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Effect of cholesterol on interaction of dibucaine with phospholipid vesicles: a fluorescence study

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Abstract

Interaction of the local anesthetic dibucaine with small unilamellar vesicles of dimyristoylphosphatidylcholine (DMPC) and dioleoyl phosphatidylcholine (DOPC) containing different mol percents of cholesterol has been studied by fluorescence spectroscopy. Fluorescence measurements on dibucaine in presence of phospholipid vesicles containing various amounts of cholesterol yielded a pattern of variation of wavelength at emission maximum and steady-state anisotropy which indicated that the microenvironment of dibucaine is more polar and flexible in membranes that contain cholesterol than in membranes without cholesterol. Experiments on quenching of fluorescence from membrane-associated dibucaine by potassium iodide showed a marked increase in quenching efficiency as the cholesterol content of the vesicles was increased, demonstrating increased accessibility of the iodide quenchers to dibucaine in the presence of cholesterol, when compared to that in its absence. Total emission intensity decay profiles of dibucaine yielded two lifetime components of ~1 ns and ~2.8–3.1 ns with mean relative contributions of ~25 and ~75%, respectively. The mean lifetime in vesicles was 20–30% smaller than in the aqueous medium and showed a moderate variation with cholesterol content. Fluorescence measurements at two different temperatures in DMPC SUVs, one at 33°C, above the phase transition temperature and another at 25°C, around the main phase transition, indicated two different mode of dibucaine localization. At 25°C dibucaine partitioned differentially in presence and absence of cholesterol. However, at 33°C the apparent partition coefficients remained unaltered indicating differences in the microenvironment of dibucaine in presence and absence of cholesterol in the phospholipid membranes.

Keywords: Local anesthetic; Phospholipid vesicle; Cholesterol; Fluorescence anisotropy; Fluorescence quenching

1. Introduction

Tertiary amine local anesthetics cause anesthesia by blocking nerve transmissions via state-dependent binding of voltage-gated Na^+-channels [1,2]. However, whether this action is a result of specific anesthetic–protein interaction or of nonspecific perturbation of the lipid bilayer structure is still an unsolved question [1]. There are two major models of anesthetic action. The first of these, called the lipid hypothesis, ascribes the effect to physical alterations of the lipid bilayer upon binding of the anesthetic to it. The second, called the protein hypothesis, explains it

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in terms of direct specific interaction of anesthetic molecules with proteins [3–5]. According to this view the interactions of local anesthetics with enzymes [6], membrane-bound acetylcholine receptor [7] and Ca^{+2}-ATPase [8], or cytoskeletal proteins which might have functional association with the Na^{+}-channel [9], are believed to be relevant to their mode of action. It is generally assumed that any one of the above mentioned interactions with lipids and/or membrane proteins can lead to the inactivation of neuronal ion channel activity. However, interactions with membrane lipids appear to be an important first step for local anesthesia, since transbilayer movement of the anesthetic molecule to the cytoplasmic side of excitable membranes is a requirement for anesthetic action [2,10].

Earlier studies on the partitioning of anesthetics, to correlate potencies, in oil/water systems indicated the site of action to be amphiphilic, and both aqueous potencies and lipid/water partition coefficients were well correlated with the hypothesis that membrane lipids are the primary site for anesthetic action [4,11]. Experimental observations of increase in surface area, surface charge [12,13] and fluidity of phospholipid membranes [14] upon addition of the anesthetics also supported the role of lipid matrix structural alteration in the mode of action. The effect of local anesthetics on the organization of phospholipid bilayers, and the motional constraints on the anesthetic molecule upon binding to lipid membranes, have been studied by several spectroscopic and theoretical techniques [15–24].

One of the most widely studied tertiary amine local anesthetics, dibucaine (2-butoxy-*N*-[2-diethylaminoethyl]-4-quinoline-carboxamine) can assume several forms such as neutral base, hydrogen-bound, monoprotonated, diprotonated and aggregated species, depending upon the pH of the solvent and the nature of the microenvironment [21]. It exists predominantly in protonated monocationic form in a hydrophilic environment, the equilibrium being shifted towards the neutral form in hydrophobic environments. NMR spectroscopic studies, supported by molecular dynamics calculations, indicate that dibucaine assumes more than two conformations and exists as dimers in phosphatidylcholine (PC) vesicles [17]. In an earlier NMR study performed in pure PC vesicles using deuterated anesthetics, multiple binding sites for tetracaine and procaine (two other tertiary amine anesthetic molecules) were observed in phospholipid bilayers [15]. These studies have also indicated two states of binding for procaine and tetracaine: a 'weakly bound' state at a low pH of 5.5 and a 'strongly bound' state at a high pH of 9.5, signifying the presence of two differently charged or aggregated species of the local anesthetics. There have also been studies showing binding of cationic dibucaine with negatively charged phospholipid vesicles being favored by electrostatic contributions [19,20]. Earlier fluorescence and NMR studies indicated dibucaine binding in the vicinity of the phospholipid glycerol moiety [16–19]. More recent studies on depth profiling by fluorescence quenching and NMR investigations in different membrane systems indicated the location of dibucaine to be at a shallow position in the membrane bilayer [22–24].

Fewer attempts have been made to study the interaction of dibucaine with specific lipid components of membranes other than PC. There has not been any study on dibucaine interaction with cholesterol, a ubiquitous component of the plasma membrane, especially when excitable plasma membranes are known to contain a relatively large amount of cholesterol. NMR study has been done to study the interaction of the local anesthetic tetracaine with membranes containing PC and cholesterol [25]. We report here a fluorescence study of the interaction of dibucaine with small unilamellar vesicles (SUV's) of dimyristoylphosphatidylcholine (DMPC) and dioleoylphosphatidylcholine (DOPC) containing different mol percents of cholesterol. Fluorescence techniques have been previously used to study the interaction of dibucaine with both zwitterionic and acidic phospholipid model membranes, sarcoplasmic reticulum vesicles and micellar systems [18–21,23,26]. Results from the present study indicate that localization of dibucaine in phospholipid vesicles containing cholesterol is different in the liquid crystalline phase from that in vesicles containing no cholesterol. The presence of cholesterol apparently creates a hindrance to the penetration of the local anesthetic molecules into the hydrophobic interior of the bilayer resulting in its possible location closer to the lipid head groups of the membranes. The present study also demonstrates differential dibucaine interaction with a membrane containing cholesterol depending

on the temperature, 25°C, when the partitioning of dibucaine is altered (near the DMPC phase transition) and at 33°C, well above the phase transition temperature of DMPC, when the apparent partition coefficient remains unaltered indicating an altered localization of the local anesthetic.

2. Materials and methods

Dibucaine hydrochloride, L-α-DMPC, L-α-DOPC, cholesterol, potassium iodide (KI), diphenylhexatriene (DPH) and 3-[*N*-morpholino]propanesulfonic acid (MOPS) were purchased from Sigma (St. Louis, MO, USA). Deionized water, twice distilled on quartz, was used for preparing solutions and buffers. Phospholipid concentrations were measured after digestion with perchloric acid following a published protocol [27]. Stock solutions of dibucaine and DPH were prepared in ethanol and dimethylformamide, respectively and their concentrations determined by absorbance measurement on a Shimadzu UV-2101PC spectrophotometer, using molar extinction coefficients of 4400 M^{-1} cm^{-1} at 326 nm for dibucaine and 88 000 $M^{-1}cm^{-1}$ at 350 nm for DPH [18,28].

To prepare SUVs of DMPC and DOPC, solutions of the phospholipids in chloroform were first evaporated under a thin stream of nitrogen. The resulting lipid films were then dried overnight in a vacuum dessicator. Lipids were swollen by adding the buffer containing 10 mM MOPS, pH 6.5, containing 50 mM NaCl to the films and the mixture was vortexed to disperse the lipids. The dispersion was sonicated for 10 min (in bursts of 1 min, while being cooled in ice) using a Braun LabSonic 1510 sonicator. The sonicated samples were centrifuged at 10 000 rpm for 15 min to remove titanium particles. To prepare cholesterol containing vesicles, appropriate quantities of cholesterol were cosolubilized with the lipids in chloroform:methanol (2:1 v/v). SUV's composed of PC alone and with cholesterol were characterized by electron microscopy and were found to have average diameters of ~300 Å. Cholesterol content in membranes is expressed as mol percent with respect to the phospholipid molar concentration.

Small aliquots of an ethanolic stock solution of dibucaine hydrochloride were added to the standard buffer to obtain typical final concentrations of 10 μM for fluorescence measurements. Dibucaine fluorescence was measured on a Hitachi F-4010 spectrofluorometer using excitation at 325 nm and slits with bandpasses of 5 nm for both excitation and emission channels. Dibucaine-free vesicles were used as reference blanks in absorption and emission measurements to cancel any contribution from scattering artifacts. Steady-state anisotropy was measured using L-format optics. All measurements were performed at 25°C (except where mentioned) and with multiple sets (3–5) of samples. Error bars were calculated as standard errors of the mean. The temperature dependences of fluorescence emission maxima of dibucaine and anisotropy of DPH in membrane environments were measured using a NESLAB RTE-110 circulating bath.

The apparent partition coefficient (so called because electrostatic effects were not taken into account) of dibucaine between the membrane and the aqueous phases (K_p) was taken as the molar ratio of lipid/drug at which the anisotropy vs. [L]/[D] plots (Figs. 3 and 4) changed over from steeply increasing to saturation behavior.

Fluorescence quenching measurements on dibucaine with iodide quencher were made by serial addition of small aliquots of concentrated (2 M) KI solution to 1 ml of sample in a cuvette. Data were fitted to the Stern–Volmer equation:

$$F_0/F_{corr} = 1 + K_{SV}\,[Q] \quad (1)$$

where F_{corr} is the quenched intensity (corrected for volume increase upon addition of quencher) and K_{SV} is the Stern–Volmer (dynamic) quenching constant. Quenching plots in presence of membranes showed saturation behavior at high quencher concentrations. For these, K_{SV} was determined by fitting the first five data points to the linear relationship given by Eq. 1. Plots exhibiting upward curvature (for dibucaine in buffer) were fitted to the following modified form of the Stern–Volmer equation using nonlinear least-squares analysis [29]:

$$F_0/F_{corr} = (1 + K_{SV}\,[Q])\,e^{V[Q]} \quad (2)$$

where V is called the static quenching constant. Designated errors in K_{SV} were the uncertainties of the best-fit parameters as given by the fitting program. From the relation $k_q = K_{SV}/\langle\tau\rangle$, the bimolecular

quenching rate constants were calculated where $\langle\tau\rangle$ is the mean excited state lifetime of dibucaine (Table 1).

Fluorescence lifetimes were determined from total emission intensity decay measurements, using a time-domain fluorometer assembled in our laboratory with components from Edinburgh Analytical Instruments (EIA, UK) and EG&GORTEC (USA) and operated in the time-correlated single photon counting mode. Excitation was provided by a pulsed high-pressure (1.5 atmosphere) N_2-lamp operating at 25 kHz repetition rate, the pulse profile having a full width at half maximum (FWHM) of 1.2 ns. The fluorescence of dibucaine was excited at 325 nm, while its emission intensity decay profiles in water and in lipid vesicles were monitored at 412 nm and 402 nm, respectively. Monochromator slits with 16 nm bandpass were used for both excitation and emission. Typically, 5000 photon counts were accumulated in the peak MCA channel for each lifetime measurement, and two sets of data were collected on each sample. Each intensity decay curve was fitted to the sum-of-exponentials series:

$$I(t) = \sum_i A_i \exp(-t/\tau_i) \quad (3)$$

where A_i is a pre-exponential factor representing the fractional contribution to the time-resolved decay of the component with a lifetime τ_i. The decay parameters were recovered using a software package supplied by EIA, implementing a nonlinear least-squares iterative fitting procedure [30]. The mean (average) lifetimes $\langle\tau\rangle$ for bi-exponential decays were calculated using the equation [29]:

$$\langle\tau\rangle = \sum_i \alpha_i \tau_i \quad (4)$$

Table 1
Quenching parameters for iodide quenching of dibucaine fluorescence at 33°C in aqueous medium and in DMPC and DOPC vesicles containing varying amounts of cholesterol at lipid/dibucaine ratios of >300 for DMPC and >400 for DOPC

Medium	K_{SV} (M^{-1})	k_q (M^{-1} s^{-1})
MOPS Buffer	42.0 ± 0.5	15.5 ± 0.3 × 10^9
DMPC	8.5 ± 0.3	3.80 ± 0.2 × 10^9
DMPC/10% cholesterol	17.3 ± 1.3	7.90 ± 0.6 × 10^9
DMPC/20% cholesterol	32.6 ± 0.8	15.0 ± 0.4 × 10^9
DOPC	4.1 ± 0.1	1.80 ± 0.4 × 10^9
DOPC/20% cholesterol	8.8 ± 0.4	4.10 ± 0.2 × 10^9
DOPC/40% cholesterol	14.9 ± 0.8	7.60 ± 0.4 × 10^9

where the fractional amplitude α_i corresponding to the lifetime τ_i is given by:

$$\alpha_i = A_i\tau_i / \sum_i A_i\tau_i \quad (5)$$

Errors in lifetime values were estimated from the spread in individual lifetimes (τ_i) obtained in repetitive experiments on each sample.

3. Results

The emission maximum of dibucaine, which occurs at 412 nm in pH 6.5 aqueous buffer (10 mM MOPS, 50 mM NaCl), shifts towards the blue in solutions

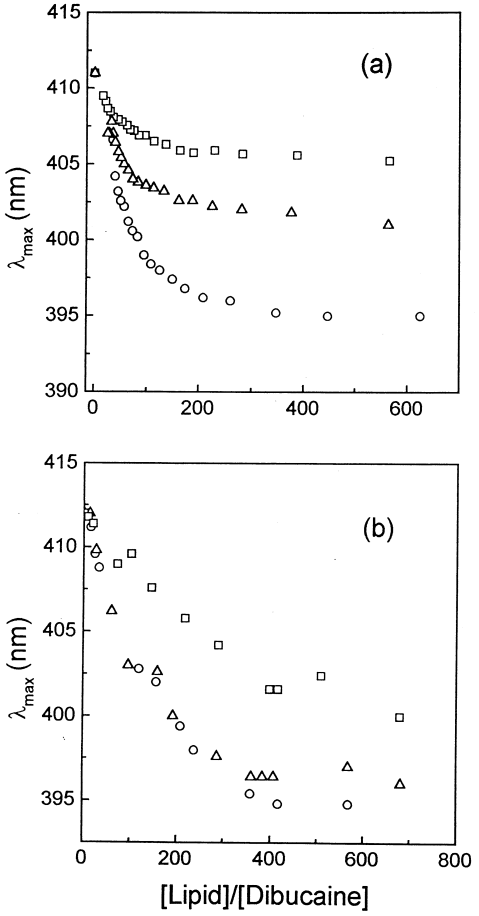

Fig. 1. Variation of wavelength at emission maximum (λ_{max}) of dibucaine at 33°C with phospholipid to dibucaine molar ratio ([L]/[D]) in SUV's composed of (a) pure DMPC (○) and DMPC with 10% (△) and 20% (□) cholesterol, and (b) pure DOPC (○) and DOPC with 20% (△) and 40% (□) cholesterol.

containing phospholipid vesicles. Fig. 1a shows the change in wavelength of the emission maximum (λ_{max}) as a function of the lipid to dibucaine molar ratio ([L]/[D]) in presence of DMPC vesicles containing different amounts of cholesterol measured at 33°C. In pure DMPC vesicles (for [L]/[D] > 400) the maximum occurred at 394 nm, signifying a blue shift of 18 nm. [L] is the molar concentration of lipid phosphate and [D] being the same for dibucaine. Incorporation of increasing amounts of cholesterol in the DMPC vesicles had the effect of progressively decreasing this blue shift and the emission maxima occurred at 401 and 406 nm when the vesicles contained 10 and 20% cholesterol ([L]/[D] > 400), respectively. At a fixed concentration of dibucaine, the blue shift increased with increasing concentration of phospholipid, i.e., with [L]/[D], to reach a saturation value. Increasing the cholesterol content of the vesicles from 0 to 20 mol percent produced a significant decrease of the blue shift, with the saturation value of λ_{max} approaching that observed in aqueous buffer. Fig. 1b shows the results of similar measurements carried out in presence of DOPC vesicles at 33°C. Again, a significant blue shift (of 18 nm) was observed with the pure lipid vesicles, which did not change even when the vesicles contained up to 20% cholesterol. At higher cholesterol contents the blue shift decreased for 40% cholesterol the saturation value of λ_{max} was 403 nm. Compared to the peak intensity of emission from bulk aqueous medium, that in presence of both DMPC and DOPC vesicles decreased by up to 50% with increasing [L]/[D] as well as with increasing cholesterol content. Fig. 2 shows the temperature dependence of λ_{max} in DMPC vesicles containing 0, 10 and 20% cholesterol. In all cases the emission maximum shifted towards the blue end with increase in temperature up to about 30°C, beyond which λ_{max} was nearly constant. All three curves run parallel and look very similar, no effect being observed of the cholesterol content on the nature of the variation.

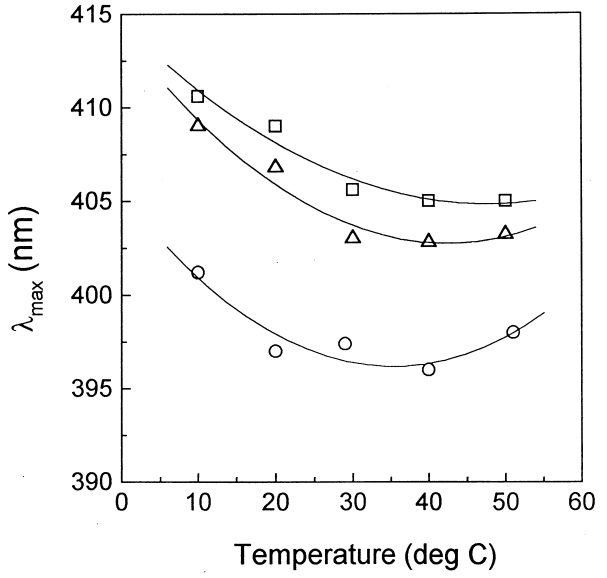

Fig. 2. Temperature dependence of fluorescence emission maxima of dibucaine in SUV's of pure DMPC (○, [L]/[D] = 400) and DMPC containing 10% (△, [L]/[D] = 300) and 20% (□, [L]/[D] = 200) cholesterol. Lines show the trends of the data.

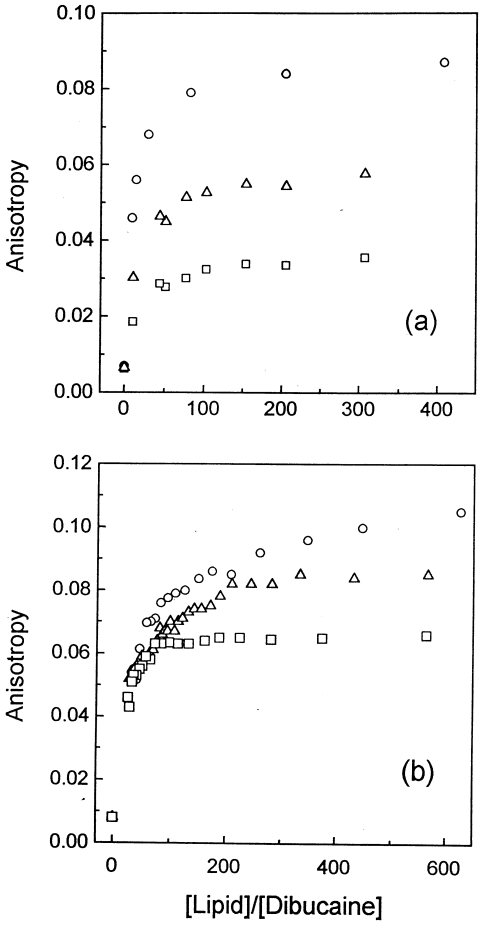

Fig. 3. Variation of steady-state anisotropy of dibucaine with [L]/[D] (a) at 33°C and (b) at 25°C in pure DMPC SUV's (○) and in DMPC SUV's containing 10% (△) and 20% (□) cholesterol. The errors in anisotropy are about the vertical size of individual data points.

Fluorescence anisotropy studies offer a convenient method for obtaining information about the rotational dynamics of a fluorophore. In aqueous solution the steady-state fluorescence anisotropy of free dibucaine was measured to be very small (0.008 ± 0.002), implying freedom of unhindered rotational motion of the fluorophore. Incorporation in DMPC vesicles produced a significant increase in anisotropy (0.09 ± 0.01) at 33°C (Fig. 3a), which showed a steep initial increase with increasing [L]/[D] before reaching saturation at higher lipid contents both in presence and absence of cholesterol. Fig. 3b shows similar anisotropy vs. [L]/[D] curves at 25°C showing higher magnitude of change in anisotropy with lower cholesterol contents, indicating that both the saturation value of the anisotropy and the value of [L]/[D] at which saturation was attained were larger for vesicles containing smaller amounts of cholesterol. Fig. 4 shows the results of similar experiments in DOPC vesicles at 25°C, with and without 20 and 40% cholesterol. As shown, the anisotropy in presence of DOPC was smaller for vesicles containing 40% cholesterol than for those with 0 or 20% cholesterol. In DMPC vesicles, T_m was determined by DPH anisotropy measurements and was found to be 24°C. Our measurements showed a broadening of the DPH anisotropy vs. temperature curve for DMPC membranes with increasing amount of cholesterol. Above 10% cholesterol we were unable to determine the T_m. A broader transition around 30°C was determined for vesicles with 10% cholesterol, while the anisotropy was unaltered in the range 13–40°C for vesicles with 20% cholesterol (data not shown).

Steady-state quenching of dibucaine fluorescence by KI was measured in the MOPS buffer (pH 6.5) and in presence of both types of lipid vesicles at 33°C, without and with cholesterol, at [L]/[D] ratios representing the saturation region of the plots in Fig. 1. Stern–Volmer plots of the quenching data are shown in Fig. 5a for DMPC and Fig. 5b for DOPC. The values of K_{SV} obtained by fitting Eqs. 1 and 2 to these data and the quenching rate constants are summarized in Table 1. In aqueous buffer a nonlinear plot with upward curvature was obtained, analysis of which yielded values of (2.8 ± 0.1) M^{-1} and (42 ± 0.5) M^{-1} for the static and dynamic (K_{SV}) quenching constants, respectively. The plot for dibucaine in pure DMPC vesicles at

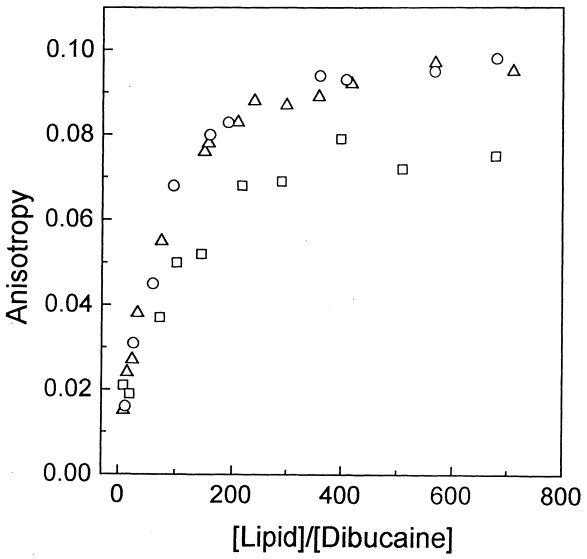

Fig. 4. Variation of steady-state anisotropy of dibucaine with [L]/[D] at 25°C in pure DOPC SUV's (○) and in DOPC SUV's containing 20% (△) and 40% (□) cholesterol. The errors in anisotropy are about the vertical size of individual data points.

Table 2
Total emission intensity decay parameters of dibucaine in aqueous buffer and in presence of DMPC and DOPC vesicles at 25°C containing various mol percents of cholesterol at lipid/dibucaine ratios of >300 for DMPC and >400 for DOPC

Medium	τ_1 (ns)	α_1 (%)	τ_2 (ns)	α_2 (%)	$\langle\tau\rangle$ (ns)
10 mM MOPS buffer, pH 6.5	3.13	78.6	1.16	21.4	2.71
Pure DMPC (no cholesterol)	2.77	71.2	0.94	28.8	2.24
DMPC+10% cholesterol	2.85	68.9	0.76	31.1	2.20
DMPC+20% cholesterol	2.92	66.0	0.84	28.6	2.17
Pure DOPC (no cholesterol)	2.77	73.7	0.77	26.3	2.24
DOPC+20% cholesterol	2.78	69.9	0.72	30.1	2.16
DOPC+40% cholesterol	2.80	62.3	0.60	37.7	1.97

Estimated errors in $\langle\tau\rangle$ were ± 0.03 ns.

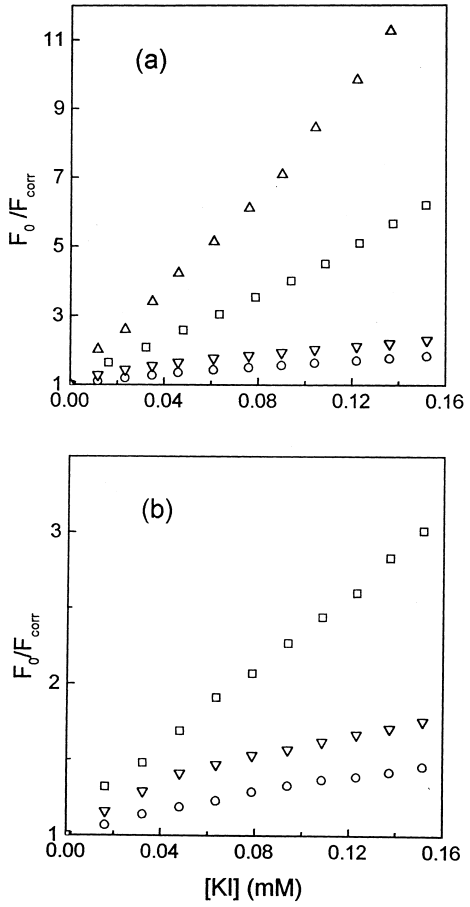

Fig. 5. Stern–Volmer plots of quenching of dibucaine fluorescence by iodide at 33°C in (a) pH 6.5 buffer (10 mM MOPS, 50 mM NaCl and 1 mM sodium thiosulfate to prevent formation of I_3^-) (△) and DMPC SUV's containing 0% (○), 10% (▽) and 20% (□) cholesterol, and in (b) DOPC SUV's containing 0% (○), 20% (▽) and 40% (□) cholesterol.

33°C was linear but the quenching efficiency of iodide was markedly lower than that in aqueous buffer, with a K_{SV} as low as (8.5 ± 0.3) M^{-1}. The quenching efficiency, however, increased with increasing amounts of cholesterol incorporated in the vesicles, as shown in Fig. 5a. In DMPC vesicles containing 20% cholesterol the value obtained for K_{SV} was about four times that obtained when the vesicles contained no cholesterol. A drastic reduction of quenching efficiency was also observed in presence of pure DOPC vesicles (Fig. 5b). However, in comparison to the case with DMPC vesicles, the effect of incorporating up to 20% cholesterol in DOPC vesicles was smaller. An appreciable increase in K_{SV} took place for vesicles containing 40% cholesterol. The quenching rate constants show similar changes with the cholesterol content in DMPC and DOPC vesicles summarized in Table 1.

Table 2 summarizes the results of time-resolved measurement of total emission intensity decays of dibucaine in buffer and in presence of the lipid vesicles, with and without cholesterol at 25°C. The intensity profiles in all cases yielded good fits to bi-exponential decays. In MOPS buffer, pH 6.5 as well as in presence of the membranes, the lifetimes were characterized by a contribution (20–30%) from a short-lived component (~1 ns) and another (70–80%) from a longer-lived one (~3 ns). The value of 2.7 ns obtained for the mean excited state lifetime of dibucaine in buffer agrees reasonably well with the lifetime in similar conditions reported by other workers [19,31]. The relative magnitudes of the two components did not change much but the mean lifetime decreased by 20% in presence of the pure phospholipid membranes, also in agreement with reported results [19]. The mean lifetime decreased by ~10% with increase in cholesterol content of both DMPC and DOPC vesicles.

4. Discussion

Previous NMR and fluorescence spectroscopic studies have concluded that dibucaine binds in the vicinity of the phospholipid glycerol moiety [16–19]. This location is in agreement with more recent studies placing the quinoline ring of dibucaine near the glycerol [22–24]. The NMR study on the interaction of the local anesthetic, tetracaine in DMPC vesicles containing 30% cholesterol clearly suggested that the location of tetracaine in a cholesterol containing system is different from that in pure PC bilayer [25]. The

Table 3
Estimates of the apparent partition coefficient (K_p) of dibucaine in different membrane systems, with and without cholesterol

Medium	25°C	33°C
Pure DMPC (no cholesterol)	350	80
DMPC+10% cholesterol	200	70
DMPC+20% cholesterol	70	50
Pure DOPC (no cholesterol)	300	
DOPC+20% cholesterol	300	
DOPC+40% cholesterol	200	

present study shows the effects of cholesterol on the partitioning and localization of dibucaine in the SUVs of DMPC and DOPC.

The increase in anisotropy of dibucaine in presence of phospholipid vesicles (Fig. 3) clearly points to the imposition of constraints on the rotational freedom of the fluorophore molecule, which in turn implies its insertion into the bilayer membrane. Table 3 summarizes the apparent partition coefficient of dibucaine between the membrane and aqueous phases (K_p), determined as mentioned in Section 2, for the different membrane systems under study. K_p was estimated to be 80, 70 and 50 in DMPC vesicles at 33°C containing 0, 10 and 20% cholesterol, respectively. In DOPC membranes at 25°C K_p was estimated to be about 300 for vesicles containing 0 and 20% cholesterol and 200 for the same containing 40% cholesterol. At 25°C, near the main phase transition temperature of DMPC, this value showed more significant change with increasing cholesterol content in the membranes. Thus K_p was estimated to be 350, 200 and 70 in DMPC vesicles at 25°C containing 0, 10 and 20% cholesterol, respectively, indicating altered partitioning of dibucaine in the presence of cholesterol. However, above the main phase transition temperatures of DMPC at 33°C and in DOPC at 25°C, K_p did not change significantly up to 20% cholesterol content, indicating displacement of dibucaine from the interior towards the surface of the vesicles by the presence of cholesterol. This is also consistent with the study on localization of tetracaine that suggested deeper partitioning in pure DMPC bilayer while the presence of 30 mol percent cholesterol 'squeezes' it closer to the aqueous interface of the bilayer [25].

Quenching of dibucaine fluorescence with iodide in membrane systems containing different amounts of cholesterol show that dibucaine is much less accessible to the iodide quencher in pure phospholipid vesicles than in vesicles containing cholesterol at 33°C (Fig. 5 and Table 1). This result implies that dibucaine is located in the solvent-accessible region around the lipid head-groups in cholesterol containing membranes, resulting in a large increase of accessibility of the (predominantly) monocationic dibucaine by the anionic quencher. This is in good agreement with the conclusion that while dibucaine is embedded in the hydrophobic region of the bilayer in pure phospholipid membranes, presence of cholesterol inhibits the penetration of dibucaine molecules deep into the bilayer.

Partitioning of dibucaine in the membrane phase is also associated with a decrease in the quantum yield of dibucaine (Table 2). This decrease in lifetime is consistent with the decrease in the peak emission intensity observed on transfer of dibucaine from buffer solution to the membranes. That the inclusion of cholesterol in DMPC vesicles does not affect the mean lifetime appreciably implies that the nature and degree of this interaction remains more or less unaltered in the process. The likely reason for the observed decrease in its mean lifetime in vesicles, measured at 25°C, can be attributed to changes in dibucaine partitioning in DMPC vesicles with and without cholesterol. However, in DOPC membranes the apparent partition coefficient does not change appreciably indicating that cholesterol brings about a rearrangement of dibucaine inside the bilayer rather than affecting the partition equilibrium between the aqueous and lipid phases, at least in DOPC membrane systems where the measurements are done well above the phase transition temperature of DOPC with T_m (−18°C) [32].

The interaction between cholesterol and PC has been extensively studied and it is known to affect the phase transition between the liquid crystalline and the ripple gel phases of PC membranes [32–36]. More recent evidence showed that there are other types of lateral organization for membrane cholesterol [37–40]. Our studies indicate that dibucaine is located close to the aqueous interface in cholesterol containing DMPC and DOPC bilayer vesicles at 33°C (above their T_m) where the partitioning of dibucaine in the membrane phase is not significantly altered due to the presence of cholesterol. The 'condensing' effect of cholesterol is presumably responsible in altering the localization site of the local anesthetic dibucaine in membranes containing cholesterol.

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