Study of physisorption of volatile anesthetics on phospholipid monolayers using a highly sensitive quartz crystal microbalance (HS-QCM)

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Received 10 December 2010; revised 5 January 2011; accepted 20 January 2011.

ABSTRACT

We have investigated the interactions between phospholipid monolayers and volatile anesthetics. Two monolayers (dihexadecyl phosphate (DHP) and dipalmitoyl phosphatidyl choline (DPPC) and two anesthetics (halothane and enflurane) were used to observe these interactions using a highly sensitive quartz crystal microbalance (HS-QCM). The concentration of each anesthetic in aqueous solution was kept at 4 mM. The frequency of QCM showed no change when halothane was added to the DHP monolayer, however, it responded and decreased when interaction occurred with DPPC monolayer. In case of enflurane addition the frequency decreased in both the monolayers of DHP and DPPC. The frequency change followed the following order of monolayer-anesthetic interactions: DHP-halothane < DPPC-halothane < DHP-enflurane < DPPC-enflurane. These results showed that the response of anesthetics to the monolayers i.e., the physisorption not only depends on the anesthetic structure, the type of anesthetic hydrate formed, but also the hydrophilic polar group structure of the monolayer or the monolayer/water interface had an important role in physisorption.

Keywords: Physisorption; Phospholipid Monolayers; Quartz Crystal Microbalance (QCM); Anesthetic Hydrate; Monolayer-Water Interface

1. INTRODUCTION

Intermolecular interactions: hydrogen bonding, hydrophobic interaction, van der Waals interaction play an important role in various cellular functions such as the construction of tertiary structures of protein, the hybridization of DNA, the molecular recognition of membranes, and the transportation of nutrients and medicines [1-3]. The phenomenon of the interaction between a biomembrane and an anesthetic is also considered to be in the category of the above mentioned interactions. Anesthetics have structures containing moieties: hydroxyl, ether, chloroform, etc. apart from known halothane and enflurane used in this work. Anesthetic phenomenon occurs at high body concentrations in the order of millimol (mM); the effect of anesthetic is reversibility depending on the medication concentration [4,5]. The anesthetic potency is also temperature dependent [6-8]. Therefore the mechanism of anesthesia has been regarded as “physisorption phenomenon” in which an anesthetic aggregation acts indirectly at the interface of biomembrane-body fluid [9].

A quartz crystal microbalance (QCM) is a powerful method to investigate the above interfacial phenomenon occurring in liquid phases. QCM can detect the mass of substances adsorbed onto a quartz crystal oscillator (QCO) in a very minute amount up to the order of nanograms [10]. Many studies on chemisorption processes such as the oxidation and redox processes on a modified self-assembled monolayers [11,12], metal ion binding to langmuir monolayers [13,14], and molecular recognition of DNA strands and lipids [15-18] have been reported over the last several decades. While there are fewer reports on the investigation of physisorption processes because of the high QCM sensitivity that restricts the use of the method. Ebara et al. [19] have investigated the complementary guest pyridine compounds and acid
binding process involving hydrogen bonding onto various cyanurate lipid monolayers formed at air/water interface using a 27 MHz QCM assembly and suggested that the microenvironment near the lipid surface in a living body is similar to hydrophobic organic medium. Sato et al. [20] have investigated the interaction between aminopurinethiol monolayers and oligonucleotides using a high sensitively improved 5 MHz QCM device that had stability range ±0.3 Hz. The authors claimed, based on the data and results obtained from highly sensitive QCM apparatus, that the hydrogen bonding interactions between the monolayers and complementary nucleic acid bases dissolved in a solution could be detected by their devices.

In this communication, we report physisorption interactions between two phospholipid monolayers DHP and DPPC and two anesthetics halothane and enflurane using a highly sensitively improved 6 MHz QCM device with a stability range ±0.5 Hz for > 12 h. A quartz crystal oscillator (QCO) was attached [21,22] horizontally to the monolayer formed at water surface. The artificially prepared phospholipid monolayer proved to be a good model that allowed to study a real living phenomenon including anesthesia. The investigation of a model physisorption in monolayer-anesthetic interactions using a QCM yielded important information for the elucidation of anesthesia mechanism.

2. MATERIALS, METHODS, AND APPARATUS

Dihexadecyl phosphate (DHP > 99%) and dipalmitoyl phosphatidyl choline (DPPC; 97%), which were used as the model membrane compounds were purchased from Sigma-Aldrich Corp. (St. Louis, USA) and Fluka Chemical Corp. Inc. (Seelze, Germany), respectively. Both the chemicals for membrane preparation were used without further purification. Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane > 99%) and enflurane ((RS)-2-chloro-1,1,2-trifluoroethyl difluoromethyl ether > 99%) which were used as volatile anesthetic were also purchased from Sigma-Aldrich Corp. (St. Louis, USA) and Abbott Japan Corp. Ltd. (Tokyo, Japan), respectively.

Figure 1 shows the molecular structures of monolayer forming compounds and anesthetics used in this work. Ultra pure water with conductance of <0.07 S/cm was obtained from a Super Water Purifying System (WL-21P; Yamato Scientific Corp. Ltd., Tokyo, Japan); the water was boiled for 10 min and subsequently cooled to room temperature [21,22]. We confirmed Langmuir type DHP and DPPC monolayers were formed spontaneously on the water surface [21,22]. The details of the experimental apparatus for HS-QCM measurement have been reported in earlier publications [21,22]. Figure 2 shows a schematic diagram of the central part of the HS-QCM apparatus. After a 6 MHz QCO (Hokuto Denko Corp., Tokyo, Japan) was attached horizontally using a micrometer (Figure 2(b)) to the DHP or DPPC monolayer (Figure 2(c)) with the surface pressures of 15 mN/m (DHP) a purified water surface by spreading method using a 100 l microsyringe (Ge-0583-04; Hamilton Corp., Nevada, USA). Surface tension was measured using Wilhelmy method by a surface tensiometer (CBVP-A3; Kyowa Interface Science Co rp., Ltd., Saitama, Japan). We confirmed Langmuir type DHP and DPPC monolayers were formed spontaneously on the water surface [21,22]. The details of the experimental apparatus for HS-QCM measurement have been reported in earlier publications [21,22]. Figure 2 shows a schematic diagram of the central part of the HS-QCM apparatus. After a 6 MHz QCO (Hokuto Denko Corp., Tokyo, Japan) Figure 2(a) was attached horizontally using a micrometer (Figure 2(b)) to the DHP or DPPC monolayer (Figure 2(c)) with the surface pressures of 15 mN/m (DHP)
and 38 mN/m (DPPC) [21,22]; anesthetics halothane and enflurane were dissolved into the water phase (Figure 2(d)). Using above explained assembly we observed the change in the QCO frequency that was in contact with the DHP or DPPC monolayer. After the addition of halothane and enflurane (4 mM), the stability of the QCO frequency before the addition of halothane and enflurane was maintained within ±0.5 Hz for longer than 12 hours [21,22]. Each measurement was carried out thrice for maintaining reproducibility in the work. The experimental error was within ±0.5 Hz range. A frequency change (Δf) of 1 Hz on the 6 MHz QCO corresponded to a mass change (Δm) 12.2 ng/cm² [10]. All experiments were conducted at temperature 26.00°C ± 0.01°C.

3. RESULTS

3.1. HS-QCM Measurements

Figure 3 shows the typical time course of the QCO frequency that was in contact with the DHP or DPPC monolayer after the addition of halothane and enflurane. The concentration of each anesthetic dissolved in an aqueous solution was 4 mM. The horizontal axis represents time (h). The vertical axis represents the frequency change (Δf) (Hz) based on the resonance frequency before the addition of each anesthetic. No change in the frequency was observed for 4 hours on the addition of halothane to the DHP monolayer. While the frequency decreased gradually until it approached an equilibrium state to the DPPC monolayer (Figure 3(b)). The value of Δf in this case was –1.4 Hz and physisorbed mass (Δm) was 17.1 ng/cm². Because the stirring bar was stirred gently in the water (1 r/s), the delayed response > 1 h is due to the time required for diffusion of the halothane molecules from the bulk solution to the DPPC monolayer/water interface [13,15,16,21]. A similar tendency of frequency decrease was observed on the addition of enflurane to the DHP and DPPC monolayers (Figures 3(c) and (d), respectively). The Δf values were –2.1 Hz for the DHP monolayer while observed Δm was 25.6 ng/cm² and –3.1 Hz for the DPPC monolayer and Δm: 37.8 ng/cm². Our recent concentration-dependence study with regard to the interaction of enflurane with the DPPC monolayer revealed that Δf of –3.1 Hz at 4 mM is nearly to the initial saturation value that corresponds to the amount of semi-saturated physisorption layer of enflurane hydrates [23]. Table 1 summarizes the Δf and Δm values of the DHP and DPPC monolayers interacting with halothane and enflurane anesthetics.

3.2. Surface Tension Measurement

Figure 4 shows the surface pressure vs molecular area DHP and DPPC monolayers form a LE (liquid expanded) and 38 mN/m (DPPC) [21,22]; anesthetics halothane and enflurane were dissolved into the water phase (Figure 2(d)). Using above explained assembly we observed the change in the QCO frequency that was in contact with the DHP or DPPC monolayer. After the addition of halothane and enflurane (4 mM), the stability of the QCO frequency before the addition of halothane and enflurane was maintained within ±0.5 Hz for longer than 12 hours [21,22]. Each measurement was carried out thrice for maintaining reproducibility in the work. The experimental error was within ±0.5 Hz range. A frequency change (Δf) of 1 Hz on the 6 MHz QCO corresponded to a mass change (Δm) 12.2 ng/cm² [10]. All experiments were conducted at temperature 26.00°C ± 0.01°C.

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3.2. Surface Tension Measurement

Figure 4 shows the surface pressure vs molecular area DHP and DPPC monolayers form a LE (liquid expanded)
state at low surface pressures and a LC (liquid condensed) state at high surface pressures [24]. The LE-LC transition occurs at 8 mN/m for DHP monolayer and 10 mN/m for DPPC monolayer, respectively. Our results of spreading method conducted in this work also showed the LE-LC transitions at the same values of surface tension. However, the shapes of the curves (Figure 4) for DHP and DPPC monolayers were a little different compared to those reported previously [24].

3. DISCUSSION

3.1. Halothane and Enflurane Structure and Properties as Anesthetics

Halothane and enflurane are generally used as inhalation anesthetics. A halothane molecule comprises fluorine, chlorine, and bromine atoms, while an enflurane molecule has fluorine and oxygen (Figure 1). The difference in their compositions can be noticed in their molecular properties such as hydrophobicity and solubility [4,25]. A characteristic common to both the anesthetics is that when dissolved in an aqueous solution, water molecules surround the anesthetic molecules and form a hydration cluster (hydrophobic hydration) [25,26]. At 4 mM of anesthetic concentration, every anesthetic molecule is surrounded by water molecules to form monomer water hydrates [9,26,27].

3.2. DHP and DPPC Monolayers

The surface tension measurements revealed that the molecular areas of DHP was 0.54 ± 0.03 nm²/molecule and surface pressure was 16 mN/m. While for DPPC surface pressure was 38 mN/m and molecular area was 0.65 ± 0.05 nm²/molecule [21,22]. The DHP and DPPC monolayers in this experiment were as close as to their maximum packing. However, the molecular areas of these monolayers were 30% larger to those of condensed monolayers (0.42 nm²/molecule for DHP and 0.47 nm²/molecule for DPPC) considering compressing method [24,28]. That is because each monolayer in our measurements was in a “semi-expanded state” and had a “fluid-rich” structure with interfacially restricted water. The difference in the molecular areas of DHP (0.54 nm²) and DPPC (0.65 nm²) monolayers, despite both DHP and DPPC molecules possessing two hydrophobic alkyl chains, is due to the degree of interaction between the hydrophilic groups and the water molecules [21,29]. The hydrophilic group of DHP is (-O-)₂-PO(OH), a typical semi-nonionic headgroup, whereas, DPPC group is,-(CO)-O-CH₂-(CO)O-)-CH-CH₂-O-POO–-O-(CH₂)₂-N(CH₃)₂, a typical zwitter ionic headgroup (Figure 1) [24]. The hydrophilic groups of DHP form a smooth hydrogen bond network of interfacially restricted water with free water molecules. In contrast, the restricted water formed on the DPPC/water interface is more complex than the one formed on the DHP/water interface [29,30]. The former’s head is complicated and larger than that of latter. In both the monolayers formed by DHP and DPPC, water molecules play an important role in maintaining the structure of the amphiphilic monolayer formed on water surface as well as in the interactions between the hydrophobic moieties of the monolayer [28,31]. In other words, the hydrophilic groups of the DHP and DPPC molecules and water molecules structured themselves in the most comfortable arrangement which is different from the behavior exhibited by compression method monolayers [24]. Therefore, the possibility exists that halothane and enflurane hydrates can physisorb on such a fluidic monolayer/water interface [32].

3.3. Monolayer-Anesthetic Interaction

Yoshino et al. [27] reported that the restricted water in a water-in-oil emulsion changed to free water by the action of enflurane hydrates. The reaction of halothane hydrates with a sodium dodecyl sulfate (SDS) micelle/water interface has also been reported [33]. In case of halothane solubilization, the –CF₃ ends of the anesthetic hydrates localized at the interface, whereas the –CHClBr ends remained dissolved in the water phase. The authors also further reported that when enflurane hydrates interacted with the above SDS micelle/water interface, the molecular axis orientation of enflurane molecule was parallel to the interface [34]. These reports indicate that anesthetics hydrates interact with the interfacially restricted water formed at the membrane/water interface and do not penetrate into the membrane’s lipid core. Furthermore the physisorption potency of enflurane hydrate physisorbed on the restricted water is higher than that of halothane hydrate being a larger molecule. In other words, the enflurane hydrate physisorbs on the restricted water more easily than the halothane hydrate because the physisorption capible sites in halothane hydrate are only the –CF₃ end groups, whereas that of the enflurane hydrate includes all the sites of enflurane molecular axis. The molecule of enflurane has one hydrophilic oxygen atom, which is different from the hydrophobic molecular structure of halothane. This “semi-hydrophobic” property of enflurane increases the strength of the interaction between enflurane hydrates and the restricted water. Based on our results and those of above mentioned references, the mechanism of the interaction of halothane and enflurane with the DHP and DPPC monolayers can be explained on the model described in Figure 5 and the results have been shown in Table 1. Halothane hydrates...
with less physisorption capable sites –CF₃ rarely physisorb on a smooth (small head group) DHP monolayer/water interface. DPPC has larger head group than DHP molecule therefore monolayer/water interface in bulkier and more spacious in former than latter. Thus more physisorption sites (acceptors) exist at the interface formed by DPPC than DHP. As a result halothane hydrates physisorb on physisorption sites at the DPPC monolayer/water interface and those were detected by sensitive QCM apparatus. The results of $\Delta f$ (Hz) and $\Delta m$ (ng/cm²) have been compared in Table 1. Enflurane hydrates, on the other hand, whose molecular axis are physisorption capable due to large size anesthetic can physisorb (Table 1) at even the smooth and smaller DHP monolayer/water interface. Many enflurane hydrates physisorb easily on the DPPC monolayer/water interface and reach to a semi saturated physisorption state [23].

In an unpublished but concluded study [35], we have found that enflurane (4 mM) does not physisorb at $n$-hexadecanol monolayer/water interface. The above monolayer was the condensed and solid with very small molecular area 0.20 nm² [36] even though it was formed by the spreading method. The interfacially restricted water in this system was stable and had poor fluidity. The effect of condensed or solid monolayer on physisorption was also noticed in the study of enflurane (4 mM) physisorption on DPPC-cholesterol mixed monolayer/water interface when DPPC-to-cholesterol ratio was 8 : 2. The physisorption frequency and volume were $\Delta f = -1.8$ Hz and $\Delta m = 21.9$ ng/cm², respectively. This mixed monolayer condensed partially by the condensation effect of cholesterol which had molecular area 0.48 nm². In this mixed monolayer the physisorption of enflurane was difficult compared to in pure DPPC monolayer. Similarly the physisorption was difficult in $n$-hexadecanol monolayer. These results also indicated that the structures of the DHP and DPPC monolayer/water interface were fluidic thus enflurane physisorption occurred.

5. CONCLUSIONS

HS-QCM apparatus were sensitive enough to measure and reveal that anesthetics hydrates physisorbed on the interfacially restricted water formed on a phospholipid monolayers/water interface. Experimental results showed that the physisorption of anesthetic hydrates was influenced not only by the physisorption potency of the anesthetic hydrates but also the form of the interfacially restricted water on the monolayer/water interface. The strength of an anesthetic (anesthetic potency) did not depend only on the type of anesthetics but the structure such as mobility, size, and orientation of molecules at monolayer/water interface were very important factors. In order to obtain better understanding of the anesthesia mechanisms such detail investigations are very important.

6. ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology (No. 15750118 and 21750143); the NITECH 21st Century COE Program “World Ceramics Center for Environmental Harmony”, DVA Medical Research and Development Funds, and Grant-in-Aid from the Sakakibara Memorial Hospital Incorporated Foundation.

REFERENCES


