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Permeability across lipid membranes☆

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ABSTRACT

Molecular permeation through lipid membranes is a fundamental biological process that is important for small neutral molecules and drug molecules. Precise characterization of free energy surface and diffusion coefficients along the permeation pathway is required in order to predict molecular permeability and elucidate the molecular mechanisms of permeation. Several recent technical developments, including improved molecular models and efficient sampling schemes, are illustrated in this review. For larger penetrants, explicit consideration of multiple collective variables, including orientational, conformational degrees of freedom, are required to be considered in addition to the distance from the membrane center along the membrane normal. Although computationally demanding, this method can provide significant insights into the molecular mechanisms of permeation for molecules of medical and pharmaceutical importance. This article is part of a Special Issue entitled: Biosimulations edited by Ilpo Vattulainen and Tomasz Róg.

1. Introduction

The transport of substances across a lipid membrane is a biological process of vital importance. Mechanisms for molecular transport across membranes can be classified into two categories: active and passive transport. While the former requires regulatory machinery (with input of energy) that transports the target molecules in the direction opposed to the concentration gradient, the latter proceeds *via* an entropy-driven, nonspecific diffusion process of the molecule across the membrane. Most of small neutral molecules and drug molecules are transported passively through the membrane. Thus, understanding the process of passive permeation is critical not only in fundamental biological science but also in medical and pharmaceutical applications [1,2].

Experimental permeability measurements have been performed for many solute molecules through various lipid membranes [3]. Permeability or leakage of a small molecule should give an experimental measure of the structural stability of the membrane because it should reflect lipid packing in the membrane core. However, experimental approaches cannot provide adequate information on the mechanism of passive transportation at the molecular level. Understanding of the regulation and/or mechanisms of molecular transport by lipid membranes requires a detailed estimation of interactions between permeants and lipid membranes.

Molecular simulation can prove to be very useful for probing the molecular mechanism of membrane permeation processes [4,5]. However, a brute-force molecular dynamics (MD) simulation is not straightforwardly useful because the typical time range of MD is too short to directly observe the complete permeation process through the membrane. Thus, an alternative approach based on a permeation model is needed. Most MD studies on membrane permeability have been carried out based on the inhomogeneous solubility-diffusion model, as explained in the next section. Using this model, one evaluates the free energy profile and diffusion coefficients along the reaction coordinate, which is typically chosen as the position of permeant along the bilayer normal. Improvements in the accuracy and sampling efficiency have been made in order to better describe permeability. Here, we discuss recent technical advances in models and simulation methods and highlight the increasing application of MD studies that treat many different penetrants through a variety of lipid membranes. In Section 2, permeation models are briefly described. In Section 3, computational and experimental measurements of membrane permeability are illustrated. Section 4 focuses on methodological development of molecular simulations, which is written specifically for the readers interested in computational and theoretical approaches to membrane permeability. Future challenges are described in Section 5.

2. Permeation model

Although several models have been developed to explain the possible permeation mechanisms, in this review, we focus on two major mechanisms for membrane permeation, namely, the solubility-diffusion mechanism and transient pore formation mechanism. Permeation of small neutral or polar molecules across lipid bilayers with different membrane thicknesses (lipid chain lengths of 14–24 carbon atoms) has been systematically investigated, [6] and results have shown that the solubility-diffusion model works well for modeling

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permeation of small molecules as long as the membrane under consideration is thick [7]. Additionally, most MD studies evaluating membrane permeability use this model; therefore, we have primarily focused on this model. However, when permeation of a polar small molecule through a thin lipid membrane is considered, transient pore-like defects in the membrane could explain the permeability better [8]. In this case, the permeation process is very different, and pore formation itself is a time-limiting step for the permeation.

2.1. Solubility-diffusion model

Overton observed that the membrane permeability coefficient of a solute is correlated with its oil/water partition coefficient [9]. This leads to a simple model wherein the lipid membrane is treated as a simple homogeneous oil slab [10]. In the solubility-diffusion model (also known as the Meyer–Overton rule) [11], the intrinsic permeability coefficient, P, is written as

$$P = \frac{KD}{d}, \tag{1}$$

where K, D, and d are the oil/water partition coefficient, the solute's diffusion coefficient in the oil slab, and membrane thickness, respectively.

As shown by many experimental and computational observations, the lipid bilayer membrane is quite heterogeneous along the bilayer normal, Z. Thus, an inhomogeneous solubility-diffusion model was proposed to provide a more realistic description of membrane permeation [12]. In this model, permeability is written as:

$$\frac{1}{P} = \int_{-d/2}^{d/2} \frac{1}{K(Z)D(Z)} dZ = \int_{-d/2}^{d/2} \frac{\exp(\Delta G(Z)/k_BT)}{D(Z)} dZ, \tag{2}$$

where $K(Z)$ and $D(Z)$ are the position-dependent partition coefficient and solute diffusion coefficient, respectively. $\Delta G(Z)$ is the free energy difference, which is related to $K(Z)$ by $K(Z) = \exp(-\Delta G(Z)/k_BT)$. This model was adopted by a pioneering MD simulation for evaluating membrane permeability [13] in which water permeability was discussed by calculating the free energy and diffusion coefficient of water along the bilayer normal. This represents the standard MD-based computational approach to evaluate the membrane permeability. As is clear from Eq. (2), precise estimation of $G(Z)$ and $D(Z)$ are required; P involves an accumulative error because of the integration along Z. Therefore, many computational schemes have been developed and tested to improve the estimates. This will be described in more detail later in this review.

Recently, an extension of the inhomogeneous solubility-diffusion model has been developed to include both the rotational and translational degrees of freedom of the solute [14]. The model has been successfully applied to the permeability of steroids, which preferably reorients in the course of permeation because of its amphiphilic nature. For fast solute reorientation, this model recovers the standard inhomogeneous solubility-diffusion equation.

2.2. Pore formation mechanism

Although the probability of spontaneous pore formation in an ordinary lipid bilayer by thermal fluctuation is quite low, once it happens, there is no doubt that the pore will provide a readily permeable pathway for hydrophilic molecules to cross the membrane. Therefore, in this case, analysis of pore formation itself is critical. Studies have also shown that transient pore formation can be induced through several methods, including electroporation [15–19], antimicrobial peptides [20,21], cationic polymers [22], external stress [15], shock waves [23,24], and sonoporation [25]. A more complete, broad review on defect-mediated transport can be found elsewhere [26–28]. We also suggest that the reader see the review paper by Böckmann in this special issue for more information on membrane pore formation [29].

Here, we describe only a few recent simulation works on pore formation in lipid membranes (or water leakage) induced by adsorption or penetration of small solvent molecules. Dimethylsulfoxide (DMSO) is thought to be a potentially pore-forming aprotic solvent. MD studies showed that DMSO strongly changes the physical properties of dipalmitoyl phosphatidylcholine (DPPC) membrane by penetrating into the hydrophobic core [30,31]. It was found that the effect of DMSO on the membrane properties was stronger than that of alcohols and sugars [30]. Furthermore, an MD simulation study using the MARTINI coarse-grained model showed that DMSO actually induces a pore formation in the DPPC bilayer membrane [32]. Upon increasing the molar ratio of DMSO in solvent, area compressibility and mean curvature moduli of the DPPC membrane are gradually lowered. At 27 mol% of DMSO, water pore formation across the DPPC membrane could be detected after 240 ns in the MD simulation. Obviously, permeability was significantly increased by pore formation. DMSO molecules, likely to be found just below the headgroup region, function as spacers/pivots that enhance lipid–lipid separation. Thus, they enable the bilayer to readily adopt a curved structure to accommodate any stress. This behavior may be common for small amphiphilic molecules.

Alcohol significantly affects membrane properties [33,34]. Indeed, an MD study showed softening of the phosphatidylcholine (PC) membrane by adding either ethanol or methanol [35]. Frequent migration of ethanol across the membrane has also been observed [35]. Generally speaking, addition of a short-chain, small alcohol enhances lipid dynamics and results in higher permeability of the PC membrane. Accelerated water permeation due to the addition of ethanol through a ceramide 2 (CER2) bilayer, as a model system of stratum corneum (SC), was also observed by an MD simulation [36]. In this case, enhanced permeability is not due to the softening of membrane by alcohol; instead, ethanol induced the formation of water-permeable defects in the CER2 membrane. In contrast to the dimyristoyl phosphatidylcholine (DMPC) membrane, the CER2 membrane exhibits higher-ordered (gel) packing of hydrophobic chains at 305 K [37]. As ethanol penetrates into the bilayer, it forms local defects that facilitate the penetration of additional ethanol molecules. This results in the formation of chains of ethanol that span from the outer membrane into the membrane core. (Fig. 1) Thus, this modification provides a pathway for water to access the membrane interior and enhances water permeation. Further addition of ethanol induced extraction of CER2 from the bilayer into the solvent phase. Even with a few dissolved CER2 molecules in the solvent, the bilayer seemed to be stable, with well-aligned packing of hydrophobic chains. The overall stability of membrane structure could be explained by the hydrogen bonding networks between CER2 headgroups; thus, ethanol acted as a small pore-forming agent in a well aligned membrane.

3. Measurement of membrane permeability

3.1. Permeation of water and small neutral molecules

The permeability of small molecules, such as water, across the membrane, can be explained by the solubility-diffusion model [7]. Several studies have examined permeability in terms of membrane properties. A very simple, three-layer model to explain passive permeability of water through lipid bilayers has been proposed based on the solubility-diffusion model, in which permeability is related to the partition coefficient of solutes in the hydrocarbon environment according to Overton's rule [38]. Based on experimental measurements of permeability and membrane structural properties, water permeability was shown to be strongly correlated with the area per lipid, although correlations with membrane thickness, curvature modulus, and area compressibility are not so clear. (See Fig. 2) In fact, a systematic permeability measurements for PC, phsophatidylserine (PS), and