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UNISOTROPIC SOLUBILIZATION OF AN INHALATION ANESTHETIC, METHOXYFLURANE, INTO THE INTERFACIAL REGION OF CATIONIC SURFACTANT MICELLES

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Proton-NMR shows that methoxyflurane ($HCCl_2$-CF_2-O-CH_3) binds hexadecyltrimethylammonium bromide micelles only at the interfacial regions and does not mix with the lipid core isotropically. The protons of the -O-CH_3 end is oriented into the hydrophobic interior, while the proton of the $HCCl_2$- end stays at the interfacial region in the close vicinity of the aqueous phase.

Introduction

It has been demonstrated that inhalation anesthetics dilate, fluidize and disorder phospholipid membranes [1–3] and depress the temperature of the thermotropic phase transition between solid-gel and liquid-crystalline states [4–12].

These observations lead to the conclusion that anesthetics fluidize the lipid core of the phospholipid bilayers by their direct action on the lipid tail of the phospholipid molecules. An isotropic mixing of anesthetic molecules with the membrane core lipids is generally assumed. The interfacial actions of anesthetic molecules are usually ignored except by Shieh et al. [7] and Yokono et al. [13] who reported that the actions of inhalation anesthetics upon the phase-transition of phospholipid membranes are directed to the interfacial region. Eyring et al. [14] and Kamaya et al. [15] emphasized that the change of the interfacial water structure may be important for the volume expansion associated with general anesthesia.

In the previous paper [16], we demonstrated that anesthetics decreased the cloud-point temperature of the non-ionic surfactant micelles. Cloud-point occurs due to the dehydration of the hydrophilic surface of the non-ionic surfactant micelles. The anesthetics favored the release of the hydrogen-bonded interfacial water molecules from the hydrophilic parts of the micelles. We also have shown [17] that methoxyflurane releases counterions from the surface of anionic surfactant micelles, indicating a decrease of the surface potential.

The present study reports the interaction between cationic surfactant micelles and an inhalation anesthetic, methoxyflurane. By the use of proton nuclear magnetic resonance spectroscopy, it will be shown that the anesthetic molecules adsorb on the surface of the micelle and that the single proton of one end of methoxyflurane molecule stays close to the aqueous environment at the micellar surface, whereas the methoxy protons of the other end of the anesthetic penetrate into the more hydrophobic interior.

Materials and Methods

Hexadecyltrimethylammonium bromide (CTAB) (Eastman, Rochester, NY) was recrystallized twice from an acetone/water (95 : 5, v/v) mixture. Its purity was checked by the critical micelle concentration (CMC) measurement. The CMC of CTAB was $1.00 \cdot 10^{-3}$ molal at 30°C, which was in good agreement with the value in the literature [18]. CTAB was dissolved at desired concentrations in 99.8% deuterium

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Abbreviation: CTAB, hexadecyltrimethylammonium bromide.

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oxide (Stohler Isotope Chemicals, Waltham, MA) which was deoxygenated by bubbling oxygen-free nitrogen gas.

The proton nuclear magnetic resonance spectra were obtained with a Varian XL-100 Spectrometer (100 MHz) at single pulse mode ($\pi/2$) and under a deuterium internal lock operating at 25°C. The pulse delay was set at 120 s and the data points were 4 096 with the spectral width 1 000 Hz. The signals were Fourier transformed after 25 accumulations.

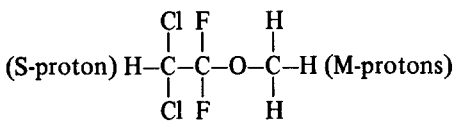
Methoxyflurane (2,2-dichloro-1,1-difluoroethyl methyl ether) was a gift from Abbott Labs. (North Chicago, IL) and contained 0.01% (w/w) 2,6-bis(1,1-dimethylethyl)-4-methylphenol as a stabilizer. The anesthetic was added to the surfactant solution by a microsyringe in a 5 mm NMR tube and the tube was capped tightly. The added amount was confirmed by weighing the tube with an analytical balance.

In order to compare the chemical shifts of the anesthetic protons in the micellar solution with those in other solvents of varying polarity, the NMR spectra of methoxyflurane in ^2H_2O, CCl_4 and C^2HCl_3 were also measured. The mixtures of methoxyflurane/CCl_4 and methoxyflurane/C^2HCl_3 (1 : 1, v/v) were introduced into 1.5 mm external diameter Pyrex capillary tubes and the openings were fused by flame. The capillaries were immersed in ^2H_2O in 5 mm NMR tubes.

Results

All peaks of the proton-NMR spectrum of CTAB in ^2H_2O were assigned by comparison with the results of Eriksson [19]. The anesthetic did not change the chemical shifts or the linewidths of any protons of the micellar surfactant in the concentration range studied.

Methoxyflurane has protons in both ends of the molecule, namely a single proton (S-proton) and methoxy protons (M-protons).



Only one resonance signal group was found for each kind of the chemically different protons of the anesthetic in the micellar solution. This is regarded as a consequence of the rapid exchange of the anesthetic molecules between the micelles and bulk water. The time scale of the NMR spectroscopy is not fast enough to resolve the anesthetic in the two phases. Therefore, a time-averaged peak appears in the spectrum frequency domain.

TABLE I

POSITION OF THE PROTON SIGNALS METHOXYFLURANE IN THREE SOLVENTS EXPRESSED BY Hz FROM THE H_2O SIGNAL

Negative values signify that the position is down-field in reference to the H_2O proton.

	^2H_2O (saturated)	CCl_4 (1 : 1, v/v)	C^2HCl_3 (1 : 1, v/v)
S-Proton	–153.3	–94.8	–102.6
M-Protons	100.3	106.7	102.7

TABLE II

PARAMETERS FOR THE SOLUBILIZATION OF METHOXYFLURANE PROTONS INTO THE CTAB MICELLAR SOLUTION

	$\nu_M - \nu_W$ (Hz)	A_M/A_W
S-Proton	32.2	1435
M-Protons	5.4	2736

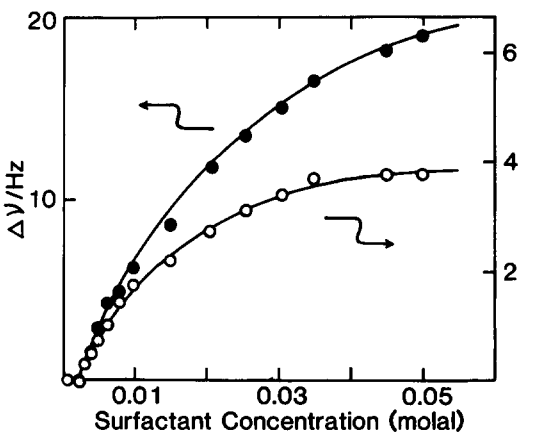


Fig. 1. The up-field shift ($\Delta\nu$) of proton signal of methoxyflurane in ^2H_2O solutions of CTAB saturated with methoxyflurane as a function of CTAB concentration. The temperature was 298.15 K. $HCCl_2$- proton (○) and -OCH_3 protons (●).

The positions of the proton signals of methoxyflurane in ^2H_2O, CCl_4 and C^2HCl_3 are shown in Table I. All values are expressed in Hz and referred to the position of the proton signal of H_2O, which was constant throughout the experiment. The negative sign signifies that the signal is down-field relative to the position of the H_2O proton signal, and vice versa. The resonance signals of both S-proton and M-protons in the organic solvents were shifted toward the higher magnetic field than those in ^2H_2O. The extent of the shift of S-proton was larger than that of M-protons.

When the ^2H_2O solution of the surfactant monomer dispersion was saturated with methoxylfurane, the proton signals of methoxyflurane did not shift until the surfactant concentration reached the CMC. An abrupt upfield shift occurred at the CMC (Fig. 1).

Discussion

As shown in Fig. 1, the positions of the proton signals of the anesthetic in the surfactant solution did not shift from those in the ^2H_2O solution until the surfactant concentration reached the CMC. The environment of the anesthetic molecule was aqueous when the surfactant was dispersed as rotating monomers. When the surfactant concentration reaches the CMC, some of the anesthetic molecules are incorporated into micelles and the signal begins to shift upfield. The fraction of the micelle-incorporated anesthetic increases as the micellar concentration increases. The signal approaches the position which corresponds to that of the anesthetic in the micellar environment.

According to the above consideration, we depict the following model for the solubilization mechanism. The anesthetic content, A_T, in the surfactant solution, the concentration of which is m molal is given by

$$A_T = \frac{1000}{M_W} A_W + A_M(m - m_0) \qquad (1)$$

where A_W is the anesthetic solubility in water expressed in mole ratio, A_M is the saturating amount of the anesthetic solubilized in the surfactant micelle in mole ratio, M_W is the molecular weight of ^2H_2O, and m_0 is the CMC. If the exchange of the anesthetic between the bulk water phase and the surfactant micelles is sufficiently rapid, as in this case, the signal of the anesthetic protons in the micellar solution appears at a position ν in the frequency spectrum as

$$\nu = \frac{50\nu_W A_W + \nu_M A_M(m - m_0)}{50A_W + A_M(m - m_0)} \qquad (m \geqslant m_0) \qquad (2)$$

where ν_W and ν_M are the signal positions of the anesthetic in ^2H_2O and in the micelle, respectively. The signal position observed with the solution below the CMC equals ν_W.

$$\nu = \nu_W \qquad (m \leqslant m_0) \qquad (3)$$

The magnitude of the shift $\Delta\nu$ measured from the signal position of the anesthetic in ^2H_2O is given by

$$\Delta\nu = \nu - \nu_W = \frac{A_M(m - m_0)}{50A_W + A_M(m - m_0)} (\nu_M - \nu_W) \quad (m \geqslant m_0) \qquad (4)$$

$$= 0 \qquad (m \leqslant m_0) \qquad (4')$$

Eqn. 4 is rewritten as follows

$$\frac{1}{\Delta\nu} = \frac{1}{\nu_M - \nu_W}\left\{1 + \frac{50A_W}{A_M}\left(\frac{1}{m - m_0}\right)\right\} \qquad (5)$$

Eqn. 5 is plotted on Fig. 2 using the data shown in Fig. 1. The plots of $\Delta\nu^{-1}$ vs. $(m - m_0)^{-1}$ showed straight lines. This indicates that the above model for the mechanism of the solubilization of the anesthetic into the surfactant micelle is reasonably realistic. From the slopes and the intercepts in Fig. 2 we obtain the parameters, $(\nu_M - \nu_W)$ and A_M/A_W, for the solubilization of the anesthetic into the micelles. The values are shown in Table II. From the comparison of the signal position in the micelle with the results shown in Table I, it is apparent that the S-proton resides in the intermediate environment between water and non-polar solvents, whereas the M-protons are in the non-polar evironment.

The value of A_M/A_W for the S-proton is approximately half of that of the M-protons. Apparently, the S-proton and the M-protons of the methoxyflurane molecule have different solubility into the micelle, the M-protons having twice stronger solubility than the S-proton.

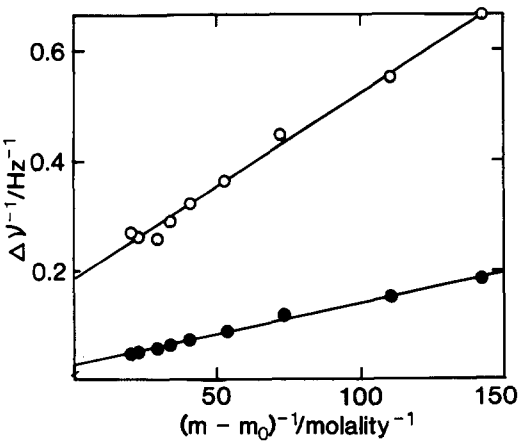


Fig. 2. The plots between the reciprocal of the shift of the anesthetic protons and the reciprocal of the excess surfactant concentration above CMC; $\Delta\nu^{-1}$ vs. $(m - m_0)^{-1}$ according to Eqn. 5 at 298.15 K. $HCCl_2$- proton (o) and -OCH_3 protons (•).

When bound to the CTAB micelles, the methoxyflurane molecules orient themselves by placing the S-proton end close to the aqueous phase and the M-protons end into the hydrophobic interior. The wedge-like shape of methoxyflurane molecule, larger at the S-proton end, probably assists this orientation.

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