequency at T_m for a/L of (*A*) 0, (*B*) 0.013, (*C*) 0.027, and (*D*) 0.053. The standard errors of the data are indicated by the vertical bars. In graph *A*, the two solid lines represent the best fit of the data to the Kolmogorov-Avrami model with $n = 1$ and $n = 2$, respectively, which shows that the data are better fit with $n = 2$ than $n = 1$ (Ye et al., 1991). In graphs *B–D*, the single solid line represents the best fit of the data to the Kolmogorov-Avrami model with $n = 1$. 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 (Ye et al., 1991).

(*b*) Residual distributions for fittings with $n = 1$, corresponding to data in (*a*). 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 (Ye et al., 1991). RMS is the root mean square of the deviation of the fit. Note the gradual change of the sign of residual as the dibucaine/$DC_{16}PC$ ratio increases. The dashed lines in graphs *A* and *D* are for visual guidance only.

TABLE 1 **Relaxation times and their one standard deviation confidence intervals, resolved from the best fit of the amplitude spectra to the Kolmogorov-Avrami model assuming $n = 1$**

No dibucaine			0.0033		
f	tau (s)	confidence interval	f	tau (s)	confidence interval
0.06	0.028	(0.013, 0.054)	0.19	0.028	(0.024, 0.031)
0.33	0.21	(0.19, 0.25)	0.25	0.1	(0.09, 0.11)
0.46	0.59	(0.45, 0.78)	0.33	0.19	(0.17, 0.21)
0.61	0.98	(0.74, 1.31)	0.44	0.39	(0.33, 0.45)
0.73	1.82	(1.31, 2.45)	0.69	1.39	(1.17, 1.65)
0.82	3.89	(2.63, 5.62)	0.84	3.2	(2.14, 4.7)
0.91	0.35	(0.32, 0.39)	—	—	—
	0.0067			0.013	
f	tau (s)	confidence interval	f	tau (s)	confidence interval
0.17	0.079	(0.056, 0.11)	0.25	0.1	(0.083, 0.11)
0.34	0.11	(0.10, 0.12)	0.34	0.089	(0.069, 0.11)
0.45	0.17	(0.15, 0.18)	0.43	0.15	(0.14, 0.17)
0.55	0.26	(0.24, 0.29)	0.56	0.28	(0.26, 0.31)
0.75	0.46	(0.39, 0.55)	0.7	1.18	(0.97, 1.44)
0.93	1.2	(0.95, 1.5)	0.83	1.81	(1.72, 1.9)
0.97	0.96	(0.75, 1.26)	0.9	0.9	(0.75, 1.09)
	0.027			0.053	
f	tau (s)	confidence interval	f	tau (s)	confidence interval
0.19	0.13	(0.08, 0.18)	0.28	0.1	(0.09, 0.11)
0.32	0.18	(0.13, 0.24)	0.52	0.16	(0.14, 0.18)
0.45	0.27	(0.20, 0.35)	0.72	0.63	(0.55, 0.86)
0.59	0.29	(0.23, 0.36)	0.85	1.13	(0.95, 1.32)
0.75	0.35	(0.28, 0.44)	0.89	1.29	(1.12, 1.45)
0.9	1.78	(1.35, 2.34)	0.92	1.62	(1.48, 1.78)
0.96	0.41	(0.33, 0.55)	0.95	1.66	(1.51, 1.78)

The times are listed with respect to the fractional completion and nominal dibucaine/$DC_{16}PC$ mole ratio at which they were measured. Not all relaxation times plotted in Figs. 4, *a* and *b* are listed in this table. Complete tables of relaxation times can be found in van Osdol (1988).

tation is also consistent with the results of Monte Carlo simulations to mimic the effects of anesthetic on the phase transition (Jorgensen et al., 1991). These results have been interpreted to mean that anesthetic molecules preferentially partition into the gel-liquid crystalline boundary regions and reduce the unfavorable Gibb's energy of interaction between lipid clusters of different phases, resulting in formation of more clusters of smaller sizes (Hill, 1974; Freire and Biltonen, 1978; Biltonen, 1990).

The interpretation discussed above must be viewed with caution because the reduction in dimensionality could also be due to kinetic heterogeneity induced by the anesthetic. This is almost certainly the case when $a/L > 0.053$ and $n < 1$ because n must always be greater than or equal to 1 for a kinetically homogenous system. Kinetic heterogeneity will occur when the relaxation process consists of two or more sequential steps of approximately the same time scale or when the anesthetic produces a distribution of vesicles whose single relaxations (of whatever dimensionality) are not identical. As we have noted previously (Ye et al., 1991), relaxation spectra of $n > 1$ cannot be fit by a sum of exponential relaxations of $n = 1$.

Our kinetic results are too limited to permit more than speculation about the relationship between anesthetic effects on the gel-liquid crystalline transition kinetics and the mechanism of anesthesia. However, the results of equilibrium and kinetic studies indicate that the major effect of an anesthetic on membrane topology in phase-coexistence regions is to shift the equilibrium distribution of clusters or domains and to stabilize cluster boundary regions with little effect on the intrinsic dynamics. This means that the average cluster size and presumably the magnitude of local fluctuations will be reduced. How such an effect may be related to anesthetic action is unknown. If, however, the function of a membrane associated enzyme is coupled with lipid structural fluctuations, then it is possible for anesthetics to modulate that function by this alteration in the magnitude of the fluctuation.

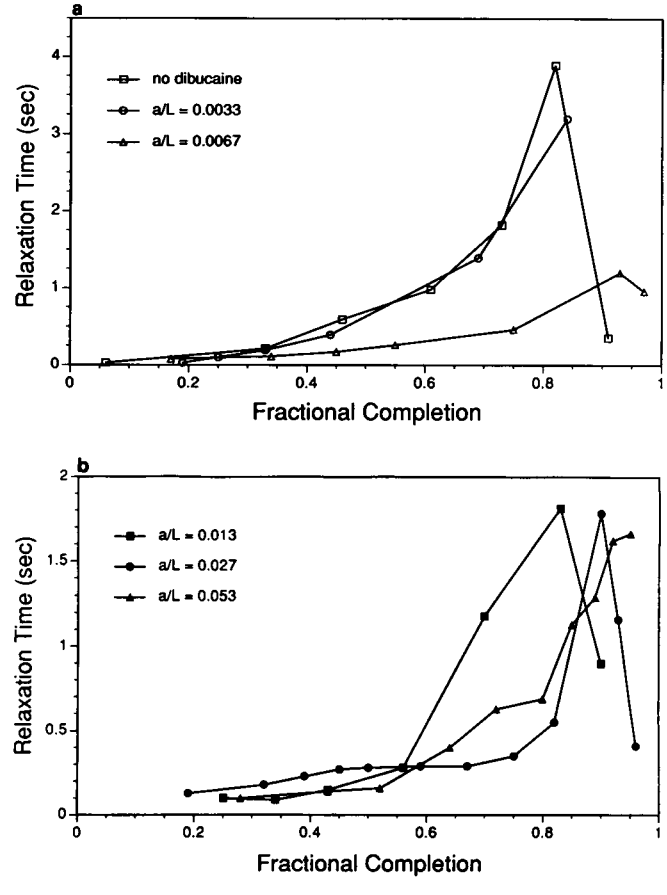

FIGURE 4 (*a* and *b*) Relaxation times resolved with $n = 1$, as a function of the fractional degree of completion of the transition for dibucaine-$DC_{16}PC$. Error bars are omitted for clarity. The relaxation times are listed in Table 1.

The effects of the general anesthetic, 1-dodecanol, on the thermodynamic and kinetics of the main transition of $DC_{16}PC$ have also been evaluated. At a nominal anesthetic/lipid ratio of 0.027 and a lipid concentration of 70 mM, the T_m was raised by 0.2°C and $\Delta T_{1/2}$ increased by $\sim$15% relative to the pure $DC_{16}PC$ system. Under these conditions, about 90% of the alcohol is estimated to be found in the lipid membrane assuming a partition coefficient of 6.9×10^4 (Franks and Lieb, 1986). However, the kinetic characteristics, including the dimensionality of the relaxation, were essentially identical to those of the pure $DC_{16}PC$ system. It thus appears that dibucaine-induced changes in the relaxation dynamics are a reflection of its effects on the details of the molecular interactions in the membrane and not simply due to the presence of a second component in the bilayer. In fact, small amounts of a second component can have a wide variety of effects on relaxation behavior of the bilayer system. For example, the results of kinetic studies on binary phospholipid systems show that in the $DC_{14}PC$-$DC_{18}PC$ system, an increase of the $DC_{18}PC/DC_{14}PC$ mole ratio from 0 to 0.06 causes a decrease of τ_{max} by two to three orders of magnitude whereas an increase of the $DC_{14}PC/DC_{18}PC$ mole ratio from 0 to 0.06 results in a decrease of τ_{max} by less than an order of magnitude (Ye and Biltonen, 1991; Ye, 1992).

This work has been supported by grants from National Institutes of Health (GM28928 and GM37658), National Science Foundation (PCM8300056 and DMB9005374), and The Office of Naval Research (N0014-88-K-03260).

Received for publication 10 April 1992 and in final form 26 June 1992.

REFERENCES

Biltonen, R. L. 1990. A statistical-thermodynamic view of cooperative structural changes in phospholipid bilayer membranes: their potential role in biological function. *J. Chem. Thermodynamics.* 22:1–19.

Eftink, M. R., R. K. Puri, and M. D. Ghahramani. 1985. Local anesthetic-phospholipid interactions. The pH dependence of the binding of dibucaine to dimyristoylphosphatidylcholine vesicles. *Biochim. Biophys. Acta.* 813:137–140.

Franks, N. P., and W. R. Lieb. 1986. Partitioning of long-chain alcohols into lipid bilayers: implications for mechanisms of general anesthesia. *Proc. Natl. Acad. Sci. USA.* 83:5116–5120.

Freire, E., and R. L. Biltonen. 1978. Estimation of molecular averages and equilibrium fluctuations in lipid bilayer systems from the excess heat capacity function. *Biochim. Biophys. Acta.* 514:54–68.

Hill, M. W. 1974. The effect of anesthetic-like molecules on the phase transition in smectic mesophases of dipalmitoyl lecithin, the normal alcohols up to c = 9 and three inhalation anesthetics. *Biochim. Biophys. Acta.* 356:117–124.

Jain, M. K., and N-Y Wu. 1977. Effect of small molecules on the phase transition in lipid bilayers of dipalmitoyl lecithin. *J. Membr. Biol.* 34:157–201.

Janoff, A. S., and K. W. Miller. 1982. A critical assessment of the lipid theories of general anesthetic action. *In* Biological Membranes. D. Chapman, editor. Academic Press, London. 417–476.

Jorgensen, K., J. H. Ipsen, O. G. Mouritsen, D. Bennett, and Zuckermann. 1991. The effects of density fluctuations on the partitioning of foreign molecules into lipid bilayers: application to anesthetics and insecticides. *Biochim. Biophys. Acta.* 1067:241–253.

Kamaya, H., I. Ueda, P. S. Moore, and H. Eyring. 1979. Antagonism between high pressure and anesthetics in the thermal phase transition of dipalmitoyl phosphatidylcholine bilayers. *Biochim. Biophys. Acta.* 550:131–137.

Landau, L. D., and E. M. Lifshitz. 1980. *In* Statistical Physics. Part 1. Third edition, J. B. Sykes and M. J. Kearsley, editors. Pergamon Press, Oxford. 333–345, 359–393, and 533–537.

MacDonald, A. G. 1978. A dilatometric investigation of the effects of general anesthetics, alcohols, and hydrostatic pressure on the phase transition in smectic mesophases of dipalmitoyl phosphatidylcholine. *Biochim. Biophys. Acta.* 507:26–37.

Mountcastle, D. B., R. L. Biltonen, and M. J. Halsey. 1978. Effect of anesthetics and pressure on the thermodynamic behavior of multilamellar dipalmitoyl phosphatidylcholine vesicles. *Proc. Natl. Acad. Sci. USA.* 75:4906–4910.

Papahadjopoulos, D., K. Jacobson, G. Poste, and G. Shepherd. 1975. Effects of local anesthetics on membrane properties I. changes in the fluidity of phospholipid bilayers. *Biochim. Biophys. Acta.* 394:504–519.

Suurkuusk, J., B. Lentz, Y. Barenholz, R. L. Biltonen, and T. E. Thompson. 1976. A calorimetric and fluorescent probe study of the gel-liquid crystalline phase transition in small single-lamellar dipalmitoylphosphatidylcholine vesicles. *Biochemistry.* 15:1393–1401.

Trudell, J. R., D. G. Payan, J. H. Chin, and E. N. Cohen. 1975. The antagonistic effect of an inhalation anesthetic and high pressure on the phase diagram of mixed dipalmitoyl-dimyristoylphosphatidylcholine bilayers. *Proc. Natl. Acad. Sci. USA.* 72:210–213.

Tsong, T. Y., M. Greenberg, and M. Kanehisa. 1977. Anesthetic action on membrane lipids. *Biochemistry.* 16:3115–3121.

van Osdol, W. W. 1988. Kinetics of the main phase transition in phospholipid vesicles. PhD dissertation. Biophysics Program, University of Virginia, Charlottesville, VA.

van Osdol, W. W., R. L. Biltonen, and M. L. Johnson. 1989. Measuring the kinetics of membrane phase transitions. *J. Biochem. Biophys. Meth.* 20:1–46.

van Osdol, W. W., M. L. Johnson, Q. Ye, and R. L. Biltonen. 1991. Relaxation dynamics of the gel-liquid crystalline transition of phosphatidylcholine bilayers: the effects of chain length and vesicle size. *Biophys. J.* 59:775–785.

Yang, C. P., and J. F. Nagle. 1988. Phase transformations in lipids follow classical kinetics with small dimensionalities. *Phys. Rev. A.* 37:3993–4000.

Ye, Q. 1992. Phase transition kinetics of multicomponent lipid membranes. PhD dissertation. Biophysics Program, University of Virginia, Charlottesville, VA.

Ye, Q., and R. L. Biltonen. 1991. Phase transition kinetics of binary lipid mixture membranes. *Biophys. J.* 59:191*a*. (Abstr.)

Ye, Q., W. W. van Osdol, and R. L. Biltonen. 1991. Gel-liquid crystalline transition of some multilamellar lipid bilayers follows classical kinetics with a fractional dimensionality of approximately two. *Biophys J.* 60:1002–1007.