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Behaviour of small solutes and large drugs in a lipid bilayer from computer simulations

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Abstract

To reach their biological target, drugs have to cross cell membranes, and understanding passive membrane permeation is therefore crucial for rational drug design. Molecular dynamics simulations offer a powerful way of studying permeation at the single molecule level. Starting from a computer model proven to be able to reproduce the physical properties of a biological membrane, the behaviour of small solutes and large drugs in a lipid bilayer has been studied. Analysis of dihedral angles shows that a few nanosecconds are sufficient for the simulations to converge towards common values for those angles, even if the starting structures belong to different conformations. Results clearly show that, despite their difference in size, small solutes and large drugs tend to lie parallel to the bilayer normal and that, when moving from water solution into biomembranes, permeants lose degrees of freedom. This explains the experimental observation that partitioning and permeation are highly affected by entropic effects and are size-dependent. Tilted orientations, however, occur when they make possible the formation of hydrogen bonds. This helps to understand the reason why hydrogen bonding possibilities are an important parameter in cruder approaches which predict drug absorption after administration. Interestingly, hydration is found to occur even in the membrane core, which is usually considered an almost hydrophobic region. Simulations suggest the possibility for highly polar compounds like acetic acid to cross biological membranes while hydrated. These simulations prove useful for drug design in rationalising experimental observations and predicting solute behaviour in biomembranes.

Keywords: Molecular dynamics simulation; Constraint; β-blockers; DPPC membrane; Permeability

1. Introduction

For most of the routes of administration, cell membrane permeation is required for a drug molecule to reach the general circulation. Even after direct injection or even if the drug can permeate via the paracellular route in the extracellular space, it soon encounters cell membranes to be crossed in order to reach its biological target which is usually represented by a protein inside the cell cytoplasm. Most drugs cross cell membranes by passive permeation without the help of protein carriers, unless they are analogues of physiological substrates. An understanding of solute behaviour inside biological membranes is then crucial for subcellular pharmacokinetics and rational drug design [1].

Functional cell membranes are fluid mosaics of proteins within a lipid bilayer matrix [2]. Experimental and theoretical models for biological membranes, especially when studying solute permeation, are therefore phospholipid bilayers. Among them, extensive data have been collected for the dipalmitoylphosphatidylcholine (DPPC) bilayer. Recently, several ns-long all-atom MD simulations have been performed in our laboratory [3,4] investigating the permeation process of eight small organic compounds in a DPPC membrane. The eight solutes represent the most common chemical functional groups: acetamide, acetic acid, benzene, ethane, methanol, methylacetate, methyla-

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mine, water. Simulation results show in general a good correlation between the free energy in the centre of the membrane with the experimental free energy of partitioning for the solutes between water and hexadecane. The notable exception to this rule is for benzene, which, because of its size, is sensitive to the lateral packing in the lipid bilayers, supporting the view that biomembranes do not always behave like bulk solvents. With the exception of water, the diffusion coefficients of the molecules are broadly similar. Surprisingly, calculated diffusion coefficients inside the bilayer are dependent on solute size to a lesser extent than in water and the size dependence shown by permeability is instead to be ascribed to the solute partitioning. Continuing those studies, the permeation of three real drugs across the DPPC bilayer has also been simulated [5]. The drugs are alprenolol, atenolol and pindolol, belonging to the class of β-adrenoreceptors antagonists. The simulations perfectly reproduce the experimental ranking of the permeability coefficients, and free energy calculations show that partition coefficients between water and 1-octanol overestimate the drug ability to dissolve into the membrane.

The advantage of MD simulations over conventional experiments is that the contributions from the different regions of the lipid bilayer, that is free energy, diffusion and local resistance as a function of depth, can be studied at a molecular level, whereas experimental models can only approximate the membrane as a uniform barrier slab. Further analyses of the simulations mentioned above are presented here. While the previous articles [3–5] focused on the calculation of the relevant physical properties, the aim of this paper is to investigate the behaviour of the drugs and the small organic compounds inside the membrane with atomistic detail. Therefore, flexibility, mean orientation, reorientational correlation times and hydrogen bonds will be described and, where possible, related to the observed partition and diffusion coefficients.

2. Materials and methods

2.1. Simulation protocol

The protocol of the simulations is described in detail elsewhere [3–5]. Briefly, the simulation box contained 72 DPPC molecules arranged in a 2×36 bilayer, together with 2094 water molecules (full hydration). Lipids and water were modelled using version 27 of the CHARMM force field for lipids [6]. An equilibrated starting structure of the lipid bilayer was kindly obtained from A. D. MacKerell and S. E. Feller, who participated in developing the force field. The simulation protocol was the same as that used in some of the latest Feller's simulations [6,7]. The LJ potential was switched smoothly to zero over the region from 10 and 12 Å. Electrostatic interactions were calculated via the Particle Mesh Ewald (PME) method using a κ value of 0.23 and a fast-Fourier grid density of $\sim 1 \text{Å}^{-1}$. The real space part of the PME summation was truncated at 12 Å. The SHAKE algorithm [8] was used to constrain all covalent bonds involving hydrogens. The leap-frog algorithm [9] was employed to solve the equation of motion with a time step of 2 fs. A neighbour list, used for calculating the LJ potential and the real space portion of the PME, was truncated at 14 Å and updated every 50 fs. Coordinates were saved every ps for subsequent analysis. Three-dimensional periodic boundary conditions were applied. Only the cell length normal to the membrane (L_z) was allowed to vary during the simulation to maintain a constant normal pressure (P_N) of 1 atm. The other cell dimensions (L_x and L_y) were kept fixed to maintain a constant surface area per lipid (A) of 62.9 Å^2. The pressure was maintained by the Langevin Piston algorithm [10] with a mass of 500 amu and a collision frequency of 5 ps^{-1}. The temperature (T) was maintained at 50 °C, well above the phase transition temperature of DPPC bilayers, by means of the Hoover thermostat [11]. A value of 1000 kcal ps^{-1} was used for the thermostat (fictitious) mass. The ensemble was therefore NP_NAT. The reliability of this ensemble in the context of these permeation calculations has been discussed elsewhere [3,4].

Small solutes and drugs were free to move on the x–y plane, but constrained at chosen distances from the bilayer centre (z depths) using the so-called z-constraint algorithm [3–5,12–14]. This allowed for the calculation of the force acting on the centre of mass of the permeants at different depths in the lipid bilayer. From that, the free energy difference between the water phase (outside the membrane) and those depths was directly accessible, and from the force instantaneous fluctuations, the local diffusion coefficients at those depths could be calculated. Eventually, the permeability coefficients of the solutes and drug molecules across the DPPC bilayer was obtained and their relative values found to agree favourably with available experimental data. The methods employed to insert solutes and drugs inside the DPPC membrane and to generate starting structures were described in the previous papers [3–5]. Those aspects of the methodology that are relevant to the results reported here will be briefly described. Different solutes and drugs were studied in separate simulations, but a few membrane depths were sampled in the same simulation to reduce the computational expenses while ensuring no solute–solute or drug–drug interaction occurred. For the large drugs, different orientations with respect to the membrane and different internal conformations of dihedral angles were also sampled. The β-blockers under study have six dihedral angles which were expected to be difficult to sample adequately. They, together with the structures of the drugs, are depicted in Fig. 1.

Therefore, representative drug conformations were carefully chosen and used as starting structures in separate simulations. Monte Carlo simulations in implicit solvents

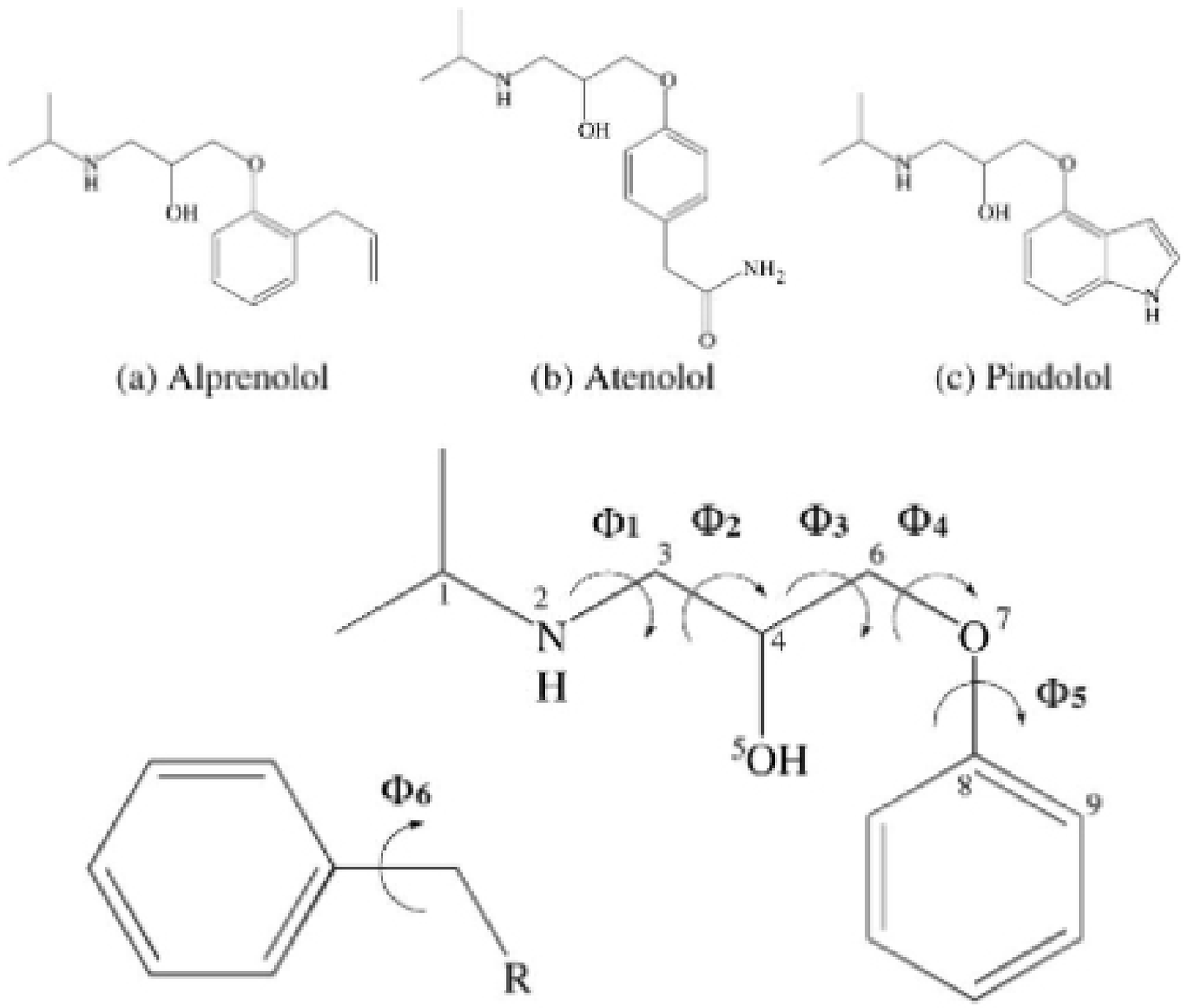

Fig. 1. Drug molecules (top) and their critical dihedral torsions (bottom). Φ1=1–2–3–4, Φ2=2–3–4–5, Φ3=5–4–6–7, Φ4=4–6–7–8, Φ5=6–7–8–9.

were used to determine the most populated conformations [5]. These conformers are reported in Table 1, with names for dihedral angles as in Fig. 1.

The β-blockers are elongated molecules and it was not expected that full rotation would be observed inside the DPPC bilayer. Therefore, each combination of drug conformer and membrane depth was sampled twice: once with the drug having the aromatic ring initially oriented towards the middle of the bilayer and the isopropylamine fragment towards the water phase, and once with the aromatic ring initially oriented towards the membrane exterior and the isopropylamine fragment towards the interior. However, the drugs were free to rotate during the simulations. If we name the aromatic ring as the *drug head* and the main chain as the *drug tail*, it is convenient to refer as *up* the orientation where the drug head is towards the membrane exterior and *down* the orientation where the drug head is towards the centre of the bilayer.[1]

Table 1
The most populated combinations of dihedral angles

Drug	Φ1	Φ2	Φ3	Φ4	Φ5	Φ6
Alprenolol	292.5	82.5	180.0	300.0	127.0	202.5
Atenolol	292.5	82.5	180.0	300.0	127.0	202.5
	292.5	82.5	180.0	300.0	127.0	23.5
	292.5	82.5	180.0	300.0	307.5	202.5
	292.5	82.5	180.0	300.0	307.5	23.5
	292.5	300.0	180.0	300.0	127.0	202.5
	292.5	300.0	180.0	300.0	127.0	23.5
	292.5	300.0	180.0	300.0	307.5	202.5
	292.5	300.0	180.0	300.0	307.5	23.5
Pindolol[a]	300.0	292.5	180.0	285.0	292.5	
	82.5	292.5	180.0	285.0	292.5	
	82.5	292.5	180.0	180.0	240.0	

[a] For pindolol, the side chain is a rigid ring and there is no Φ6.

All the simulations were run in parallel with 4 processors, using version 27 of the CHARMM software package [15], which was modified to introduce the *z*-constraint described above. The simulations were run on different Linux PC clusters with either 1 GHz Pentium III, 1.5 GHz Pentium IV, or 1.4 GHz AMD Athlon.

3. Results and discussion

3.1. Four region model

Since the membrane has a very inhomogeneous character when moving from one side to the other, each individual

[1] We are aware that the nomenclature usually applied is drug scaffold and drug side chain, but in the context of these simulations the use of drug head and tail is preferred. There are several reasons. Such terms recall those commonly employed for the surrounding lipids and this is useful when it comes to investigate drug–lipid interactions. Given the shape of the three β-blockers , the head and tail definitions are straightforward to understand and visualise. The name side chain here would create misunderstanding because of the presence of the second shorter chain on the aromatic ring of the drugs. Since the part of the drug molecule which is in common between β-blockers in general comprises the (3-(N-isopropyl)amino-2-idroxy)-propyl chain together with the aromatic ring, the term scaffold would not be as effective for defining the two fragments separately as head and tail. We therefore ask the reader to accept our more physical nomenclature.