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Effect of liposome type and membrane fluidity on drug–membrane partitioning analyzed by immobilized liposome chromatography

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Abstract

Immobilized liposome chromatography (ILC) has been proven to be a useful method for the study or rapid screening of drug–membrane interactions. To obtain an adequate liposomal membrane phase for ILC, unilamellar liposomes were immobilized in gel beads by avidin–biotin binding. The retardation of 15 basic drugs on the liposome column could be converted to membrane partitioning coefficients, K_{LM}. The effects of small or large unilamellar liposomes and multilamellar liposomes on the drug–membrane partitioning were compared. The K_{LM} values for both small and large liposomes were similar, but higher than those for the multilamellar liposomes. The basic drugs showed stronger partitioning into negatively charged liposomes than into either neutral liposomes or positively charged liposomes. The membrane fluidity of the immobilized liposomes was modulated by incorporating cholesterol into the liposomal membranes, by changing the acyl chain length and degree of unsaturation of the phospholipids, and by changing the temperature for ILC runs. Our data show that K_{LM} obtained using ILC correlated well with those reported by batch studies using free liposomes. It is concluded that negatively charged or cholesterol-containing large unilamellar liposomes are suitable models for the ILC analysis of drug–membrane interactions.

Keywords: Membranes; Immobilized liposomes; Liposomes; Basic drugs; Beta-blockers

1. Introduction

Drug partitioning into biomembranes is essential for drug transport through lipid bilayers and thus is an important pathway for drug absorption. The partitioning of drugs between cell membranes and aqueous solutions has been widely studied using liposomes, which are model membranes with a lipid bilayer structure. Multilamellar liposomes (MLVs) have been used in a sedimentation method to study drug–membrane interactions and to determine the membrane partition coefficient for drugs [1]. This method usually requires a long time for solute molecules to equilibrate with the membrane due to heterogeneity in the MLV size and multilamellarity. Therefore, unilamellar liposomes of relatively homogeneous size have been used to determine drug–membrane partitioning using equilibrium dialysis [2] and ultrafiltration [3]. When the solute–membrane interaction is weak, the partitioning coefficients are smaller and are difficult to determine precisely [3]. Immobilized liposome chromatography (ILC) has recently been developed as a convenient

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and rapid method for analysis of solute–membrane interactions [4–8]. We recently developed an avidin–biotin binding method to immobilize unilamellar liposomes, with excellent immobilization stability and only slight leakage of the encapsulated content [7]. Liposomes immobilized by site-specific binding [9] show no deformation [10]. More importantly, unilamellar liposomes expose all of their membrane surfaces for interaction with solutes and thus have an advantage over immobilized multilamellar liposomes, where only some bilayer membranes are exposed for interaction. Furthermore, calculation of the membrane partition coefficient requires measurement of the volume of the stationary membrane phase. Although this is obscure for multilamellar liposomes, it can be estimated for unilamellar liposomes [11].

In this study, we determined the membrane partition coefficients (K_{LM}) for 15 pharmaceutical drugs using the ILC method and stable immobilized unilamellar liposomes. K_{LM} can be calculated from the retention volume of solutes measured by ILC [11]. To obtain an adequate membrane stationary phase for ILC analysis of drug–membrane interactions, the liposomes were immobilized in gel beads by avidin–biotin binding. We then studied the effects of liposome size, lamellarity, surface charge, membrane fluidity, and temperature on K_{LM}. Comparison of the K_{LM} values that we measured for several β-blockers on ILC columns with those reported in the literature for free liposomes [12,13] shows that large unilamellar liposomes which are negatively charged and contain cholesterol are most suitable for the ILC analysis of drug–membrane interactions.

2. Experimental

2.1. Materials

TSK gel G6000PW was purchased from Tosoh (Tokyo, Japan). Sephacryl S-1000 (Superfine) was obtained from Amersham Pharmacia Biotech (Uppsala, Sweden). Egg-white avidin (M_r 66 000) was obtained from Bio-Rad (Hercules, CA, USA). From Avanti Polar Lipids (Alabaster, AL), we purchased egg yolk phosphatidylcholine (EPC, >99%), phosphatidylserine from brain extract (BPS, >99%), 1,2-dimyristoylphosphatidylcholine (DMPC, >99%), 1,2-dipalmitoylphosphatidylcholine (DPPC, >99%), 1,2-distearoylphosphatidylcholine (DSPC, >99%), 1,2-dipalmitoleoylphosphatidylcholine (DPOPC, >99%), dioleoylphosphatidylcholine (DOPC, >99%), 1,2-dioleoylphosphatidylethanolamine-*N*-(cap biotinyl) (biotin-cPE) and 1,2-dioleoyldipalmitoylphosphatidylethanolamine-*N*-(cap biotinyl) (biotin-cDPPE). From Dojindo Labs. (Kumamoto, Japan), we obtained (2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES). The following chemicals were supplied by Sigma (St. Louis, MO, USA): (−)-isoproterenol, isoproterenol, salbutamol, octopamine, synephrine, bupivacaine, disopyramide, clenbuterol, metoprolol, atenolol, alprenolol, oxprenolol, pindolol, acebutolol, DL-propranolol, Fiske & Subbarow reducer, and stearylamine (STA). Cholesterol (Chol) was obtained from Nacalai Tesque (Kyoto, Japan). Other chemicals were of analytical grade.

2.2. Immobilization of biotinylated liposomes in avidin-coupled gels

Avidin was coupled to chloroformate-activated Sephacryl S-1000 gel or TSK G6000PW gel (denoted hereafter as Sephacryl and TSK, respectively) to a concentration of 3 mg protein/ml gel as described in Ref. [7]. The avidin-gels were stored at 4°C in buffer H (10 m*M* HEPES, 150 m*M* NaCl, 0.1 m*M* Na_2-EDTA, pH 7.4) supplemented with 3 m*M* NaN_3 before use. Small unilamellar liposomes and large unilamellar liposomes (SUVs and LUVs, respectively) containing 2 mol% biotin-cDOPE or biotin-cDPPE in the presence of buffer H were prepared by probe sonication and extrusion on two stacked polycarbonate filters of 100-nm pore size as described elsewhere [7]. The mean sizes of the SUVs and LUVs were 29±11 nm ($n = 2$) and 104±11 nm ($n = 12$). The biotinylated LUVs composed of DMPC, DPPC, and DSPC were always prepared at 30, 50, and 60°C, respectively, which are above their respective phase transition temperatures. For immobilization [7], the biotinylated LUVs were gently mixed with moist avidin-gel of TSK or Sephacryl by rotation for 2–3 h at 23°C or overnight at 4°C under nitrogen. Considering the phase transition temperature, DMPC, DPPC and DSPC liposomes were mixed with the avidin-gel for 3 h at 25, 45, and

55°C, respectively. The amounts of immobilized liposomes in the gel beads were determined by phosphorus assay [14,15].

2.3. Chromatography of drugs on immobilized liposomes

The gel beads containing avidin–biotin-immobilized liposomes were packed into a 5–5.5 cm×5 mm I.D. gel bed in a glass column (HR 5/5, Pharmacia Biotech). Fifteen basic pharmaceutical drugs (1 μg/μl, 5 μl) were applied to the immobilized-liposome gel bed and eluted with buffer H at a flow-rate of 0.5 ml/min for TSK and 0.3 ml/min for Sephacryl. The chromatographic runs were performed using a Tosoh HPLC System 1 equipped with an HPLC pump (CCPM-II, Tosoh), a UV detector (UV-8010, Tosoh) set at 220 nm, and a recorder, which was interfaced with an IBM computer. Columns were placed in a column oven (CO-8020, Tosoh) equipped with a sample injector. The temperature for the chromatographic runs was kept at 25°C, except for DSPC liposomes at 55°C. For the temperature dependence of drug retention by ILC, the chromatographic runs were carried out at 25–45°C for EPC and 25–50°C for DPPC. The temperature in the column oven was varied from 4 to 55°C with an accuracy of ±0.1°C. The injector connected to the oven allowed the sample to be loaded in a 0.1-ml loop in the oven; the injector was equilibrated to the oven temperature for 10 min before the injection. The buffer reservoir was placed in a water-bath to maintain the buffer solution at the same temperature as the run temperature in the oven. The time required for temperature equilibration in the oven was always more than 1 h. Before reaching the liposome column, the buffer solution passed through a 200-cm coiled tube mixer, and a 2-ml loop, which were all placed inside the oven. The running buffer was thus equilibrated to the desired temperature before passing through the column for the ILC run.

2.4. Membrane partition coefficients

The membrane partition coefficient (K_{LM}) for a solute partitioning between the aqueous phase and the liposome membrane phase can be calculated as [11]:

$$K_{LM} = (V_r - V_0)/0.755A \quad (1)$$

where V_r is the retention volume (ml) of the drug on the liposome column, V_0 is the retention volume (ml) of the small hydrophilic reference (NaN_3) (ml) on the same liposome column, and A is the amount of immobilized liposomes (mmol of phospholipid). Note that Eq. (1) is derived using the lipid bilayer of the immobilized liposomes as the stationary phase volume. The derivation of this equation has been described elsewhere [11].

3. Results and discussion

3.1. Effect of liposome size and lamellarity on K_{LM}

Sterically entrapped multilamellar liposomes (MLVs) [6,16,17] and avidin–biotin-coupled unilamellar liposomes of both small and large sizes [7] were used for ILC. Fifteen pharmaceuticals were applied to the immobilized liposome column. Fig. 1 shows the linear correlation between drug partitioning into the liposomal membranes of LUVs with MLVs and SUVs. A good correlation ($r = 0.963$) was obtained between $\log K_{LM}$ values for MLVs and LUVs, suggesting that the same interaction exists for the drug partitioning into the liposomes, despite differences in size and lamellarity as well as differences in immobilization methods. The values of $\log K_{LM}$ for the bilayer (Fig. 1A) were calculated by assuming that the drugs were distributed both in the outer and inner leaflets of the liposomes, and those for the outer layer (Fig. 1B) were calculated by assuming that the drugs were distributed only in the outer monolayer. For both layers, the $\log K_{LM}$ values obtained for the MLVs column were significantly lower than those for either the LUVs or SUVs column. One possible explanation for these lower values is that equilibrium of the drugs partitioning into the multilamellar vesicles that are heterogeneous in size and lamellarity might not have been reached during the chromatographic runs. Thus, only the surface parts of the bilayer membrane of the MLVs were accessible to the drugs. Studies [1,18,19] show that, in sedimentation experiments for drug partitioning in multilamellar liposomes, the time needed for the partitioning to reach equilibrium is as long as

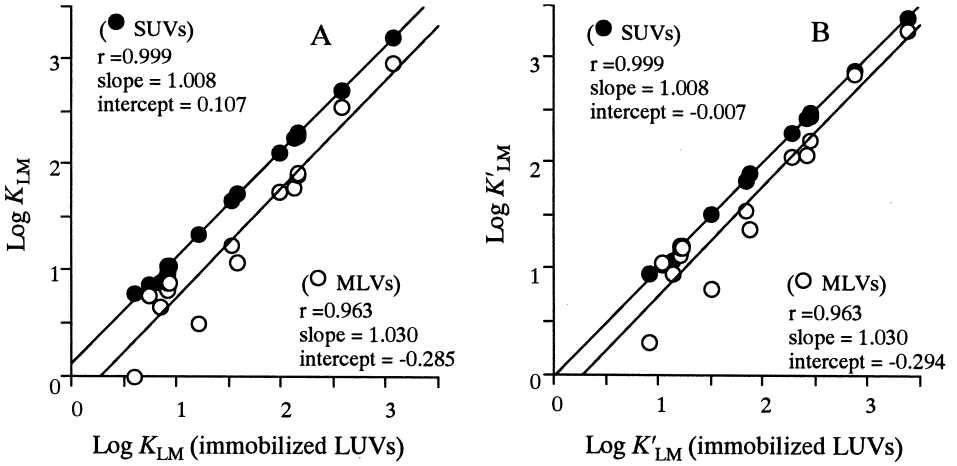

Fig. 1. Correlation of drug partitioning into immobilized LUVs and SUVs (●), and MLVs (○) composed of biotinylated EPC. (A) Drugs were assumed to interact with the outer and inner leaflets of the liposomal bilayer. (B) Drugs were assumed to interact only with the outer leaflet. Fifteen drugs (see Section 2.1) were applied to liposomes immobilized in a TSK gel bed at 25°C. MLV liposomes were prepared by re-hydration of the lipid film in buffer H in the presence of gel beads followed by freezing and thawing to entrap liposomes of sufficiently large size (compared to the bead pore size) according to the protocol described in Ref. [4]. LUVs and SUVs were immobilized in gel beads by avidin–biotin binding as described in Section 2.2.

overnight and that the volume of the lipid phase cannot be measured precisely. Such heterogeneous liposomes have not yet been successfully used as reference model systems for the characterization of lipophilic solute–membrane partitioning [2]. Therefore, ILC analysis using immobilized unilamellar liposomes will be more accurate than the MLV model for analysis of drug–membrane partitioning.

A good correlation ($r = 0.999$) was also found between $\log K_{LM}$ obtained for the LUV column and that for the SUV column (Fig. 1). However, when the inner and the outer leaflets of the lipid bilayers were assumed to be accessible to the drugs, $\log K_{LM}$ values for SUVs were slightly higher than those for LUVs (Fig. 1A). It is well known that the asymmetric bilayer packing and membrane curvature of SUVs of small radius result in a large area and low density of the outer membrane surfaces [20]. One possible explanation is that the low density makes the SUV membrane more fluid, which is preferable for drug partitioning [21]. Another possible explanation is the difference in surface area between SUVs and LUVs, which requires recalculation of the membrane partition coefficients K_{LM} to take this difference into account. By using a molar ratio of the phospholipid molecules in the outer and inner leaflets of 2.1 to 1 for SUVs [20] and considering that drug partitioning to the liposomal bilayers might be restricted to the outer lipid membrane, Eq. (1) becomes $K'_{LM} = (V_r - V_0)/0.51A$, whereas assuming that the ratio is 1:1 for LUVs and MLVs, Eq. (1) becomes $K'_{LM} = (V_r - V_0)/0.38A$. Fig. 1B shows that the $\log K'_{LM}$ values obtained for the LUV column were similar to those for the SUV column, because the slope of the $\log K'_{LM}$ plot of LUVs vs. SUVs was very close to 1 (slope 1.008) and the intercept was very close to 0 (intercept -0.007). We recently reported that hydrophobic phosphonium cations likely interact with only the outer leaflet of the liposomal lipid bilayer, but do not pass through the membrane [3]. In that study, the log K'_{LM} values for the hydrophobic cations partitioning in the SUV column were similar to those in the LUVs column during the ILC operation [3]. We thus propose that such drugs are likely to partition only into the outer liposome membranes, and that the $\log K'_{LM}$ values are relatively independent of the size of the unilamellar liposomes.

3.2. Effect of liposome surface charge on K_{LM}

A lipid bilayer membrane is a type of interfacial phase that is fundamentally different from a bulk

solvent phase [22,23]. The surface properties of the membrane phase affect the extent of drug–membrane partitioning. Therefore, in addition to the liposomal size and lamellarity, we measured the K_{LM} values using ILC on neutral, negatively charged, and positively charged LUVs. Comparison of these measured values (as in Fig. 2) shows that the correlation between the negatively charged liposomes and the neutral liposomes ($r = 0.993$) was better than that between the positively charged liposomes and the neutral liposomes ($r = 0.975$). Furthermore, $\log K_{LM}$ values for the partitioning of basic drugs into negatively charged liposomes were significantly higher than those for partitioning into either positively charged liposomes or neutral liposomes. These results agree with those in the literature [13,24] and with ILC studies using entrapped MLVs [16] and clearly show that electrostatic interactions between the drugs and the liposome surfaces are involved in drug–membrane partitioning. For the basic drugs studied here, drug partitioning into liposomes by ILC decreased in the following order: negatively charged>neutral>positively charged liposomes. For the negatively charged liposomes, an increase in the negative charge density in the liposomal membranes, measured by varying the BPS content from 25 to 50 mol%, resulted in only a slight increase in drug partitioning (Fig. 2). A typical biological membrane contains approximately 10–30% of negatively charged phospholipids. To mimic the membrane surface charge, immobilized unilamellar liposomes that contain negative charges should be adequate models for analysis of basic drug–membrane partitioning, even for drugs that are not charged in the physiological environment.

3.3. Effect of membrane fluidity on K_{LM}

3.3.1. Effect of cholesterol incorporation

In addition to the surface charge effect, membrane fluidity, described also as ''surface density'' [23,25], is known to play an important role in drug partitioning when free liposome suspensions are used [18,19,25–27]. In the present work, we systematically studied this effect using ILC. The membrane fluidity of the immobilized unilamellar liposomes was modulated using ILC under various compositions of liposomes and at various temperatures. Fig. 3 shows the effect of cholesterol, where 20–40 mol% cholesterol is incorporated into EPC. The $\log K_{LM}$ values decreased as the cholesterol content increased. Furthermore, for less hydrophobic drugs

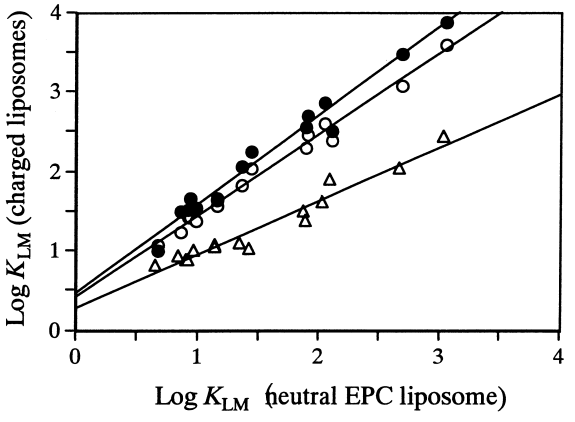

Fig. 2. Correlation of drug partitioning into the bilayer of neutral EPC with negatively charged BPS–EPC (circles) and positively charged STA–EPC (triangles) immobilized in Sephacryl S-1000. The molar ratios of BPS to EPC are 1:1 (●) and 1:3 (○), while STA to EPC is 1:3 (△). Fifteen drugs (see Section 2.1) were applied to liposome-gel beds and eluted with buffer H as described in Section 2.2.

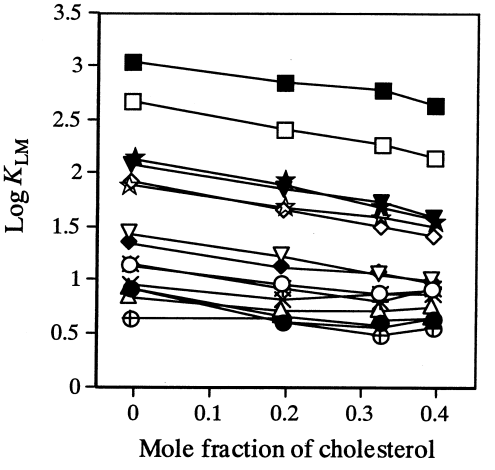

Fig. 3. Effect of cholesterol mole fraction in the liposomal bilayers on log K_{LM} for drug partitioning into EPC LUVs immobilized in Sephacryl S-1000 gel beads. The drugs are (○) (−)-isoproterenol, (*) (γ)-isoproterenol, (●) salbutamol, (×) DL-octopamine, (△) synephrine, (★) bupivacaine, (▲) disopyramide, (▼) clenbuterol, (▽) metoprolol, (⊕) atenolol, (□) alprenolol, (◊) oxprenolol, (☆) pindolol, (♦) acebutolol, (■) DL-propranolol.

with lower log K_{LM} values, the cholesterol effect was less pronounced than for drugs with higher log K_{LM} values. This difference is probably due to the fact that interaction between the less hydrophobic drugs and the membrane is restricted to the region near the outer membrane surface, and thus membrane fluidity plays a smaller role than for more hydrophobic drugs with higher log K_{LM} values. Incorporation of cholesterol into liposome bilayers increases the orientation and conformational order of the phospholipid chains in EPC–chol bilayers and decreases the partitioning of small molecules [23,25]. Our ILC results agree with these results and suggest that solute partitioning into the bilayer membrane is caused by interactions at the interfacial phases.

3.3.2. Effect of the fatty acyl chain

Similarly to the effect of cholesterol on membrane fluidity, increasing the chain length or decreasing the degree of saturation of the hydrocarbon chain results in a decrease in solute–membrane partitioning, as was previously studied using the free liposome system [18,26,27]. To diminish this acyl chain effect on the ILC analysis of drug–membrane partitioning, we performed chromatographic runs on immobilized liposomes composed of PC of various chain lengths at temperatures above the main transition temperature. Fig. 4 shows that the log K_{LM} values for drugs partitioning into liposomes composed of different lipids are not significantly different, indicating that the fluidity of lipid membranes provides a similar environment for interaction with drugs. For β-adrenergic agonists (isoproterenol, (−)-isoproterenol, salbutamol, octopamine, synephrine) and certain β-blocker drugs (DL-propranolol, alprenolol, pindolol, oxprenolol), the log K_{LM} values decreased in the order DMPC>DPPC>DSPC, DOPC>DSPC, DPOPC>DPPC, similar to the order reported by Wright [18]. This order can be rationalized by noting that when the temperature is above the membrane phase transition temperature, lipids with short chains or more unsaturation provide a more fluid membrane

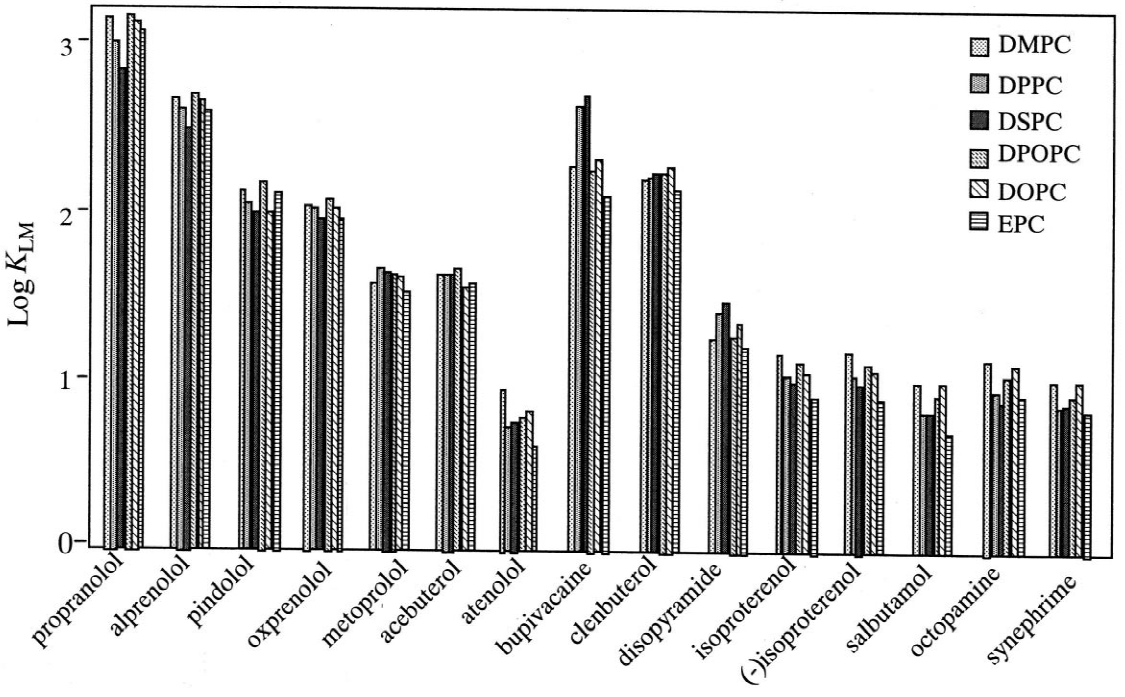

Fig. 4. Effect of the fatty acyl chain of phosphatidylcholine on log K_{LM} for drug partitioning into LUVs immobilized in TSK gel beads. The values of K_{LM} were evaluated in the fluid state of the liposomal lipid bilayer at 25°C for EPC, DMPC, DPOPC, and DOPC, at 45°C for DPPC, and at 55°C for DSPC as described in Section 2.2.

environment, which favors drug partitioning. For bupivacaine and disopyramide, the $\log K_{LM}$ values increased significantly with increasing temperature. The $\log K_{LM}$ values were similar for DMPC, DPOPC, DOPC, and EPC at 25°C, but were significantly higher upon ILC for DPPC at 45°C and DSPC at 55°C, suggesting that partitioning of these kinds of drugs depends on the temperature rather than on membrane fluidity. The $\log K_{LM}$ values of drug partitioning into EPC (which, according to the manufacturer's specifications, was mainly a mixture of C_{16} and C_{18} saturated acyl chains at C-1 and C_{18} unsaturated acyl chains at C-2) were between those of the pure disaturated (DPPC) and diunsaturated (DOPC) forms. Due to the natural membrane structure of the mixing lipid composition, immobilized EPC membranes might be suitable models for the analysis of drug–membrane interactions.

3.3.3. Effect of temperature

The temperature dependence of $\log K_{LM}$ for drugs partitioned into gel-bead-immobilized EPC LUVs and DPPC LUVs is given in Fig. 5A and B, respectively. As shown in Fig. 5A, this dependence is similar to that reported by Beigi et al. (Fig. 1C in Ref. [16]) for five β-blockers partitioning into immobilized EPC, which was in the fluid phase over the observed temperature range. For EPC, the $\log K_{LM}$ values for 11 of the 15 drugs studied here increased slightly with increasing temperature, except for disopyramide, the $\log K_{LM}$ values of which increased three-fold (Fig. 5A). In contrast, the $\log K_{LM}$ values for β-blockers such as propranolol, alprenolol or pindolol decreased with increasing temperature, consistent with the results of Beigi et al. [16]. Similar behavior was previously observed for other compounds, such as lindane [26] or teniposide [18]; the partitioning coefficient decreased as the temperature of fluid liposomes increased. The water solubility of hydrophobic drugs increases with increasing temperature, thus partitioning of these drugs into the hydrophobic regions decreases [18]. Another possible explanation for the temperature-related effects includes thermotropic geometrical factors imposed by the molecular structure of the lipid according to the ''shape hypothesis'' [28], which is assumed during the interaction between anesthetics and membranes.

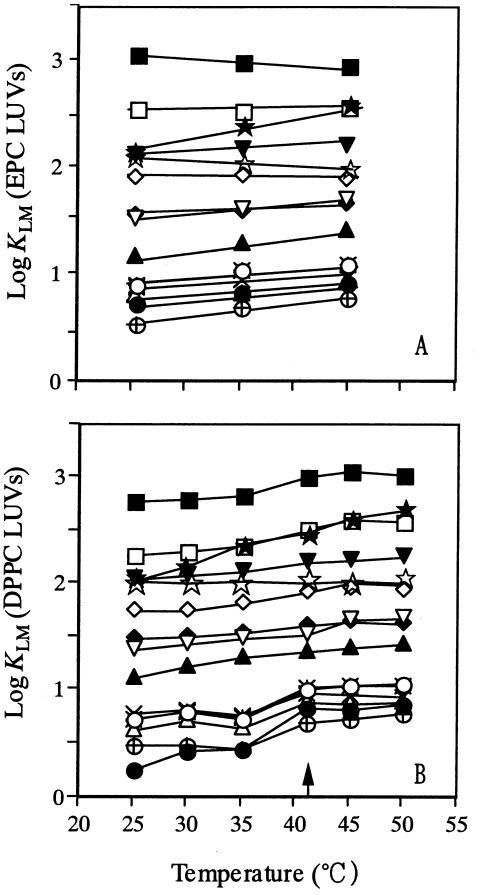

Fig. 5. Effect of temperature on $\log K_{LM}$ for drug partitioning into EPC LUVs (A) or DPPC LUVs (B) immobilized in TSK gel beads. The phase transition temperature for DPPC (42°C) is indicated by the arrow. The drugs are denoted in Fig. 3.

On the other hand, the DPPC lipid phase can be a gel or a solid phase and can assume ripple and fluid or liquid-crystalline states depending on the temperature range [29]. Therefore, we designed our experiments to determine the temperature sensitivity of drug partitioning into DPPC by changing from the sub-transition temperature (25°C), past the pre-transition temperature (35°C), and then through the main phase transition temperature (T_c, 41°C). Fig. 5B shows that the $\log K_{LM}$ values of drugs in DPPC LUVs increased with increasing temperature in the range 25–50°C. The K_{LM} values were low when the temperature was below the phase transition temperature in which the lipid bilayers were in the gel phase.

This can be explained by noting that a more organized structure of the lipid bilayer leads to an increase in lipid density, which decreases drug partitioning. The log K_{LM} values gradually increased between the pre- and main transition temperatures (35–42°C). In this temperature range, a step-like change in the partitioning coefficient occurs, where DPPC is in a ripple state [27]. Therefore, above the phase transition temperature, the higher membrane fluidity caused by the disordered packing of the lipid bilayer might promote drug partitioning.

ILC can be performed at various temperatures, which allows one to study the thermodynamics of drug partitioning into liposomal membranes by linear van't Hoff plots (unpublished results). The membrane partitioning coefficient for lipophilic cations was previously measured by ILC [11]. Furthermore, the thermodynamics of cation partitioning into immobilized unilamellar liposomes was investigated recently [3], indicating that thermodynamic analysis is also applicable to drugs. In general, the different mechanisms of interaction between drugs and membranes could be conveniently investigated by ILC using immobilized unilamellar liposomes of various compositions.

3.4. Comparison of partitioning coefficients measured by ILC and the free liposome system

In our previous work [7], a good correlation was obtained between the log K_s values of β-blockers measured by ILC and the apparent partition coefficients (log K'_m) in liposome suspensions [1], indicating that drug partitioning between the homogenous membrane stationary phase and the aqueous mobile phase is similar to that between the liposome and the aqueous phase. For comparison with the ion-corrected membrane partition coefficients (K_m) of β-blockers obtained in a free liposome system [1,12], we corrected the partition coefficients for the β-blockers in an elution buffer (pH 7.4) for ILC to $K_m = K_{LM}(1 + 10^{pK_a - 7.4})$ by using published pK_a values of each β-blocker [1]. In Fig. 6, we compare the K_m values of β-blockers partitioned into immobilized LUVs with those of a DMPC–CHOL–DCP (7:2:1 molar ratio) liposome suspension measured by Betageri and Rogers [12] and Choi and Rogers [13]. The K_m values for the ILC system were slightly higher than those for a free liposome system.

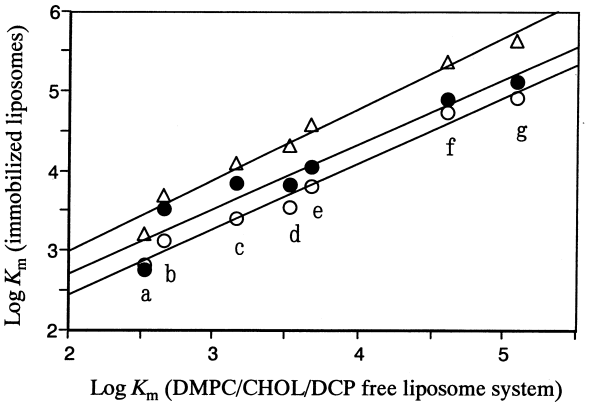

Fig. 6. Correlations of log K_m for seven β-blockers partitioning into a free DMPC–CHOL–DCP (7:2:1, molar ratio) liposome system and into lipid bilayers of immobilized LUVs composed of EPC (●), EPC–BPS (△) and EPC–chol (○). The values of K_m in the free liposome system are from Refs. [12,13], and the drugs were a = atenolol, b = pindolol, c = acebuterol, d = metoprolol, e = oxprenolol, f = alprenolol, g = propranolol. The chromatographic runs on the LUV-TSK column were carried out at 25°C.

This was probably caused by the fact that not all of the phospholipids in the multilamellar liposomes used in the free liposome system interacted with the drugs, similar to the situation for unilamellar liposomes. To evaluate a suitable ILC membrane mode for drug partitioning studies, we compared the K_m values (data not shown) from ILC columns of various liposome models with those from a free liposome model of DMPC–CHOL–DCP. We found that negatively charged or cholesterol-containing LUVs produced the best correlation ($r = 0.990$, Fig. 6), better than that for EPC LUVs ($r = 0.962$, Fig. 6) with the free liposomes. Betageri et al. reported [12] that this DMPC–CHOL–DCP liposome model yielded the best results with pharmacokinetic parameters in humans [30]. These results indicate that the pharmacokinetic behavior of β-blockers can be reasonably predicted when negatively charged and more ordered lipid structure liposomes are used in ILC studies. Therefore, ILC can be effective in quantitative structure–activity relationship studies.

4. Concluding remarks

The membrane partition coefficient of drugs can

be easily and reliably obtained by ILC. Note that there was no loss of lipid after chromatographic runs of 15 drugs on the same liposome column. Even after 60 chromatographic runs at different temperatures, only 7% of the immobilized EPC LUV was lost. The stability of the liposomes was demonstrated in Ref. [18]. Furthermore, the membrane partition coefficient calculated using Eq. (1) was independent of the dimensions of the gel beads and the amount of immobilized liposomes [11]. This provides a practical advantage for ILC for the quantitative analysis of solute–membrane partitioning. Chromatographic results of drug partitioning into liposomes of various compositions might provide information on the mechanisms involved in drug–membrane interactions and thus allow better prediction of drug absorption. For ILC analysis of drug–membrane partitioning, negatively charged or cholesterol-containing large unilamellar liposomes are good models to predict drug–membrane interactions in the cell membrane.

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