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1-Alkanols and membranes: A story of attraction

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Abstract

Although 1-alkanols have long been known to act as penetration enhancers and anesthetics, the mode of operation is not yet understood. In this study, long-time molecular dynamics simulations have been performed to investigate the effect of 1-alkanols of various carbon chain lengths onto the structure and dynamics of dimyristoylphosphatidylcholine bilayers. The simulations were complemented by microcalorimetry, continuous bleaching and film balance experiments. In the simulations, all investigated 1-alkanols assembled inside the lipid bilayer within tens of nanoseconds. Their hydroxyl groups bound preferentially to the lipid carbonyl group and the hydrocarbon chains stretched into the hydrophobic core of the bilayer. Both molecular dynamics simulations and experiments showed that all 1-alkanols drastically affected the bilayer properties. Insertion of long-chain 1-alkanols decreased the area per lipid while increasing the thickness of the bilayer and the order of the lipids. The bilayer elasticity was reduced and the diffusive motion of the lipids within the bilayer plane was suppressed. On the other hand, integration of ethanol into the bilayer enlarged the area per lipid. The bilayer became softer and lipid diffusion was enhanced.

Keywords: Molecular dynamics (MD) simulation; DMPC; Alkanol; Anesthetic; Penetration enhancer; Elasticity

1. Introduction

Since the demonstration of the phenomenon of anesthesia in the middle of the 19th century by William Morton, there has been a keen research interest to elucidate the underlying mechanism. Special interest arises from the fact that there is a vast number of structurally and chemically different molecules – amongst them the 1-alkanols – which all cause anesthesia (see review [1]). To account for this variety of anesthetics, the hypothesis of a nonspecific or physical mode of action of anesthesia, mediated by lipid bilayers, rather than a chemical reaction mechanism with binding of anesthetics to membrane proteins was proposed (see, e.g., [2–4]). This hypothesis is supported by the fact, that the Meyer–Overton rule, after which the anesthetic effect of a drug correlates with its lipophilicity, is the only relation which was found to be valid for almost all general anesthetics [1].

Many different theories explaining the mode of action of anesthetics were suggested and investigated. Indirect lipid-mediated theories proposed anesthetic action to be exerted by a change of membrane properties upon insertion of anesthetics — like the volume of the membrane [2] or the volume that anesthetics occupy within a membrane [5], the phase transition temperature [6], the lipid chain order, the thickness of the membrane, the lateral phase separations in membranes [7], or the lateral pressure profile [8]. The latter changes may influence the function of proteins embedded in the membrane, e.g., induce a shift of the conformational equilibrium between the closed and the open state of membrane channels [7,8]. A similar mechanism of protein function regulation by bilayer elasticity was also suggested (for a review, see [9]). However, up to now there is no consensus neither about the site of action of anesthetics nor about the mechanism of their action.

Apart from anesthesia, an important application of aliphatic alkanols is their use as penetration enhancer in transdermal drug delivery [10]. Like in the case of anesthetics, neither the mechanism of action of penetration enhancers nor the exact site of action is known yet [10]. An interesting parallel between the

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potency of 1-alkanols as anesthetics and as penetration enhancers can be found for the so-called cutoff-effect for anesthetics: the potency of a homologous series of anesthetics – e.g., the 1-alkanols – is increasing until a certain chain length is reached. 1-Alkanols with a chain length above the cutoff length show no anesthetic potency anymore. Similarly, the permeation enhancing effect of 1-alkanols increases with increasing chain length up to decanol and decreases again for 1-alkanols with longer carbon chains [11]. Also, the potency of alcohols as anesthetics as well as penetration enhancers was found to decrease with branching of the carbon chain [10,3]. For monounsaturated 1-alkenols the cutoff in potency for anesthesia was found to be shifted to longer carbon chains [12]; the same effect can be found for 1-alkenols as penetration enhancer [10]. Apart from the general interest in the mechanisms of anesthesia and penetration enhancement caused by alcohols, an understanding of these effects would possibly allow an improved design of anesthetizing and permeation enhancing drugs.

Up to now there have been only a few computational studies targeting the influence of anesthetics or alcohols on lipids. A recent study by Patra et al. [13] examined the influence of methanol and ethanol onto dipalmitoylphosphatidylcholine (DPPC) and palmitoyloleoylphosphatidylcholine (POPC) lipid bilayers by molecular dynamics (MD) simulations, reporting a decreased order for lipids bound to ethanol and an increased fluidity of the bilayers upon insertion of ethanol. In two successive studies, Chanda and Bandyopadhyay [14,15] studied the influence of ethanol onto dimyristoylphosphatidylcholine (DMPC) bilayers at moderate and high concentrations. Despite a short simulation time of 5 ns and pre-insertion of ethanol molecules into the bilayer according to experimental results, a change in the distribution of lipid headgroup dipoles and an increase of the in-plane and out-of-plane mobility of the lipids could be observed. The mobility of interfacial water was raised due to preferential hydrogen bonding of ethanol to the lipids. In a combined experimental and theoretical study of POPC bilayers with ethanol at low hydration, Feller et al. [16] found an interaction between the ethanol molecules and the lipid phosphate groups via the formation of hydrogen bonds and a predominant localization of the ethanol molecules at the bilayer/water interface. Kranenburg et al. and Venturoli et al. [17–19] applied coarse-grained simulations to investigate the influence of alcohols on the phase diagrams and especially on the interdigitated phase of lipid bilayers. Concerning anesthetics, the influence of halothane on a pure DPPC bilayer [20] and on a simple transmembrane channel (gramicidin A) [21,22] has been studied by means of MD simulations. Halothane molecules preferentially resided at the channel–lipid–water interphase. At physiologically relevant concentrations, only minimal effects on the gramicidin A structure, but profound changes in the channel dynamics were reported.

Here, we use MD simulations to investigate the effects of 1-alkanols of different chain lengths (below and above the cutoff length) on the structure and dynamics of lipid bilayers. Although lacking physiological components as, e.g., integral membrane proteins, phospholipid bilayers can be considered as a first approximation to understand the behavior of cell membranes [13]. Applying the technique of MD simulations allows to monitor the insertion of the 1-alkanols into the bilayer as well as modifications of the bilayer properties induced by the 1-alkanols in atomic detail. Special emphasis has been put on the analysis of the bilayer elasticity, volume changes of the bilayer, as well as the lipid ordering before and after addition of 1-alkanols, as these effects have been proposed to be central to the mechanism of action of anesthesia. The simulation results were endorsed by continuous bleaching, film balance and microcalorimetry experiments.

2. Materials and methods

2.1. Molecular dynamics simulation

2.1.1. Simulation setup

MD simulations have been carried out using the GROMACS software package version 3.3 [23–25]. For the starting structure, a hydrated DMPC bilayer consisting of 128 lipids (kindly provided by Peter Tieleman) was used. This bilayer was placed into solutions of different alkanols – ethanol, octanol, decanol and tetradecanol – with water. The initial coordinates of the 1-alkanols were created with the help of the Dundee PRODRG2 Server [26] and then the 1-alkanols were randomly added to the water phase. The concentrations of the water–alkanol solutions were in the range of 0.0 to approximately 0.6 mol kg^{-1} (0, 8, 24, and 72 1-alkanols). Suggested values for full hydration of lipid bilayers range from 20 to 32 water molecules per lipid [27–29]. To ensure full hydration, we chose a minimum total number of 5000 water molecules for the systems with 128 lipids, corresponding to a minimum ratio of 39 water molecules per lipid (compare Table 1). Additionally, for octanol and ethanol, systems at larger 1-alkanol concentrations of about 0.9 to 1.3 mol kg^{-1} were studied. Starting

Table 1
The systems studied in the simulations

System name	Number of lipids	Number and type of 1-alkanol molecules	Number of water molecules	Simulation time (ns)
C1	128	None	5673	100
C2	512	None	22,692	52
C3	2048	None	90,768	23
E1	128	8 Ethanol	7470	100
E2	128	24 Ethanol	7409	100
E3	128	72 Ethanol	9394	100
E4	128	128 Ethanol	6995	100
E5	512	288 Ethanol	26,800	31
E6	2048	1152 Ethanol	107,200	23
E7	128	72 Ethanol	6146	100
E8	128	72 Ethanol	9322	100
O1	128	8 Octanol	5237	100
O2	128	24 Octanol	5140	100
O3	128	72 Octanol	6656	100
O4	128	128 Octanol	7945	100
O5	128	185 Octanol	7955	100
O6	512	96 Octanol	20,560	40
O7	512	288 Octanol	26,624	33
O8	2048	1152 Octanol	106,496	26
D1	128	8 Decanol	5229	100
D2	128	24 Decanol	5092	100
D3	128	72 Decanol	7474	100
D4	512	288 Decanol	29,896	31
TD1	128	8 Tetradecanol	5212	100
TD2	128	24 Tetradecanol	5027	100
TD3	128	72 Tetradecanol	7307	100
TD4	512	288 Tetradecanol	29,228	31

For the simulation E7, the Gromos 53A6 [37] forcefield was used instead of the Gromacs forcefield. The simulation E8 contained ions in the aqueous phase.