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Partitioning behaviour of 1-hexanol into lipid membranes as studied by deuterium NMR spectroscopy

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Deuterium nuclear magnetic resonance (NMR) spectroscopy was used to study the partitioning behaviour of 1-hexanol specifically deuterated in the α-position into model lipid bilayers. In all systems studied, the observed deuterium NMR lineshapes were time-dependent. Initially, 1-hexanol-d_2 gave rise to an isotropic deuterium resonance with a different chemical shift from that of aqueous 1-hexanol-d_2. After equilibration over a period of days, a broader spectral component characteristic of a spherically-averaged powder-pattern was observed. The quadrupole anisotropy of the 1-hexanol-d_2 giving rise to the broad spectrum depended upon the cholesterol content of the membrane. From quantitation of the anisotropic to isotropic deuterium NMR spectra, the partition coefficients of 1-hexanol-d_2 in a number of bilayer systems (asolectin and phosphatidylcholine bilayers (the latter with and without cholesterol)) were determined. The partitioning of 1-hexanol-d_2 into red blood cell membranes, and a suspension of lipids extracted from red blood cell membranes, was also examined. It is suggested that 1-hexanol, and probably other lipophiles, can partition to either the bilayer surface or the bilayer interior in a time-dependant manner.

Introduction

There is little information on the molecular mechanisms by which lipophiles partition into bilayer membranes. An understanding of this mechanism and disposition of lipophiles in membranes is a necessary requirement for the analysis at a molecular level of biological phenomena such as anaesthesia, drug–membrane interactions and membrane solubilization. The hypotheses by which lipophilic anaesthetics, general and local, produce physiological effects are constantly subject to challenge by experiment and speculation [1–5].

Frequently, the only information about the behaviour of a lipophile in a membrane-water system is the magnitude of its partition coefficient determined by centrifugation and filtration methods [6]. In some cases, fluorescence can be used if the lipophile possesses an intrinsic fluorophore [7]. Changes in the order of the bulk membrane produced by partitioning can be revealed by spin-labelled lipids probing the hydrophobic interior of the membrane [8].

The published values of partition coefficients (P) for various compounds differ greatly in the literature depending on the membrane system into which they are partitioned, the temperature, the ionic strength and pH of the aqueous phase and the method of determination. In addition, the explicit definition of 'partition coefficient' gives a value which is dimensionless since it is a ratio of like quantities. The most common definition employed in the literature is the ratio of the molarity of compound in the lipid phase to the molarity of compound in the aqueous phase [6]. However, other definitions used include expressions to describe solute concentration, such as mole fraction [9,10], molality [11] and Langmuir absorption isotherm behaviour [12]. For example, Kamaya et al. [10] quote a value for P of 23 in terms of mole fraction of hexanol in DPPC bilayers, but employing the definition in terms of molarities, the value of P is 963 in the same system.

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Many methods for determining partition coefficients suffer from shortcomings, such as the presence of membrane-associated water not being available as solvent to the lipophile and absorption of lipophiles to glass and apparatus [6] or depletion of compounds with high relative values of P (such as the long chain alcohols) into the membranes, leaving small amounts of radioactive impurities to be determined in the supernatant [13]. If the lipophile is ionizable, its partitioning behaviour may depend on bulk pH. For example, P for tetracaine in DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine)/cholesterol (7:3, mol/mol) is 110 at pH 9.5 (uncharged form) and 8 at pH 5.5 (charged form), where the buffer was 0.1 M NaCl/10 mM phosphate [14]. A value of P for 1-hexanol in DPPC (1,2-palmitoyl-*sn*-glycero-3-phosphocholine) bilayers in water has been reported to be 23, with P for 1-hexanol in egg phosphatidylcholine bilayers being approx. 70 [15].

Few determinations of P have relied on non-perturbing or relatively non-perturbing methods. An example is that of Frezzati et al. [16], where the ratio of two electron paramagnetic resonance (EPR) linewidth parameters of a spin-labelled derivative of a local anaesthetic was used as a measure of the partition coefficient of the labelled anaesthetic, together with the decrease in EPR spectral linebroadening of spin-labelled phosphatidylcholine (12-PCSL) by partitioning tetracaine. In another EPR study, partition coefficients determined optically were compared with changes in the EPR spectrum of spin-labelled local anaesthetic [17]. Optical methods relying on a shift in absorbance maximum of a lipophile have occasionally been reported [18]. Recently, NMR studies have reported on the orientation and partitioning of lipophilic (and anaesthetic) gases such as methoxyfluorane [19,20]. Information on the disposition of alcohols in lipid bilayers has been provided by a deuterium NMR study of the interactions of a range of deuterated primary alcohols with DMPC membranes [21]. Deuterium and phosphorus-31 NMR may be used to follow perturbations of DPPC and DPPE by local anaesthetics [12,22]. Other deuterium NMR studies have been performed using ethanol-d_6 added to sonicated DPPC vesicles [7]. The changes in ^1H resonances of lipid molecules in rat brain neuronal membranes upon addition of ethanol [23] have also given rise to estimates of the partition coefficient and effects of ethanol on membrane 'order'. Paramagnetic probe ions such as the lanthanide ion, Pr^{3+}, have also been employed to investigate anaesthetic-induced perturbations in membrane structure [24]. However, a single, widely applicable magnetic resonance method has not been described to date.

Here, a novel, non-perturbing method for determination of the partition coefficient of 1-hexanol in lipid bilayers is presented. Deuterium nuclei are incorporated chemically into the lipophile of interest, leading to minimal chemical and physical differences between deuterated and non-deuterated molecular species. From the nature of the spectral lineshapes in deuterium NMR spectra of deuterated 1-hexanol-d_2 added to various membrane dispersions, estimates can be made of the partitioning behaviour and partition coefficient of the deuterated lipophile. The method may have general application to drugs, anaesthetics and other membrane active molecules.

Methods

Synthesis of a specifically deuterated 1-hexanol. Hexanoic acid was reduced with sodium borodeuteride in diethyl ether at room temperature. The final reaction product, [1,1-^2H]hexan-1-ol (abbreviated henceforth as 1-hexanol-d_2, was pure upon distillation (as determined by thin-layer chromatography (TLC) and proton NMR) and was specifically deuterated (as determined by loss of the α-proton resonance in the proton NMR spectrum).

Preparation of lipid vesicles. DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine), DMPC, cholesterol and asolectin (Sigma Chemical Co. and Lipid Products, U.K.) were used without further purification. Thin films of lipid were prepared by drying down a solution of the appropriate lipid or lipid mixtures in chloroform/methanol (2:1, v/v) using dry nitrogen. Residual solvent was removed under high vacuum (3 h; room temperature).

Large multilamellar vesicles (LMV) were prepared by dispersion of a thin lipid film in deuterium-depleted phosphate-buffered saline (PBS) buffer (50 mg for 1ml). The PBS had the following composition; 150 mM NaCl, 10 mM Na_2HPO_4 in deuterium-depleted water, adjusted to pH 7.4). Large unilamellar vesicles (LUV) were prepared by subjecting LMVs to six freeze-thaw cycles.

Red blood cell lipids were obtained from red cell ghosts which were pre-extracted with Triton X-100 to remove band 3 protein. Residual detergent was removed by incubation of the membranes with Bio-Beads SM-2 (a Biorad product). The membranes were then subjected to a Folch-Lees acid extraction into chloroform/methanol (2:1, v/v). Both LMVs and LUVs were prepared from this lipid as described above.

Preparation of red blood cells. Intact red blood cell membranes were prepared from outdated human blood. The coagulation factors and lymphocytes were removed by washing, centrifugation and resuspension in isotonic PBS buffer (four times). Residual HDO was removed by washing the cells in deuterium depleted PBS buffer (four times).

Sample preparation. The appropriate amount of undiluted 1-hexanol-d_2 was rapidly added to membrane suspensions which were then sealed directly in

10-mm diameter NMR tubes (with no air space above the suspension), vortexed briefly (30 s) and allowed to equilibrate at 4°C. Prior to the recording of NMR spectra, a given sample was equilibrated for 30 min at the temperature at which the spectrum was to be acquired.

Deuterium NMR. Spectra were recorded on a home-built 360 MHz (^{1}H) spectrometer, equipped with a Nicolet processor. The spectra were acquired with a single pulse experiment using a high power amplifier, 900 pulse of approx. 7 ms (10 mm insert) or a quadrupole echo sequence. The FID were Fourier transformed using a line broadening of 50 Hz. The temperature was controlled to an accuracy of 1 C° with a Bruker variable temperature control unit and unless otherwise stated, a temperature of 30°C was set.

Results

Deuterium NMR spectra of 1-hexanol-d_2 added to large multilamellar vesicles (LMVs) of DMPC

For comparison with later results, the deuterium NMR spectra of 1-hexanol-d_2 in aqueous buffer was first recorded (Fig. 1a). A sharp isotropic resonance was observed, with a width at half-height of 50 Hz. The deuterium NMR spectra of large multilamellar (LMV) DMPC vesicles were recorded at various times after addition of 1-hexanol-d_2 (1-hexanol-d_2/DMPC mol/mol ratio of 1:2) at 30°C. The spectral lineshapes varied with time. At timepoints longer than 15 min after the addition of 1-hexanol-d_2 to LMVs, the deuterium NMR spectrum comprised two isotropic resonances with a difference in chemical shift of approx. 150 Hz (Fig. 1b). The highfield resonance was identical to that observed immediately after addition of 1-hexanol-d_2 to the dispersion, with a linewidth at half-height of 50 Hz, whilst the low-field resonance was broader with a width at half-height of 100 Hz.

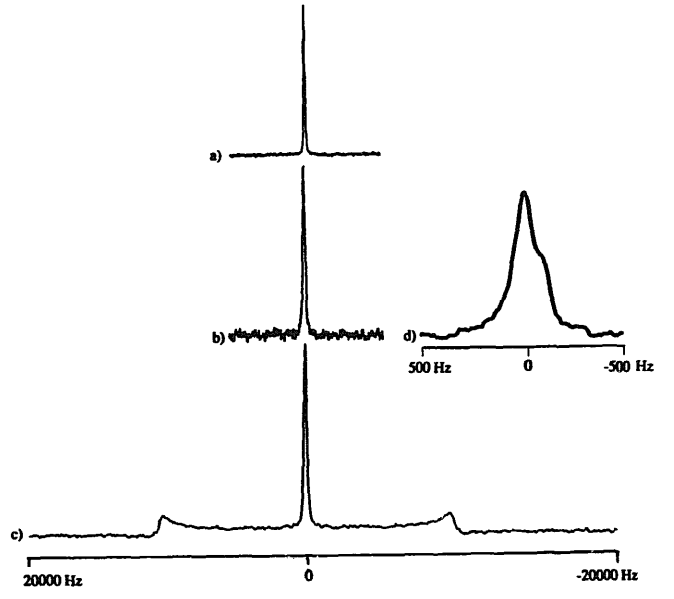

Fig. 1. Deuterium NMR spectra of 1-hexanol-d_2; (a) in PBS buffer alone and (b–d) after 1-hexanol-d_2 addition to an aqueous dispersion of DMPC LMVs (hexanol/DMPC (mol/mol) ratio of 1:2); (b) 15 min after addition to the dispersion; (c) after incubation of the dispersion for 72 h at 4°C and (d) an expansion of the central region of spectrum (b). All spectra were recorded at 30°C.

After 72 h incubation of 1-hexanol-d_2 with DMPC LMVs, the NMR spectral lineshape lineshape of the central region of the spectrum had changed. The low-field isotropic resonance decreased in intensity, almost to zero, whilst the intensity of the high-field isotropic resonance remained constant. In addition, the deuterium spectra revealed the presence of a third component which resembled a spherically-averaged powder pattern resonance for deuterium undergoing fast local motion [25] (Fig. 1c). The quadrupole splitting of this spectral component was approx. 19 kHz.

The resolution of such a multicomponent deuterium NMR spectrum from 1-hexanol-d_2 in DMPC LMVs indicates that 1-hexanol-d_2 is in slow exchange (on the deuterium NMR quadrupole anisotropy averaging timescale, $\nu_{ex} < 10^4$ Hz) between motionally distinct environments. An upper limit for this exchange rate may be estimated [26] from the spectral separation of the isotropic and powder pattern resonances ($\nu_{ex} \approx 8$ kHz). The broad spectral component has the same chemical shift as the high-field isotropic component since it is axially symmetric around this isotropic resonance. No further changes in the deuterium NMR lineshapes were observed with time.

One complication of our method could be that not all the intensity of the powder-pattern component, which has a shorter T_1 relaxation time than the isotropic component, may be observed in a single pulse experiment due to the initial receiver delay (12 μs). However, the use of a solid state quadrupole echo sequence with a dwell time of 1 μs did not give significantly different spectral lineshapes or intensities.

The ratio of intensities of the isotropic to broad spectral components was used to determine the partition coefficient for 1-hexanol-d_2 in DMPC vesicles. It is possible to quantify the relative proportions of isotropic and powder-pattern spectral components by integration of the spectral intensity of each component as a result of the good separation of the resonances. This approach is only valid when the exchange rate between components is judged to be slow ($\nu_{ex} < 10^4$ Hz) on the deuterium NMR timescale. The central isotropic resonances were deconvoluted to determine the proportions of membrane-absorbed and aqueous 1-hexanol-d_2. It was found that the component corresponding to absorbed 1-hexanol-d_2 decreases in intensity after 72 h. The molar partition coefficient, P_{mol} was defined as the ratio of absorbed (with spectral intensity P_{abs}) and partitioned hexanol (with spectral

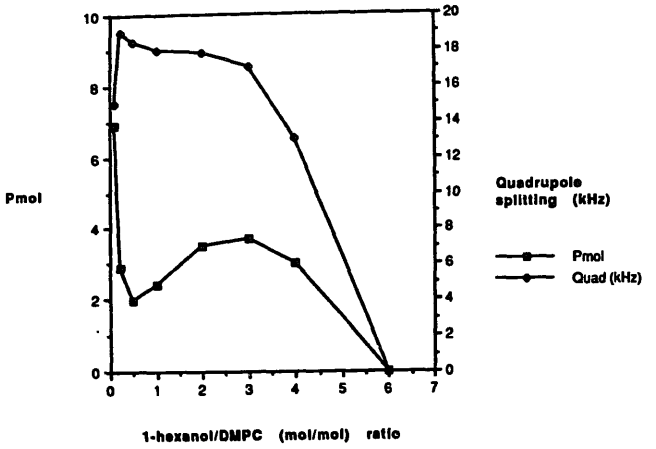

Fig. 2. Graph showing the relationship of P_{mol} (□) (ratio of powder-pattern resonance intensity to isotropic resonance intensity) and quadrupole splitting (♦) of powder-pattern resonance for 1-hexanol-d_2 added to DMPC LMV dispersions with varying 1-hexanol-d_2/DMPC (mol/mol) ratio. P_{mol} was estimated as described in Results. All spectra were recorded at 30°C.

intensity P_{part}) to aqueous hexanol (with intensity P_{aq}) as judged from the relative intensities in the deuterium NMR spectrum of 1-hexanol-d_2 added to a suspension of lipid. That is, $P_{mol} = (P_{abs} + P_{part})/P_{aq}$. For 1-hexanol-$d_2$ added to a hexanol/DMPC mol/mol ratio of 1:2, P_{mol} was determined to be 2.0 after 72 h incubation at 4°C.

A range of values for P has previously been offered for 1-hexanol in a series of systems (see Introduction). Our values for P_{mol}, in terms of mole fractions, may be related to P in terms of molarities by multiplication by a factor of 20; in our samples, the volume occupied by lipid molecules is approximately 20 times less than the aqueous volume. Thus, we would quote a value for P_{mol} of 7 for 1-hexanol-d_2 added to DMPC LMVs (with a 1-hexanol/DMPC mol/mol ratio of 1:10 (approximately 1 mM 1-hexanol)) giving a value for P of approx. 140, which falls in the range of previous estimates.

The effect of varying hexanol / lipid (mol / mol) ratio on the deuterium NMR spectrum of 1-hexanol-d_2

Various amounts of 1-hexanol-d_2 were added to DMPC LMVs to give varying final hexanol/DMPC (mol/mol) ratios (H/L ratios). Both the proportion and quadrupole splitting of the powder-pattern component changed with H/L ratio (Fig. 2). The molar partition coefficient, P_{mol}, was found to decrease as H/L ratio increased, to a point (H/L > 4:1) where the lipid vesicles were observed macroscopically to have solubilised. Deuterium and phosphorus-31 NMR spectra of samples with high H/L ratios showed only an isotropic lineshape. It is suggested that hexanol solubilised the lipid bilayer at and above this H/L ratio. At H/L ratios approaching solubilisation, the variations in P_{mol} suggested that phase changes of the lipids occurred. The phosphorous-31 NMR lineshape characteristic of lipids in the bilayer state was progressively distorted to a more isotropic lineshape, characteristic of micellar lipid [27].

Deuterium NMR spectra of 1-hexanol-d_2 added to large unilamellar vesicles (LUVs) of DMPC

Large unilamellar vesicles (LUVs) of DMPC exhibit similar time-dependent deuterium NMR spectra upon addition of 1-hexanol-d_2 (spectra not shown). Initially after addition of 1-hexanol-d_2, two isotropic signals were observed. The low-field resonance was ascribed to membrane associated 1-hexanol-d_2 while the high-field resonance was ascribed to aqueous 1-hexanol-d_2. The relative intensities of the two signals could be quantified by integration as above, leading to a ratio equivalent to P_{mol} (moles membrane-associated hexanol relative to aqueous hexanol) but prior to equilibrium. This value was determined to be 12.8 for 1-hexanol-d_2 measured at 3 h after addition to LUVs of DMPC. A value of 4.9 for LMVs of DMPC was determined under similar conditions. After 72 h, a broad spectral component was present with a quadrupole anisotropy of approx. 20 kHz, which resembles strongly the spectral lineshape observed from 1-hexanol-d_2 in DMPC LMVs at equilibrium. At this point P_{mol}, the molar partition coefficient defined above, was determined to be 2.2 which is similar to that observed in the equivalent situation with DMPC LMV's (2.0).

Deuterium NMR spectra of 1-hexanol-d_2 added to large multilamellar vesicles (LMVs) of other lipid types

Deuterium NMR spectra were also recorded for hand-shaken lipid dispersions of DOPC (with and without cholesterol) and asolectin (Fig. 3). Time-dependent deuterium NMR spectral line-shapes were observed. The quadrupolar anisotropy of the motionally restricted 1-hexanol-d_2 varied with membrane lipid composition. The quadrupolar splitting of the broad powder-pattern spectral component varied for different lipid types (Table I). All the experiments were performed at similar temperatures (25–37°C), well above the respective lipid membrane gel-to-liquid crystalline phase transition temperatures.

To investigate whether the cholesterol content of a membrane influences the quadrupolar splitting of the membrane-partitioned 1-hexanol-d_2, additional experiments were performed using large multilamellar vesicles (LMV) of DOPC containing 25 and 50 mol% cholesterol (Figs. 3c and d). The quadrupolar splitting of the broad powder pattern spectral component is reduced from 17 kHz for pure DOPC bilayers to 16 kHz for DOPC bilayers with 25 mol% cholesterol and was completely collapsed in the presence of 50 mol% of cholesterol. By integration of the spectral lines, the

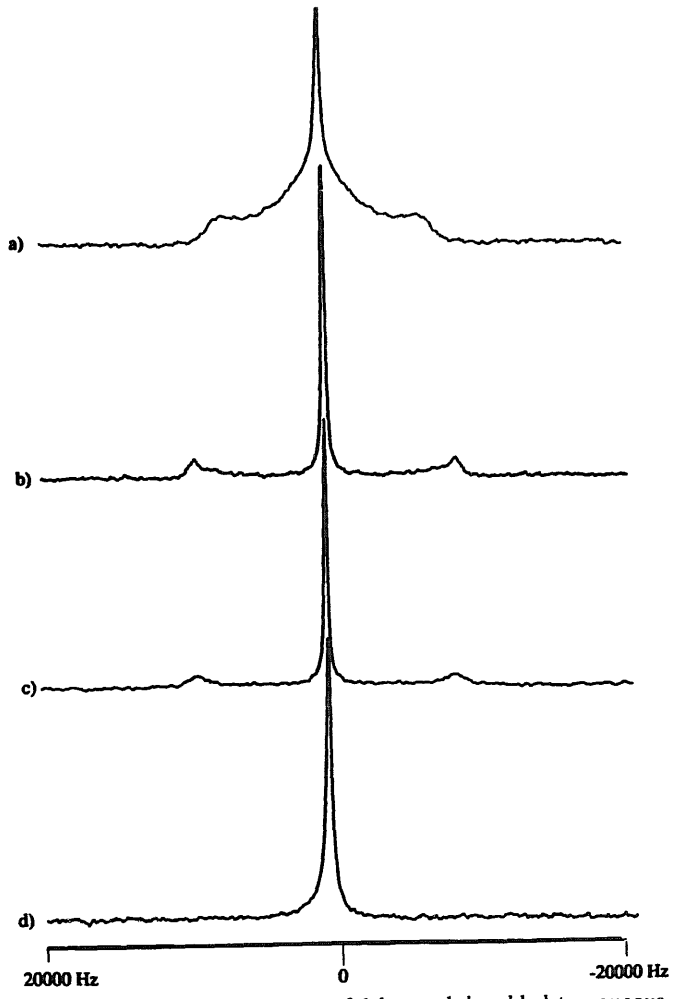

Fig. 3. Deuterium NMR spectra of 1-hexanol-d_2 added to aqueous dispersions of (a) asolectin (b) DOPC (c) DOPC with 25 mol% cholesterol and (d) DOPC with 50 mol% cholesterol. The hexanol/lipid (mol/mol) ratio in each case was 1:2 and all spectra were recorded at 30°C. All dispersions were LMVs.

TABLE I

Quadrupolar splitting of the broad powder-pattern component

Data derived from deuterium NMR spectra recorded at 30°C of 1-hexanol-d_2 added to aqueous suspensions of lipid (in all cases, hexanol/lipid (mol/mol) ratio of 1:2)). All suspensions were LMVs unless otherwise stated. P_{mol} is defined in the text. (n.d.; not determined due to spectral overlap of component).

Vesicle type	P_{mol}	Quadrupole splitting (kHz) of powder-pattern component
DMPC (LMVs)	2.0	19.0
DMPC (LUVs)	2.2	19.0
DOPC	3.0	17.0
DOPC + 25% cholesterol	3.2	16.0
DOPC + 50% cholesterol	n.d.	≈ 0
Asolectin	6.0	8.0
Red blood cells	n.d.	≈ 0
Red blood cell lipids	n.d.	≈ 0

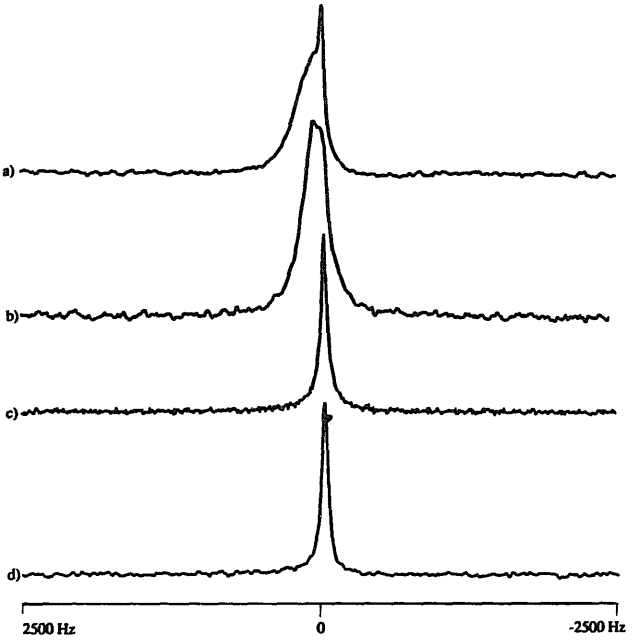

Fig. 4. Deuterium NMR spectra of 1-hexanol-d_2 added to suspensions of human red blood cells to a concentration of 6 and 16 mM ((a) and (b), respectively), and added to an aqueous dispersion of extracted human red blood cell lipids, to a concentration of 6 and 16 mM ((c) and (d), respectively). All spectra were recorded at 37°C.

partition coefficient of 1-hexanol-d_2 in DOPC bilayers with 25 mol% cholesterol (3.2) was very similar to the value for pure DOPC bilayers (3.0). This value could not be determined in DOPC bilayers with 50 mol% of cholesterol, due to the close overlap of resonances.

Deuterium NMR spectra of 1-hexanol-d_2 added to a suspension of human red blood cells and extracted red blood cell lipids

The methods described above were applied to study the partitioning of 1-hexanol-d_2 into human red blood cells and suspensions of extracted red blood cell membrane lipids. Typical deuterium NMR spectra, recorded at 37°C, of red blood cells with various amounts of 1-hexanol-d_2 are shown in Fig. 4(a) and (b). The presence of residual HDO after four centrifugation cycles is not significant (spectra not shown). When 1-hexanol-d_2 was added to RBC to a concentration of 6 mM, two isotropic spectral components were observed with different chemical shifts. By similar reasoning to that employed above for DMPC, the low-field resonance was ascribed to membrane-partitioned 1-hexanol-d_2. The degree of motional freedom of this population of 1-hexanol-d_2 molecules must be much less than that experienced by the equivalent population of 1-hexanol-d_2 in DMPC, as a spectral component resembling a spherically-averaged powder pattern is not observed. Larger amounts of 1-hexanol-d_2 (to 16 mM) produce an increase in the width of this low-field resonance in the deuterium NMR spectra with respect to the high-

field signal. The ratio of high- to low-field signal intensity was also changed upon the addition of 1-hexanol-d_2 to 16 mM but the value could not be estimated by integration across the spectrum, as the spectral overlap of the two resonances was too great. An accurate estimate for the partition coefficient of 1-hexanol-d_2 in red blood cells could not therefore be evaluated. Spectra of 1-hexanol-d_2 added to suspensions of extracted red blood cell lipids (Fig. 4(c) and (d)) were also recorded. Spectral overlap again prevented the derivation of P_{mol}, although the spectral line-shapes were clearly different from those obtained in equivalent samples of red blood cell suspensions. It is possible that spectral deconvolution techniques could be applied to such data.

Discussion

The dynamic properties of 1-hexanol-d_2 in an aqueous suspension of lipid may be inferred from the deuterium NMR spectral lineshapes. Assignment of the three observed spectral resonances to molecular environments for distinct populations of hexanol molecules is suggested.

On addition of 1-hexanol-d_2 to an aqueous suspension of lipid vesicles, the isotropic spectral component immediately observed by deuterium NMR is similar to that observed for 1-hexanol-d_2 in aqueous buffer at a concentration of 2 mM (Fig. 1a). The resonance, with a half-width of approx. 50 Hz, is ascribed to 1-hexanol-d_2 in a highly mobile environment on the deuterium NMR anisotropy averaging timescale and exchanging slowly with any partitioned 1-hexanol-d_2 in the bilayer core, with an exchange rate ($\nu_{ex} < 10^4$ Hz). All intensity observed in the spectra must be due to 1-hexanol-d_2; the presence of any significant amount of HDO was excluded because the lipids were dried under high-vacuum and redissolved in deuterium depleted PBS buffer solution (pH = 7.4). Thus, as this isotropic resonance persists in the fully equilibrated sample of 1-hexanol-d_2 with DMPC large multilamellar vesicles (LMVs) it may ascribed to 1-hexanol-d_2 remote from the membrane, in aqueous solution.

The lowfield isotropic spectral component observed at later timepoints (1–72 h) after addition of 1-hexanol-d_2 to DMPC LMVs, with an increased linewidth (width at half-height of 100 Hz) relative to the high-field signal, may be ascribed to 1-hexanol-d_2 in a different chemical environment to that 1-hexanol-d_2 in aqueous solution (hence the shift) and with less motional freedom (hence the broadening). Such a lineshape might originate from hexanol molecules absorbed onto the membrane surface, perhaps in a loose association with the polar lipid headgroups. The difference in chemical shift of this less mobile component could be due to the less efficient dipolar shielding that deuterium nuclei near or in the lipid bilayer would experience relative to an aqueous environment. However, the isotropic nature of the resonance indicates that hexanol molecules in weak association with the membrane have a high degree of motional freedom. From the two separate deuterium NMR resonances, it is suggested that 1-hexanol-d_2 is in slow exchange ($\nu_{ex} < 10^4$ Hz) between membrane-absorbed and aqueous environments and that this phenomenon is most clearly observed by deuterium NMR at time points prior to full equilibration. The low-field isotropic resonance is absent or insignificant in fully equilibrated samples.

The third spectral component, that resembling a spherically averaged powder-pattern, is ascribed to 1-hexanol-d_2 which has partitioned into the lipid bilayer. From previous studies, it has been suggested that the short aliphatic chain of hexanol is intercalated into the hydrophobic domain of the membrane [21] whilst the hydroxy group resides at the level of the glycerol backbone of the lipid molecules forming the bilayer. The well-resolved powder-pattern component observed after 72 h indicates that those molecules of deuterated hexanol which have partitioned into the lipid bilayer are restricted in their amplitude of motion but moving quickly within this environment on the deuterium NMR timescale. Additionally, the slow exchange rate between partitioned and free hexanol suggests that the hydroxyl group of the 1-hexanol-d_2 is situated near the glycerol backbone of the DMPC lipids to which it may be hydrogen bonded. Deuterons in lipid chains that reside in this region give rise to quadrupole splittings in the order of 20–30 kHz [25], with the quadrupole splitting of the deuterons in 1-hexanol-d_2 measured to be 19.0 kHz when added to DMPC LMVs at a 1-hexanol-d_2/lipid (mol/mol) ratio of 1:10. The slow exchange rate of hexanol between the motionally distinct environments explains the time needed for complete equilibration in the hexanol/lipid system.

Previous NMR studies have offered methods for determination of the partition coefficient of the anaesthetic concerned. Kreishman et al. [7] describe the loss in intensity of a sample of ethanol-d_6 added to a suspension of sonicated DPPC liposomes. This was interpreted as corresponding to the partitioning of the ethanol-d_6 into the bilayer with exchange that was slow on the deuterium NMR timescale. It was proposed that the transverse relaxation time (T_{2B}) of the methyl group was short compared to the preexchange lifetime (τ_B) and that the resonance from the population of 'bound' molecules was line-broadened beyond detection. The remaining signal corresponding to 'free' ethanol-d_6 could lead to calculation of the partition coefficient. However, to use this technique more widely one would have to be assured of the validity of the line-broadening assumption. For the spectra of 1-

hexanol-d_2 added to suspensions of lipid described in this paper, it is clear that the 'bound' population is directly observed.

Comparison of the spectral data for DMPC LMVs and LUVs provides further information concerning the process of 1-hexanol-d_2 partitioning. The observation of a greater P_{mol}, prior to equilibrium, when 1-hexanol-d_2 is added to DMPC LUVs, compared to the equivalent situation for LMVs, suggests that P_{mol} is an accurate reflection of the distribution of 1-hexanol-d_2 at the membrane surface. In LUVs, a far greater membrane surface area is initially available for the association of aqueous hexanol. However, the similar values of P_{mol} for fully equilibrated samples of 1-hexanol-d_2 with either LUVs or LMVs (2.2 and 2.0, respectively) indicate that the observed slow exchange of 1-hexanol-d_2 between the partitioned and aqueous environments (and thus the value of P_{mol}) is predominantly a function of the total amount of lipid present and is not uniquely observed in multi-lamellar, closely opposed bilayer structures.

The smaller quadrupole splitting observed in membranes with a greater heterogeneity of lipid type and increased unsaturation of the acyl chains (Table I) may indicate that increased internal motion at the lipid bilayer surface in such mixed bilayers results in a greater motional amplitude of the partitioned 1-hexanol-d_2 molecules. According to results with headgroup deuterated lipids the degree of mobility is ranked DMPC ≈ DOPC > asolectin > red blood cell lipids [28]. When 1-hexanol-d_2 was added to those lipid bilayers that contained a large proportion of cholesterol, a powder-pattern type resonance with a measurable quadrupolar splitting for membrane-partitioned 1-hexanol-d_2 was not observed. Previous studies have suggested that cholesterol spaces the acyl chains of the hydrophobic domain. Therefore, as the mobility in the lipid headgroup and glycerol backbone region is increased with increasing cholesterol concentration [28], the 1-hexanol-d_2 headgroup will similarly undergo increased amplitude of motion which would result in a collapse of the deuterium quadrupolar splitting. Cholesterol is equally reported as modulating the effect of lipophiles on membranes containing spin-labelled lipids [29]. A trend of increasing P_{mol} for vesicles with greater heterogeneity of lipid type and increased unsaturation of the acyl chains (Table I) may indicate a greater number of sites for 1-hexanol-d_2 partitioning or a greater affinity of 1-hexanol-d_2 for the bilayer core and/or surface. Previous data follow the same trend [15].

Here an attempt has been made to estimate the partitioning coefficient of hexanol in membranes by deuterium NMR observation of 1-hexanol-d_2. The values obtained were comparable to the range of values quoted in the literature. Information about the magnitude and timecourse of partitioning was obtained most easily in membranes that contained one defined phospholipid type and little or no cholesterol. However, the lowest hexanol concentrations employed in this series of experiments (1–2 mM) are similar to those needed to produce pharmacological effects in biomembranes, such as the desensitization to agonist of the nicotinic acetylcholine receptor of *Torpedo* electroplax organ [30]. The values of P that we derive, may therefore be applicable to biological systems. The technique presented here, especially if coupled with spectral deconvolution techniques, may find wider application in the investigation of lipophile–membrane interactions.

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