Chemical Physics Letters 462 (2008) 112–115

Contents lists available at ScienceDirect

Chemical Physics Letters

journal homepage: www.elsevier.com/locate/cplett

Free-energy change of inserting halothane into different depths of a hydrated DMPC bilayer

P.-L. Chau [a,*], K.-M. Tu [b], K.K. Liang [c], S.L. Chan [d], Nobuyuki Matubayasi [e,f,*]

[a] Bioinformatique Structurale, CNRS URA 2185, Institut Pasteur, 75724 Paris, France
[b] Department of Physics, National Taiwan University, Taipei, Republic of China
[c] Division of Mechanics, Research Centre for Applied Sciences, Academia Sinica, Taipei, Republic of China
[d] Chemical Computing Group, Inc., Suite 910,1010 rue Sherbrooke Ouest, Montréal, Québec H3A 2R7, Canada
[e] Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan
[f] Japan Science and Technology Agency (JST), CREST, Kawaguchi, Saitama 332-0012, Japan

ARTICLE INFO

Article history:
Received 24 May 2008
In final form 11 July 2008
Available online 16 July 2008

ABSTRACT

Using the method of energy representation, we have calculated the free-energy change of inserting a halothane molecule into different depths of a hydrated dimyristoylphosphatidylcholine (DMPC) bilayer at pressures of 10^5 Pa to 4×10^7 Pa. Our results show that the free-energy change is more negative within the membrane than in bulk water. The halothane distribution is diffuse, and the preferred location is near the headgroup. The pressure effect is found to be minimal within the pressure range examined, which corresponds to previous biological experimental conditions.

1. Introduction

Clinicians and scientists have been trying to locate the site and mechanism of action of general anaesthetics ever since its invention. At the turn of the last century, Meyer [1] and Overton [2] independently discovered that the logarithm of the efficacy of an anaesthetic was related to the logarithm of its hydrophobicity. This became known as the famous Meyer–Overton rule. The structure of these anaesthetic molecules differed greatly, so a hypothesis was formulated, that there was a unified mechanism of action for general anaesthetics.

A clue to the mechanism of action of these agents came with the discovery of pressure reversal. Johnson and Flagler found that, by increasing ambient pressure, general anaesthesia by ethanol could be reversed in the tadpole [3,4]. These results were confirmed by other scientists using a variety of general anaesthetics on other species, including the mouse [5–8] and the human [9].

Trudell et al. prepared a mixture of spin-labelled phosphatidylcholine in water and organic solvents, added halothane to this suspension, and measured the electron spin resonance (ESR) spectra of the system. Halothane is 1,1,1-trifluoro-2-chloro-2-bromoethane, and was a commonly-used general anaesthetic. They defined an order parameter:

$$S'_n = \frac{3\langle \cos^2 \theta \rangle - 1}{2} \quad (1)$$

where θ is the angular deviation of the nitroxide $2p\pi$ orbital axis from the axis of magnetic symmetry. The nitroxide group was used as a label of the terminal CH_3 group in these ESR experiments [10]. There was a concomitant decrease of the order parameter S'_n as the concentration of anaesthetic increased [11]. On application of pressure up to 274 atm by helium, a non-anaesthetic gas at these pressures, in the container, these changes were reversed: S'_n increased and the spectra shifted back [12].

The work described led to two key concepts: that the phospholipid cell membrane is involved in general anaesthetic action, and any attempt to define the mechanism of general anaesthetic action must also explain pressure reversal. Recently, Chau and his coworkers suggested that halothane aggregation in the membrane at high pressures can be a key to pressure reversal [13]. They note that the site of action of general anaesthetics is probably the transmembrane portion of a protein [14–19]; this putative binding site is only large enough to accommodate one halothane molecule [20]. They hypothesise that the membrane acts only as a conduit, and propose that pressure reversal occurs when halothane aggregates, so there are fewer monomeric halothane molecules to bind to the putative binding site.

When the membrane acts as a conduit, the knowledge of the binding strength and location is an important first step for the molecular understanding of general anaesthetic action. The key quantity is the free-energy profile of permeation. In the present work, we determine the free-energy change of inserting a halothane molecule into different depths of a hydrated DMPC membrane at a number of clinically relevant pressures. The free-energy calculation is performed using the method of energy representation [21–23].

* Corresponding authors.
E-mail addresses: pc104@pasteur.fr (P.-L. Chau), nobuyuki@scl.kyoto-u.ac.jp (N. Matubayasi).

0009-2614/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.cplett.2008.07.037

2. Methods

All simulations were carried out with version 2 of DL_POLY [24], using the CHARMM potential [25] for the membrane, the TIP3P potential [26] for water and a special potential for halothane (the parameter set II was chosen in this work) [27].

As described previously [28], molecular dynamics simulations were carried out for hydrated membrane systems with and without a solute molecule. The former is called the 'solution' system and contains the solute at full coupling of the solute–solvent interaction. The latter is called the 'reference solvent' system and the solute is treated as a test particle. The solvation free energy of halothane in the membrane system was obtained from the simulations of the solution and reference solvent systems and the resulting energy distribution functions according to Eqs. (19)–(28) of a previous publication [28]. For both the solution and reference solvent systems, 100 DMPC molecules and 2742 water molecules were located in the simulation cell. The unit cell is rectangular with the periodic boundary condition. The x, y, and z-axes for the coordinate system were identified with the edges of the rectangular cell. The z-axis was set to the direction normal to the plane of the DMPC bilayer, and $z = 0$ was taken to be the centre of mass of the 100 DMPC molecules.

The equation of motion was integrated with the SHAKE procedure by fixing all the bond lengths [29]. The time step of integration was 2 fs, and the electrostatic interaction was handled by the Ewald method with the surrounding medium of infinite dielectric constant. The Lennard-Jones interaction was truncated at 13 Å. The Nosé–Hoover–Melchionna NPT scheme [30] was used at 310 K and at the different pressures with a thermostat time constant of 1 ps and a barostat time constant of 5 ps.

For the solute position in the membrane system, four regions were examined in terms of the z-coordinate of the solute center of mass by dividing the domain of $|z| \leqslant 20$ Å with equal intervals of 5 Å, and treating an interval $z_1 \leqslant z \leqslant z_2$ in the positive z-domain and the corresponding interval $-z_2 \leqslant z \leqslant -z_1$ in the negative z-domain as a single region [28]. The 'solution' and 'reference solvent' systems were sampled for each z region of the solute. Fig. 1 shows the division of the hydrated membrane into slabs.

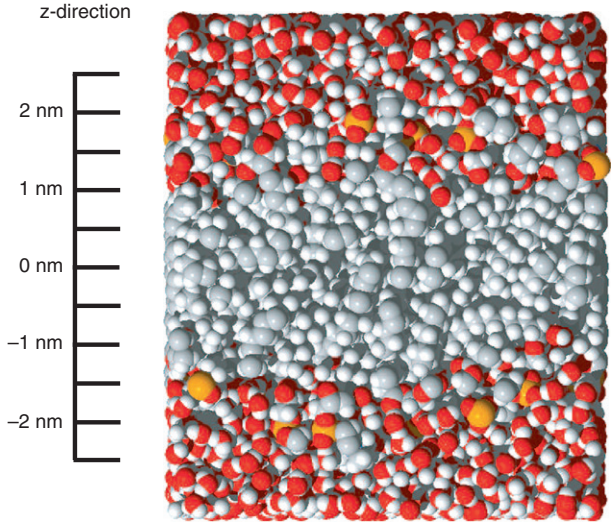

Fig. 1. Diagram showing the simulated system, with the atoms coloured according to the CPK scheme. In the centre of simulation box lie DMPC molecules, with the water molecules on either side. The distance scale in the z-direction is shown on the left.

To sample the solution system (the membrane system with halothane) the simulation was performed separately for the two subregions with positive and negative z. In each simulation for the subregion, the restriction on the solute position was implemented with a restraining potential between the coordinates $z_{\min}$ and $z_{\max}$. If the z-coordinate of the halothane centre of mass, z_h, is such that $z_{\min} < z_h < z_{\max}$, then there is no restraining force. However, if $z_h > z_{\max}$, then the restraining force is $F_z = -k(z_h - z_{\max})$, where $k = 20\,000$ kJ/mol/Å^2. If $z_h < z_{\min}$, then the restraining force is $F_z = k(z_{\min} - z_h)$. The simulation length of the solution system was 250 ps in each subregion and the total length of simulation was 500 ps for each of the four z-regions. The solute–solvent pair interaction energy was sampled every 10 fs and used to construct the energy distribution function.

When the reference solvent is sampled the simulation was carried out only with DMPC and water. The solute was inserted as a test particle into the region of interest at random orientation with the uniform probability over the z value contained in the region. The simulation of DMPC and water was performed for 100 ps, and (instantaneous) configuration of the hydrated DMPC was sampled every 100 fs. At each configuration sampled, the solute insertion was performed 1000 times. The computational procedure is similar to that of [28], and more detailed explanations are found in that paper with the assessment of the reliability of the method. The 'outer' membrane regions of $|z| > 20$ Å were not treated in the present work, because it was found in [28] that a hydrophobic solute is not present in the region of $|z| > 20$ Å with appreciable probability.

The simulation was also conducted for halothane solvated in pure water. In this case, the number of water molecules was 2742 and a single solute molecule was involved in the solution system. The unit cell was cubic, and the simulation length was 100 ps and 50 ps for the solution and reference solvent systems, respectively. Lastly, the procedure described above was repeated with every system at 2×10^7 Pa (200 atm) and 4×10^7 Pa (400 atm); the only difference is the molecular dynamics simulation length for the solution system of halothane in DMPC and water, which is 300 ps (150 ps for each of the positive and negative z-subregions) at higher pressures.

In order to characterize the effects of pressure as well as of the presence of anaesthetics in the membrane on the structure of the DMPC tails, we calculated the order parameter of the lipid tail carbon atoms S_{CD}. The order parameter S_{CD} of a given carbon atom of the hydrocarbon lipid tail can simply be calculated as [31]:

$$S_{CD} = \frac{\langle 3\cos^2\theta - 1\rangle}{2} \tag{2}$$

where θ is the angle between the C–H bond and the membrane normal, and the angled brackets denote ensemble averaging over all the DMPC molecules, over the C atoms located at the same position in the two tails, and over all C–H bonds belonging to the same C atom. Higher values of S_{CD} indicate that the C–H bonds in the lipid tails are more parallel to the membrane.

3. Results

In Fig. 2, the density profile of the membrane is shown at 10^5 Pa (1 atm), 2×10^7 Pa (200 atm) and 4×10^7 Pa (400 atm). It can be seen that there is no discernible effect of pressure on the density profile of membrane atoms within the pressure range examined.

In Fig. 3, we display the bond order parameter S_{CD} of the membrane DMPC molecules at 10^5 Pa, 2×10^7 Pa and 4×10^7 Pa. The pressure effect is also small. The orientational structure represented by S_{CD} is not appreciably affected by the pressure elevation