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Association of ethanol with lipid membranes containing cholesterol, sphingomyelin and ganglioside: a titration calorimetry study

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Abstract

The association of ethanol at physiologically relevant concentrations with lipid bilayers of different lipid composition has been investigated by use of isothermal titration calorimetry (ITC). The liposomes examined were composed of combinations of lipids commonly found in neural cell membranes: dimyristoyl phosphatidylcholine (DMPC), ganglioside (GM_1), sphingomyelin and cholesterol. The calorimetric results show that the interaction of ethanol with fluid lipid bilayers is endothermic and strongly dependent on the lipid composition of the liposomes. The data have been used to estimate partitioning coefficients for ethanol into the fluid lipid bilayer phase and the results are discussed in terms of the thermodynamics of partitioning. The presence of 10 mol% sphingomyelin or ganglioside in DMPC liposomes enhances the partitioning coefficient by a factor of 3. Correspondingly, cholesterol (30 mol%) reduces the partitioning coefficient by a factor of 3. This connection between lipid composition and partitioning coefficient correlates with in vivo observations. Comparison of the data with the molecular structure of the lipid molecules suggests that ethanol partitioning is highly sensitive to changes in the lipid backbone (glycerol or ceramide) while it appears much less sensitive to the nature of the head group.

Keywords: Alcohol–membrane interaction; Partitioning coefficient; Calorimetry; Lipid bilayer; Lipid composition

1. Introduction

Since Meyer and Overton [1,2] made their observations about anaesthetic potency and the correlation with the solubility of anaesthetic compounds in olive oil, a large number of models regarding the hydrophobic lipid–membrane core as the target for anaesthetic drugs have been proposed. However, it is still an unsettled question as to which extent specific membrane bound receptors are involved in the toxic action of ethanol or if the lipid bilayer is a target for ethanol [3]. The role of lipid composition in the function of biological membranes is not fully understood either, but the well-established regulation of lipid composition in cells combined with the increasing elucidation of the diversity of lipid physical properties provides strong evidence for the importance of

Abbreviations: DMPC, dimyristoyl phosphatidylcholine (14:0); GM_1, monosialoganglioside (C 18:0); sphingomyelin (C 16:0); ITC, isothermal titration calorimetry

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the unique physical properties of individual lipid species for specific membrane functions [4]. Further systematic studies on well-defined lipid bilayer model systems of various compositions are a prerequisite for a quantitative characterisation of the effects of ethanol on the physicochemical properties of the lipid bilayer part of the membrane.

Numerous investigations have indicated that an ethanol-induced perturbation of the mechanical and structural properties of the cell membrane are important mechanisms underlying alcohol intoxication [5–7]. Focus has been on the membrane interior as the putative region of ethanol action, because ethanol-induced disordering of the membrane interior occurs at pharmacologically relevant concentrations of ethanol [5,8,9]. However, detailed structural information on alcohol–membrane interactions from NMR studies [10–13] and fluorescence spectroscopy [14] has shown that ethanol resides predominantly in the lipid-water interface near the glycerol backbone and it has been suggested [15,16] that alcohol as well as anaesthetics compete with water for the same hydrogen bonding sites in membrane systems. This structural information is clearly of immediate importance but also raises new questions about the thermodynamics of ethanol–liposome interactions.

Intermolecular interactions are generally a concept of microscopic nature and information on molecular behaviour is usually obtained by spectroscopic techniques. Thermodynamics, on the other hand, does not depend on explicit details of molecular behaviour but is often visualised in this way. Koga and coworkers have extensively described the evaluation of molecular interactions in solution from precise measurements of partial molar quantities such as partial molar enthalpies or chemical potentials [17–20]. In a recent study we used the same methodology to evaluate the interactions of short-chain alcohols with unilamellar DMPC liposomes [21]. This work showed that the alcohols have approximately the same enthalpy in the lipid bilayer as in the pure liquid alcohol. In the present paper we have investigated the enthalpic interactions of ethanol with liposomes of different lipid composition, including cholesterol and ceramides, by isothermal titration calorimetry in order to elucidate the possible role of lipid specificity in alcohol–membrane interactions.

2. Materials and methods

Dimyristoyl phosphatidylcholine, DMPC (purity $>99\%$), cholesterol (purity $>98\%$) and sphingomyelin (egg, purity $>99\%$) were purchased from Avanti Polar Lipids (Birmingham, AL). Monosialoganglioside, GM_1 (bovine brain, purity $>99\%$) was supplied by Matreya (Pleasant Gap, PA). All lipids were used without further purification. Anhydrous ethanol (purity $>99.9\%$) was obtained from Merck (Darmstadt, Germany).

2.1. Preparation of unilamellar vesicles

For each series of calorimetric measurements a concentrated (50–70 mg/ml) suspension of unilamellar liposomes was prepared. The mixtures studied were as follows: pure DMPC, DMPC with cholesterol (30 mol%), DMPC with sphingomyelin (10 mol%) and DMPC with ganglioside GM_1 (10 mol%). After weighing, the dry lipids were co-solubilised in a chloroform/methanol 2:1 mixture (or hydrated immediately in the case of pure DMPC). Chloroform/methanol was driven off by a nitrogen stream, and the samples were stored under vacuum for at least 72 h. The resulting dry lipid films were then dispersed in a 50 mM Hepes buffer (pH 5.1) with 10 mM NaCl and 60 μM EDTA. The temperature was kept at 40°C for 1 h during which the suspensions were shaken vigorously several times. Unilamellar vesicles were produced by standard extrusion techniques [22] (Lipex Biomembranes, Vancouver, Canada). Twelve repeated extrusions through two stacked polycarbonate filters were performed (Nucleopore, 0.1 μm pore size) using a hydrostatic pressure of 25 atm at 40°C. The final concentration of lipid was determined gravimetrically by freeze drying 100 μl aliquots of the suspensions.

A second set of experiments was performed for preparations of DMPC and DMPC/sphingomyelin (10 mol%) in phosphate-buffered saline (pH 7.4). No effect of the pH change was observed.

2.2. Preparation of ethanol solutions for ITC method II

Ethanol–buffer solutions were prepared from aqueous buffer (50 mM Hepes at pH 5.1, or phos-

phate-buffered saline at pH 7.4) by weighing. The solutions were mixed using a stirrer magnet and then sonicated for 20 min to ensure complete mixing. After mixing, the apparent pH of the ethanol solutions was measured and if necessary readjusted to pH 5.1 (or pH 7.4).

2.3. Isothermal titration calorimetry

ITC were conducted by two different methods.

Method I. The excess partial molar enthalpy of ethanol in solutions with various liposome contents was measured directly by heat conduction ITC (TAM 2277, ThermoMetric, Järfälla, Sweden). Small aliquots (3 μl) of neat ethanol were titrated into 3-ml solutions of a fixed liposome concentration. These experiments were done with increasing liposome concentrations (0, 66, and 100 mmol/kg H_2O).

Method II. The excess partial molar enthalpy of liposomes at infinite dilution in dilute ethanol-buffer mixtures was measured by high sensitivity ITC (MSC-ITC, MicroCal, Northampton, MA).

To measure the enthalpy change associated with transferring liposomes from buffer (the standard state) to an ethanol–buffer mixture, Hess' law is used. The overall enthalpy change is the sum of the reaction enthalpies of the individual reactions into which the reaction may be divided. In Fig. 1A–D, the four-step procedure adopted to measure this enthalpy change is illustrated. The procedure is described in further detail elsewhere [21].

The heats measured in each step (q_A, q_B, q_C, and q_D) are used to calculate the transfer enthalpy, ΔH_{trans},

$$\Delta H_{trans} = (M/\rho w_L)(q_A/V_A + q_B/V_B + v_w(q_C/V_C + q_D/V_D)) \quad (1)$$

V_i is the volume titrated in step i (i: A, B, C, or D), M is the molar mass of the lipids, ρ is the density of the concentrated lipid solution removed in step A, and w_L and v_w are, respectively, the weight fraction of lipid and the volume fraction of buffer in the concentrated solution. The value of $\rho = 0.99$ g/ml was measured for DMPC (100 mmol/kg H_2O) by densitometry. We assume the density to be independent of the lipid composition. The contributions from step A and D to the cycle are found to be negligible. Thus, the difference between the heats in step B and C represents the total amount of heat absorbed by transferring liposomes from aqueous solution to the ethanol–buffer mixture. As argued below this is the partial enthalpy of the liposomes with respect to a standard state in which liposomes at infinite dilution are dispersed in pure water.

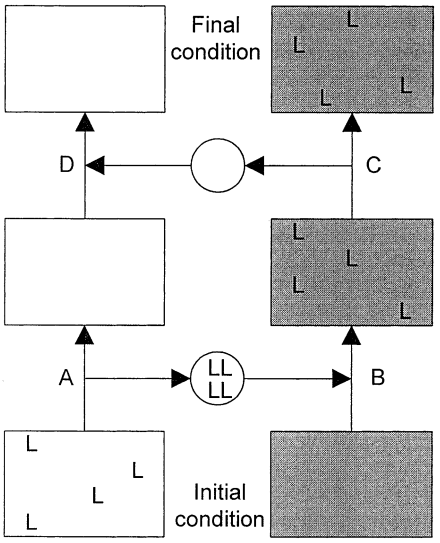

Fig. 1. Schematic illustration of the ITC procedure. Four separate measurements (A–D) are made in order to determine the transfer enthalpy of liposomes (L) at infinite dilution, from water (white) to an ethanol solution (shaded). The heats of process A (q_A) and B (q_B) are obtained by titrating the concentrated liposome solution into water and ethanol solutions, respectively. Similarly, q_C and q_D are measured by titration of water and the water–ethanol–liposome mixture with pure water.

Further, we have adopted the method developed by Zhang and Rowe [23] to determine partitioning coefficients of ethanol into DMPC and DMPC/sphingomyelin bilayers. The method is based on the so-called solvent null method. The syringe is loaded with a lipid–ethanol–buffer suspension and the reaction cell with a ethanol–buffer solution. When aliquots from the syringe are added to the sample cell heat is absorbed if the free ethanol concentration in the cell is higher than in the suspension; when the concentration in the cell matches the free ethanol concentration in the syringe, no heat is generated, and when the concentration in the syringe is higher than in the sample cell heat is released (see Zhang and Rowe [23], for a detailed discussion of these

effects). The amount of partitioned ethanol is calculated by subtraction of the free ethanol concentration in the bulk solution from the total ethanol concentration of the suspension.

3. Data treatment

It has previously been argued [17] that since the excess partial molar enthalpy[1] of a component A in solution, H_A^E, constitutes the enthalpic contribution of A to the total (integral) excess enthalpy of the system,

$$H_A^E = (\partial H^E/\partial n_A)_{T,p,n_B} \quad (2)$$

the sign of the derivative

$$H_{A-A}^E = (\partial H_A^E/\partial n_A)_{T,P,n_B} \quad (3)$$

may be used to elucidate intermolecular interactions. In general, composition derivatives of partial molar functions are referred to as interaction functions or interaction parameters. These interaction functions are macroscopic (average) quantities but have previously been used for the understanding of the structural nature of simple solutions of non-electrolytes [18] as well as to investigate the intermolecular interactions of small organics with macromolecules [20]. Recently, the application of the approach to membrane partitioning has been discussed [21].

The slope in a plot of H_A^E against the concentration of another component, m_B, (for practical reasons molalities, m_B, is used throughout this study) indicates how additional B-molecules enhance or decrease the average enthalpy of species A in the solution. Hence $\partial H_A^E/\partial m_B$ (i.e., H_{A-B}^E in a system containing 1 kg of solvent water) is a measure of the enthalpy change generated from mutual A–B interactions. A situation where $H_{A-B}^E > 0$ implies that additional B makes the contribution of A to the total enthalpy of the solution more positive; in other words, A–B interactions are associated with a positive enthalpy change. In such a case it may be said that A–B interactions are unfavourable in terms of enthalpy, i.e., endothermic. Since this is a purely thermodynamic approach, which makes no assumptions about the underlying molecular mechanism, derivatives similar to Eq. 3 may be applied to homogenous (A–A type) as well as heterogeneous (A–B type) interactions. In the following we will use such slopes, $\partial H_{\text{lipid}}^E/\partial m_{\text{ethanol}}$ and $\partial H_{\text{ethanol}}^E/\partial m_{\text{lipid}}$ (i.e., $H_{\text{alcohol-lipid}}^E$ or $H_{\text{lipid-alcohol}}^E$), to elucidate enthalpic effects of membrane–ethanol interactions.

4. Results

The results of adding ethanol to solutions of liposomes of different lipid composition at 26°C are shown in Fig. 2 (method I). In reasonable agreement with previous reports on mixtures with pure water [24,18], the partial enthalpy of ethanol in buffer is about −9.7 kJ/mol in the limit of infinite dilution. This value increases (i.e., becomes less negative) in solutions containing liposomes indicating endothermic liposome–ethanol interactions. The effect of liposomes on the partial enthalpy of ethanol depends strongly on the lipid composition. Cholesterol acts as to decrease H_{ethanol}^E from −9.2 kJ/mol in solutions

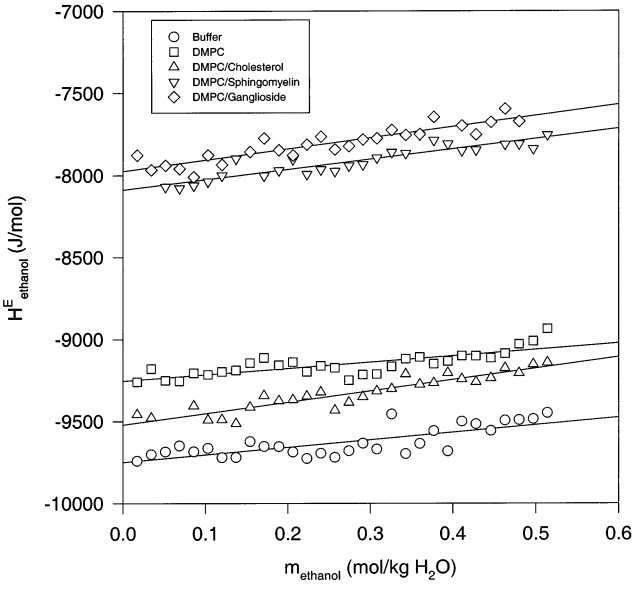

Fig. 2. The partial enthalpy of ethanol in solutions of liposomes of different lipid composition as a function of the ethanol molality, m_{Ethanol} at 26°C. (○) Buffer; (□) DMPC; (△) DMPC/cholesterol; (▽) DMPC/sphingomyelin; (◇) DMPC/ganglioside. The concentration of lipid in all liposome solutions are 100 mmol/kg H_2O.

[1] For convenience we will refer throughout to the excess partial molar enthalpy as the partial enthalpy.

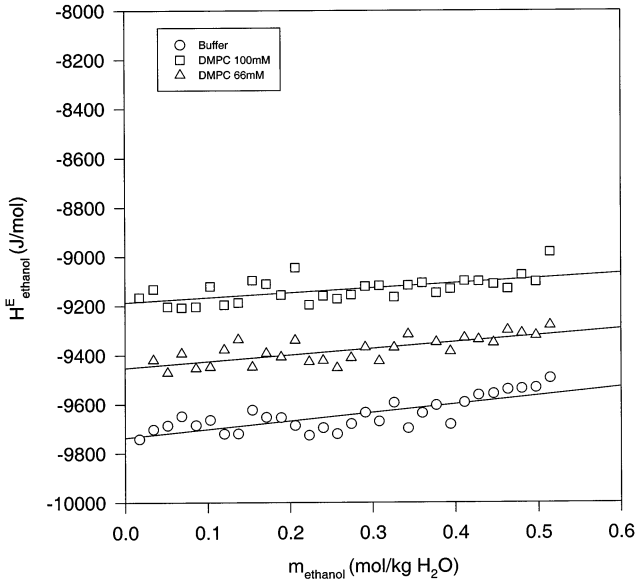

Fig. 3. Partial enthalpy of ethanol in solutions of pure DMPC liposomes as a function of the ethanol molality, m_{Ethanol}. (○) Buffer; (△) DMPC 66 mmol/kg H_2O; (□) DMPC 100 mmol/kg H_2O.

with pure DMPC liposomes to −9.5 kJ/mol in solutions with liposomes containing 30 mol% cholesterol. Conversely, incorporation of sphingomyelin or ganglioside to the DMPC bilayer (both 10 mol%) strongly increases H^E_{ethanol} to values of −7.9 and −8.1 kJ/mol, respectively. The linear fits to the data in Fig. 2 are nearly parallel. This suggests that the enthalpy of ethanol–liposome interaction is independent of the ethanol concentration over the range studied (0–500 mmol/kg H_2O). Thus, there is no sign of saturation of ethanol 'binding sites' in 100 mmol/kg H_2O liposome solutions corresponding to physiologically relevant ethanol concentrations. A similar behaviour is obtained from the data in Fig. 3 where the partial enthalpy of ethanol is plotted as a function of the ethanol concentration for different concentrations of pure DMPC liposomes. It appears that the large, negative enthalpy of ethanol becomes gradually less negative with increasing liposome concentration.

In Fig. 4 the partial enthalpy of ethanol in the limit of infinite dilution as a function of the lipid concentration is illustrated. The graphs were obtained by extrapolation of the linear fits in Fig. 3 and similar fits for the other lipid compositions (not shown) to $m_{\text{ethanol}} = 0$ and normalised to $H^E_{\text{ethanol}} = 0$ for $m_{\text{ethanol}} = 0$. The slope for all types of liposomes (DMPC, DMPC/cholesterol, DMPC/sphingomyelin, and DMPC/ganglioside) is positive, $H^E_{\text{ethanol-lipid}} > 0$, i.e., ethanol–liposome interactions are unfavourable in terms of enthalpy. In accordance with the results discussed above, this suggests that the well-documented association of ethanol with lipid membranes generates an increase in the enthalpy of the system (the associated complex is energetically less stable than the dissociated components). The slopes of the curves in Fig. 4, $H^E_{\text{ethanol-lipid}}$, may be considered as a measure of the average enthalpy of ethanol–liposome interactions. Slopes for the four investigated types of liposomes are listed in Table 1. For liposomes containing cholesterol the enthalpy of ethanol–liposome interaction is reduced by a factor of about 3 in comparison with pure DMPC liposomes. Incorporation of sphingomyelin or ganglioside in the liposomes, on the other hand, enhances the unfavourable interactions between liposomes and ethanol by a factor of about 3.

Enthalpic effects of liposome–ethanol interactions are further elucidated in Fig. 5, which shows the partial enthalpy of liposomes, H^E_{lipid}, as a function

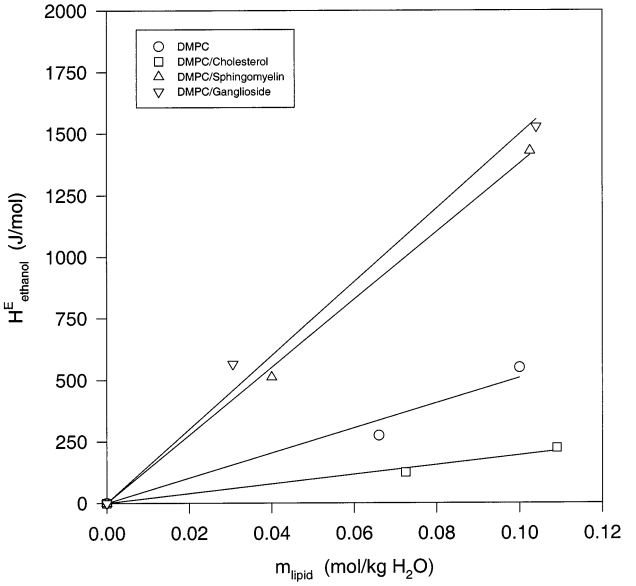

Fig. 4. Partial enthalpy of ethanol in solutions of liposomes of different composition as a function of the lipid concentration, m_{lipid}, obtained by method I. The enthalpies are calculated by extrapolating linear curves to graphs like those in Fig. 5 to $m_{\text{Ethanol}} = 0$. (○) DMPC; (□) DMPC/cholesterol; (▽) DMPC/ganglioside; and (△) DMPC/sphingomyelin.

Table 1

	% Bound ethanol at 100 mM liposome	K_p	$H^E_{ethanol-lipid}$ (kJ/mol^2)	$H^E_{lipid-ethanol}$ (kJ/mol^2)
DMPC	5.2	28 (29)	4.8	4.2
Cholesterol (30%)	2.3	12	1.9	1.7
Sphingomyelin (10%)	16	85 (83)	13.7	12
Ganglioside (10%)	18	87	14.9	10

Partitioning coefficients, K_p, and interaction functions, $H^E_{ethanol-lipid}$ and $H^E_{lipid-ethanol}$, for the four different lipid compositions studied.

of the alcohol concentration at 26°C (method II). For all four lipid compositions, transfer of liposomes from water into the most dilute ethanol solution is accompanied by an increase in the partial enthalpy of the liposomes. Hence, as pointed out in the data analysis section, the contribution of liposomes to the enthalpy of the entire solution is higher in dilute ethanol solution than in pure water. In other words, the interaction between liposomes and ethanol is unfavourable in terms of enthalpy, $H^E_{lipid-ethanol} > 0$. Consequently, any binding of ethanol to liposomes must be entropy driven in the range of temperature and composition studied. Comparison of Figs. 4 and 5 shows $H^E_{ethanol-lipid} \cong H^E_{lipid-ethanol}$ (see Table 1).

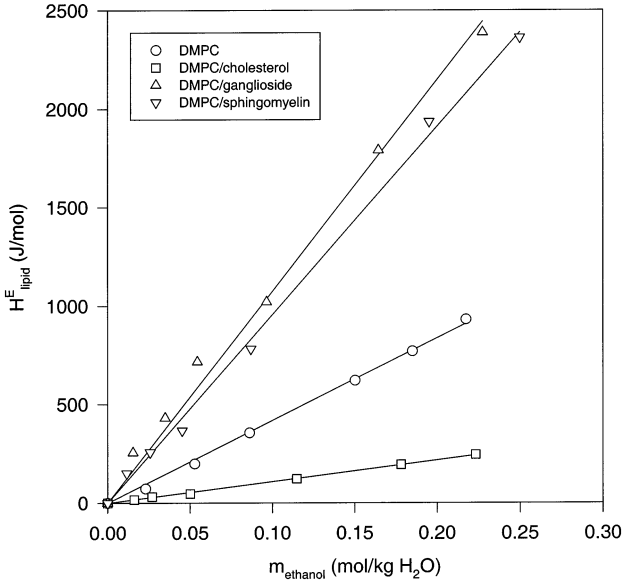

Fig. 5. Measured values of the partial enthalpy of liposomes of different lipid composition, H^E_{lipid} in ethanol solutions calculated according to Eq. 1 and plotted as a function of the ethanol molality, $m_{Ethanol}$. (○) DMPC; (□) DMPC/cholesterol; (△) DMPC/ganglioside; (▽) DMPC/sphingomyelin. The data are obtained by method II.

Clearly, for symmetry reasons, these two derivatives should be equal since they can both be expressed as $\partial^2 H/(\partial n_{lipid}\partial n_{ethanol})$, where H is the total enthalpy of the system. The fact that two independent methodologies (methods I and II) give the same result supports the consistency of the experimental methods.

Fig. 6a shows the titration calorimetry results obtained by applying the method of Zhang and Rowe [23]. Sixty-three mg/g (90.4 mM) DMPC suspensions containing 27.3 mg ethanol pr. gram solvent water (0.59 mmol/kg H_2O) was injected into the reaction cell, which contained different concentrations of ethanol from 10 mg/g to 40 mg/g (22 and 87 mmol/kg H_2O) at 26°C. Heat is released for ethanol concentrations below 26 mg/g (0.56 mmol/kg H_2O) and heat is absorbed for concentrations above 26 mg/g (0.56 mmol/kg H_2O). The null concentration (or free concentration of ethanol in the lipid suspension in the syringe) is therefore at 26 mg/g. The amount of ethanol partitioned into the DMPC bilayer is determined by subtraction of the free concentration from the total concentration, and the partitioning coefficient is calculated from

$$K_p = X_L/X_W \quad (4)$$

where X_L is the mole fraction of ethanol in the lipid phase and X_W is the mole fraction of ethanol in the solvent. This gives a partitioning coefficient of $K_p = 29$. In a similar fashion $K_p = 83$ is determined for the partitioning of ethanol into DMPC/sphingomyelin bilayers (10 mol%) in Fig. 6b.

5. Discussion

The main purpose of the present study is to discuss molecular interactions between ethanol and model

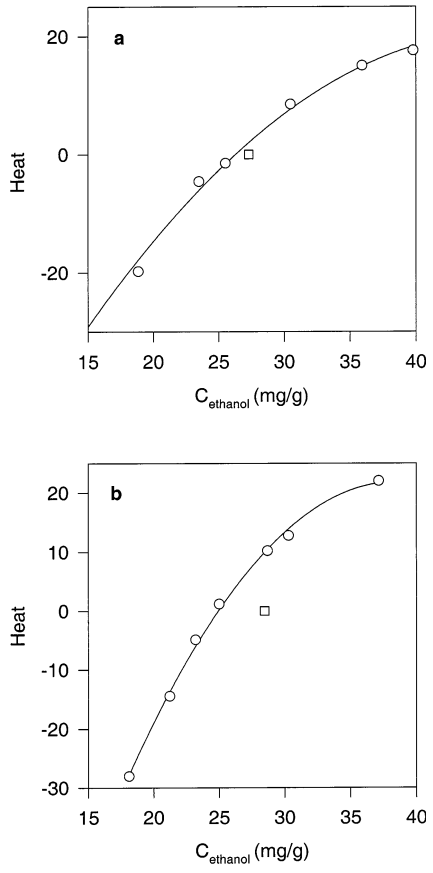

Fig. 6. Measured values of the heat of reaction when (a) DMPC suspensions containing 27.3 mg ethanol/g buffer is injected into the sample cell which contained different concentrations of ethanol solutions from 15 to 40 mg/g at 26°C; (b) DMPC/sphingomyelin suspensions containing 28.47 mg ethanol/g buffer is injected into the sample cell which contained different concentrations of ethanol solutions from 15 to 40 mg/g at 26°C. The squares (□) in each figure indicates the concentration of ethanol in mg/g solvent buffer in the liposome suspensions in the syringe.

membranes as well as to determinate partitioning coefficients of ethanol into lipid bilayers even at physiological relevant ethanol concentrations. The discussion will be based on estimated values of the interaction functions, Eqs. 2 and 3. The results demonstrate that calorimetry can be an effective tool for the investigation of weak binding of solutes to macromolecules.

In Table 1 interaction functions, $H^E_{\text{alcohol-lipid}}$, signifying the overall enthalpic effect of ethanol–liposome interactions are summarised. The data show that $H^E_{\text{alcohol-lipid}}$ depends strongly on the lipid composition. Incorporation of 30% cholesterol in the DMPC bilayer reduces the interaction function by a factor of 2–3. This result suggests that the interaction enthalpy of ethanol with DMPC liposomes is significantly reduced by the addition of cholesterol to the bilayer. This reduction could either be due to changes in the standard enthalpy of association or to reduced affinity of the bilayer for the alcohol. Conversely, addition of 10% sphingomyelin or ganglioside to the bilayer increase the interaction functions by a factor of 3, suggesting an enhanced affinity of ethanol for the bilayers. Since both sphingomyelin and ganglioside have a ceramide backbone, the difference in the way these two lipids respond to ethanol compared to pure DPMC bilayers, indicates that ethanol interaction with the liposomes may be sensitive to the bilayer surface configuration. Previous structural studies [11,25] have shown that the binding of ethanol to lipid–bilayer surfaces is enhanced by gangliosides, presumably due to the hydrophilic sugar residues located in the head group, whereas the effect of sphingomyelin was found to be only negligible [11]. In contrast to the calorimetric experiments where dilute aqueous lipid suspensions are used, NMR experiments are traditionally performed with only a small excess of bulk water. These differences in sample preparation may lead to a different distribution and position of ethanol in the lipid bilayer.

The membrane to water partitioning coefficient is the equilibrium constant for the distribution of a solute between the membrane and aqueous phases. The partitioning of ethanol into the lipid bilayer has been studied by several direct and indirect methods, including differential scanning calorimetry [26], radioactive labelling [27,28] and fluorescence spectroscopy [14]. Recently, Rowe and co-workers [23,29] introduced titration calorimetry for the measurement of partitioning coefficients of long chain alcohols into lipid bilayers. The simplest and most commonly used approach for a thermodynamic characterisation of the process is based on the assumption that alcohol can be in only two states, bound or in aqueous solution. In a recent work [21] we applied titration calorimetry to the study of molecular interactions of small chain alcohols with pure DMPC bilayers. One of the essential results of this work was that the variation of enthalpy for the process of transferring alcohol from water into the lipid bilayer was

found to be of same magnitude but opposite sign as the enthalpies of transferring alcohol from the pure liquid into buffer. This indicates that dehydration of the alcohol upon association with the membrane governs the energetics of the interaction. In other words, partitioned alcohol has approximately the same enthalpy as pure liquid alcohol. Based on this result and the partitioning model mentioned above, we have used the data in Figs. 4 and 5 to estimate partitioning coefficients of ethanol into liposomes of different lipid composition. The amount of ethanol bound to liposomes is simply calculated as the relative reduction in the partial enthalpy of ethanol in solutions of liposomes. In the case of pure DMPC the data in Fig. 4 thus indicate that approximately 5% of the total amount of ethanol molecules have been removed from the buffer upon an increase in the liposome concentration from 0 to 100 mmol/kg H_2O. Under this assumption the partitioning coefficient of ethanol into pure DMPC bilayers is estimated to be 28. This value is in reasonable agreement with results of radiolabelled tracer studies by Katz and Diamond [30] who reported $K_p = 17$. Partitioning coefficients for ethanol into mixed lipid bilayers based on the data in Figs. 4 and 5 were found to be 12, 85 and 87, for DMPC/cholesterol, DMPC/sphingomyelin and DMPC/ganglioside, respectively (see Table 1). The applicability of methods I and II to estimate K_p values was further tested by the calorimetric technique introduced by Zhang and Rowe [23]. By this technique, partitioning coefficients are calculated from estimates of the free (bulk) ethanol concentration in a liposome suspension and do not rely on any assumptions about the enthalpy of the partitioned solute molecule. The observation that this method yielded K_p values very close to those estimated from Figs. 4 and 5 (see the values in the brackets in Table 1) further supports the validity of the assumption that the enthalpy of a partitioned ethanol is similar to that of neat ethanol. One of the advantages of methods I and II introduced in this study is that they allow for the determination of partitioning coefficients even at very low (physiological relevant) ethanol concentrations. This can be seen, for example, in Fig. 5 where the slope of the linear fits (i.e., the value of $H^E_{\text{lipid}-\text{alcohol}}$) can be reasonably quantified at ethanol concentrations down to 20–40 mM (0.1–0.2%).

Table 1 lists the partitioning coefficients estimated from Fig. 4 for the four different liposome systems examined in this study. Comparison of the chemical structure of the classes of lipids used in this study can provide some indication of the location of partitioned ethanol in the lipid bilayer. The partitioning coefficient of ethanol into bilayers containing sphingomyelin or ganglioside is three times as large as for pure DMPC bilayers. All three lipids have two fatty acid chains. Sphingomyelin and DMPC on the one hand have the same choline head group, but differ by having a ceramide and a glycerol backbone, respectively. Sphingomyelin and ganglioside, on the other hand, have different head groups but the same backbone. The observation that the two ceramides have high, approximately equal K_p values, while no correlation between K_p and the chemical nature of the head groups can be established suggests that ethanol interaction with lipid bilayers may be sensitive to the bilayer interface configuration.

The composition dependence of ethanol interaction with bilayer preparations suggests the potential importance of a complex interplay between ethanol association and membrane composition and structure. Ample precedence exists in the literature of this relationship. It is well known that cholesterol acts as a natural antagonist against the perturbing effect of ethanol on the physical properties of the membrane [31], and that gangliosides sensitise synaptic plasma membranes to the effect of ethanol [25]. In addition, an enhanced content of cholesterol reported in membranes in in vivo experiments after chronic ethanol treatment has been reported [32]. Our results show a strong correlation to in vivo studies in the sense that cholesterol on the one hand reduces the sensitivity of the membrane to ethanol, and ganglioside, on the other hand, enhances this sensitivity.

The thermodynamics and the temperature dependence of ethanol partitioning into lipid bilayers are important aspects of ethanol–membrane association, and a well-established way of evaluating alcohol–membrane interactions relies on an assumption where the partitioning coefficient is considered being the equilibrium constant for the distribution of a solute between the aqueous phase and a homogeneous lateral bilayer, respectively. However, there might be some limitations to this approach. First,

the structural state of the lipid system, and hence the partitioning coefficient, is dependent on the temperature. Experimental observations have shown that the passive ion permeability as well as the partitioning of small organics are enhanced in the vicinity of the main gel to fluid phase transition [33], and computer simulation studies have demonstrated that the formation of heterogeneous lateral bilayers composed of fluctuating lipid domains at temperatures close to the main phase transition enhances the partitioning of small molecules [34]. The partitioning equilibrium is linked to such structural equilibria in the lipid membrane (i.e., the measured enthalpy is a product of the number of partitioned alcohol molecules and the enthalpy of partitioning). Hence, temperature-induced changes in $H^E_{\text{lipid}-\text{alcohol}}$ cannot be directly interpreted as signifying ΔC_p of the partitioning process as the case would be for partitioning of small organics between oil and water [24]. This has recently been illustrated [21,35] in a study of the partitioning of small alcohols into pure DMPC bilayers. It was found that $H^E_{\text{lipid}-\text{alcohol}}$ decreases strongly in a non-linear fashion with increasingly temperature from 26°C to 40°C [35]. This reflects primarily structural changes in the membrane leading to an enhanced affinity of the bilayer for alcohols at temperatures close to the phase transition temperature [36]. Hence, measured thermal effects signify a complex interplay between several linked equilibria and cannot be readily described to a simple model comprising only one equilibrium constant (K_p). Thermodynamic parameters will differ from those found, just assuming that the association process is equivalent to the partitioning into bulk non-polar phases.

We have discussed the association of ethanol with lipid bilayers of different natural membrane components and shown that compositional differences in the membranes alter the membrane–ethanol interaction. The influence of different lipids on bilayer response to ethanol has been reported before [11,31], and an interesting hypothesis to further explore concerns the possible long-term adaptive response in the composition of the membranes during long-term administration of alcohol. Such a change in the lipid composition might be regarded as an attempt to compensate the perturbing effect of ethanol on the membrane properties, trying to keep a balance between order and function.

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