Annals of Biomedical Research
An open access journal
ISSN 2817-2094
eSciRes
Mini Review
ABR-6-131

Cholesterol in Virus-Cell Membrane Fusion – A Perspective from Computational Chemistry

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Abstract

In this short article, we discuss several recent studies that applied molecular dynamic (MD) simulations to the questions in the mechanisms for viral-cell membrane fusion. We focus on the roles of the cholesterol as a membrane component. Cholesterol can change basic membrane properties and, in addition, modify membrane properties through regulation of protein distribution and peptide/protein conformation. Clearly, MD simulations may well be utilized in a wide range of settings of this area in the future. On the other hand, while the structural plasticity of peptides/proteins is likely to play key roles in virus-induced membrane fusion and the structural changes occur depending on the membrane lipid composition, further improvements in computational methods appear necessary for significant contributions in the area related to such structural plasticity.

Introduction

The mechanism for virus-cell membrane fusion has been under intensive study. The studies of atomistic details of the molecular machinery serving in such membrane fusion has deepen our understanding how specific lipid-protein interaction support such membrane fusion [1]. In the following, we review the experimental findings on membrane lipids relevant to the fusion and then provide some computational perspectives with a focus on cholesterol and conformational changes of fusogenic peptides regulated by membrane cholesterol. For broad aspects of viral infection and MD simulation approaches, excellent review articles are recommended [2,3].

Cholesterol is necessary as a membrane constituent in virus-host cell membrane fusion

Cholesterol is known to be important in viral infection. Cholesterol is associated with the successful entry of influenza virus [4] and SARS-CoV2 [5] into the host cell. In general, cholesterol induces a negative curvature to the membrane due to its smaller headgroup compared to the hydrophobic tail. This feature is considered to support the formation of stalk intermediate, and promoting membrane fusion [6,7]. In addition to such direct effects of cholesterol on general properties of membranes, cholesterol also exerts effects on membranes though modifying structural dynamics of membrane proteins [7,8].

Recent studies focusing on the effects of cholesterol on (protein-free) membrane include Fiorin et al. that used MD simulations to measure directly the free energy of membrane bending [9]. The authors' approach allows quantification of the membrane-stiffening effect of cholesterol. More recently, Pöhnl et al. used protein-free membranes to analyze membrane bending and cholesterol redistribution using MD simulations [10]. The authors observed that, while cholesterol stiffens and thickens the membrane of the saturated acyl chain lipids, in some settings with DOPC (dioleoylphosphatidylcholine) membranes, cholesterol rather softens membranes. This calls for deeper understanding of cholesterol effects on mechanical membrane properties and also highlights the usefulness of MD simulations. We previously observed that the sharp curvature in the branching membranes mimicking the hemifusion state often coincided with the boundary of L_o (liquid ordered) and L_d (liquid disordered) microdomains, suggesting that cholesterol can contribute to lipid sorting through microdomain formation and through curvature-driven lipid sorting [11]. In the future, more simulation-based studies may address the question regarding whether L_o/L_d boundary assists the formation of strong curvature enabling the budding of virus particles from cellular membranes.

Evidence shows that cholesterol also promotes membrane fusion through regulation of localization of membrane proteins. The ability of cholesterol and phospholipids to form microdomains is considered to play important roles in this aspect. Using a pseudovirus, Yang et al. showed that the boundaries between the L_o and L_d coincided the sites of the virus docking and fusion [12]. Importantly, the edge of microdomain has a unique feature; the existence of a line tension that has been proposed to control membrane deformation, budding and fission [13]. Influenza M2 channel is known to form clusters in the cholesterol-rich microdomain, and Kolokouris and coworkers showed that the clusters are localized at the L_o/L_d boundary [14]. Using coarse-grained (CG) MD simulations, the authors found that cholesterol fills the gap between adjacent M2 channels, thereby stabilizing the clusters. Assisted by the wedge-like structure of the unit 'TM-AH', where TM represents transmembrane (TM) helix and the AH represents amphipathic helices, the M2 cluster is considered to induce negative curvature necessary for virus budding [14]. Recent studies on M2 channel include Lincoff et al. [15]. In their unbiased atomistic simulations, the conformation of the AH domain was dynamic and the fourfold symmetry observed for

M2 protein was easily broken. Their further simulations, in which the M2 protein was restrained to each of the three structures with differing levels of symmetry, all the structures induced membrane deformation, with pronounced curvature generation in the extracellular leaflet and strong lipid tilt around the amphipathic helices in the intracellular leaflet. Overall, their findings showed that M2 channels can sense the membrane curvature and fits to the membranes with negative curvature [15].

We recently used metadynamics (metd) simulations to analyze the free energy landscape of phospholipid protrusion near the fusogenic peptide CpreTM derived from HIV gp41 MPER (membrane-proximal external region). Metd is a method that expedites sampling by adding a repulsive history-dependent potential energy along a set of reaction coordinates called collective variables (CVs). Our results showed that the peptide facilitates the POPC (1-palmitoyl-2-oleoyl-sn-glycero-phosphocholine;) protrusion in the cholesterol-containing POPC membrane but not in cholesterol-free POPC membrane [16]. In a broad sense, such phospholipid protrusions can induce negative curvatures of membrane, as the phospholipid protrusion can reduce the lateral pressure of the outer monolayer. In future atomistic (AT) or CG MD simulations addressing the peptide/protein effects on membrane curvature, fusogenic peptides/proteins may be placed in the membranes which have free ends, so as to reduce the artifacts arising from the small membrane size (and the membrane continuity to the adjacent simulation box), which obscure the intuitive understanding of the curvature-inducing effects of peptides/proteins. We also reported in [16] that, in the cholesterol-containing membrane, the peptide CpreTM resided at shallower positions in the membrane-water interface compared to the case with cholesterol-free membrane. It may be that the well-known cholesterol-phospholipid interaction tends to bring most amphiphilic peptides to shallower positions regardless of their conformation. This may bring the aromatic amino acid residues to shallower positions as well, thereby promoting the POPC protrusion [16]. This may explain the reason why cholesterol is necessary for the CpreTM-mediated membrane fusion, which seems rather puzzling given that cholesterol generally stiffens POPC membranes.

Fusion peptides derived from viral proteins have been a major focus of research. Such studies have provided important insights into the mechanisms for the peptide-induced membrane fusion [6]. Viral fusogenic peptides can modulate basic membrane properties. To name but a few studies, the influenza virus fusion peptide has been shown to increase the lipid order of the membrane [17]. The increased order is considered to generate negative curvature in the membrane, which in turn increases the propensity for membrane fusion [17]. More recently, Basso et al. analyzed the effects of the two SARS-CoV fusion peptides named $SARS_{FP}$ and $SARS_{IFP}$, and observed that both peptides can increase lipid packing and headgroup ordering.They further showed that the peptides also reduce the water content for anionic membranes, likely increasing membrane curvature [18].

Free simulations aided by high-performance computing have recently been used, allowing sufficient sampling in systems with large membranes focusing on the effect of peptide oligomer formation on the membrane properties [19]. Although this is not a study focusing on cholesterol, Valério et al. performed AT and CG MD simulations along with biophysical experiments to study the mechanism of the parainfluenza fusion peptide-mediated membrane fusion [20]. Their CG MD showed oligomer formation of the fusion peptide in a membrane-spanning configuration, which assumed a pore-like structure, in support of the experimental results showing pore formation. Their AT MD including metd showed dynamic helicity of the peptide in support of the experiments that showed that, upon interaction with membranes, random-coil conformation changed to α-helical conformation. This study underscores the usefulness of the multiscale simulations as well as the importance of the combination of experiments and simulations.

Conformational plasticity as a key feature of fusogenic peptides

It should be reminded that the overall fusogenicity of a peptide is determined by a number of steps, not only by the ability to perturb the membrane. The current consensus postulates that structural plasticity is important for the overall fusogenicity of the peptide and that such plasticity reflects the presence of several distinct steps critical in membrane fusion. In the following, we first discuss several experimental studies supporting such structural plasticity of peptides.

The fusion peptide of influenza virus (HAfp1-20) has been analyzed by many research groups. Its inverted V (boomerang-like) shape has been considered important for its fusogenicity [1]. However, Worch et al. showed that the fusion peptide (HAfp1-23), which was longer by three amino acid residues than HAfp1-20, can promote the membrane fusion more efficiently than HAfp1-20 [21]. Intriguingly, HAfp1-23 assumed a helical hairpin structure [21], suggesting structural plasticity of the HA fusion peptide. In the case of the fusion peptide (M770-L788) of SARS-CoV S protein, it assumed a V-shaped helical structure in dodecylphosphocholine micelles [22], but, when the structure of the longer peptide (R758-E821) was analyzed, discontinuous helical and extended conformation was observed [23]. In the case of HIV gp41 fusion peptide, the peptide formed an α-helix in membranes that contained low levels of cholesterol but formed β-sheet secondary structure in cholesterol-rich membranes [24], indicating a role for cholesterol in the conformational change of peptides. Further analyses led the authors to suggest that both α-helix and β-sheet conformations induce membrane fusion [24]. These findings argue that structural plasticity is a common feature of fusogenic peptides derived from viruses. It is also important to consider the possibility that changes in the oligomerization state can take place in association with the conformational changes. For instance, Meher et al showed that membrane cholesterol can induce oligomerization of the N-terminal fusion peptide of the SARS-CoV S protein [25] and that the

oligomer showed higher efficiency in promoting membrane fusion relative to monomers [25].

The peptides derived from the MPER-TMD (transmembrane domain) of gp41 are another example that showed diverse conformations and oligomeric states depending on the membrane composition and the peptide density [26,27]. Among them, the helix-rich structures were categorized into three groups [27]. Moreover, a fusogenic peptide (CpreTM) derived from MPER-TMD can assume an extended β-strand conformation in a cholesterol-rich membrane [26].

In the future, we expect that more simulation analyses will address the questions regarding to what extent peptide oligomerization can promote the lipid perturbation, dehydration, budding, and curvature formation. On the other hand, we feel that it has been generally difficult to use MD simulations to analyze slow processes such as the helix-coil transitions of peptides in the water-membrane interface due to the high energetic barriers between different conformers. It seems relevant to ask whether current methods for enhanced sampling can be a remedy for this issue.

Can enhanced conformational sampling methods help membrane fusion mediated by proteins/peptides?

Recently developed enhanced sampling techniques in MD simulations have been proved to be valuable tools for analyses of protein folding. Such techniques have been applied to membrane/protein systems [28,29], but, in our view, only a limited number of studies employed metd and accelerated MD (aMD) in protein (peptide)-mediated membrane fusion. In the following, let us consider potential benefits and challenges in these techniques when applied to protein/peptide-mediated membrane fusion studies.

Metd has been a powerful tool for protein folding studies [30]. At least to our knowledge, compared to the free energy analyses of protein folding, metd application to protein-ligand binding has been limited especially when the size of the protein is large. From our experience in which the three-dimensional free energy landscape of the ligand (in our case, POPC) position around a membrane-bound peptide was analyzed using atomistic metd simulations with a 5 × 5 nm bilayer, 50 × 500 ns simulations were necessary to obtain good convergence, which costs eight months using 30 intel Core i5 PCs despite our use of the united-atom model (Berger model) for the membrane lipids [16].This cost was mainly caused by the slow diffusion of the ligand in the membrane and in the membrane/water interface. It may thus be suggested to use a smaller ligand, which has a similar function. For example, a phospholipid molecule with short acyl chains, which diffuses more rapidly, may suffice for preliminary analyses of the peptide effect on phospholipids protrusion.

In our view, conformational changes of the peptides relevant to virus-mediated membrane fusion have not been analyzed intensively by metd. Despite the general interest in conformational changes of fusogenic peptides as we discussed above, at least so far, metd does not appear suitable to this issue. This may be mainly because conformational changes of peptides longer than ~20 amino acids are difficult to control and tract in the current metd technique because the number of CVs necessary for this handling exceeds three, which makes analyses difficult. Another issue is that the presence of lipids slows the convergence, increasing the computational cost to a prohibitive degree for analyses of conformation changes. Thus, it would be highly challenging to compute various conformers within one trajectory. In contrast, the quantification of peptide effect on membrane lipid dynamics seems relatively easy for a given conformer. Therefore, computation using a particular peptide conformer and later comparison across different conformers may become an approach widely used in near future.

Because of its merits in sampling, the accelerated MD (aMD) is drawing researchers' attention. In this method, the potential energy landscape is modified by adding a non-negative bias potential to the energy wells below a certain threshold, whereas the energy barriers above the threshold are unchanged [31]. This procedure makes the sampling of the conformational space efficient. Wang et al showed that aMD can accelerate the trans-gauche isomerization and lateral diffusion of phospholipids in atomistic bilayer simulations [32]. This procedure produced speedup in trans-gauche isomerization and lipid lateral diffusion, resulting in a 2-3 fold speedup compared to conventional MD. Therefore, aMD is likely to be applied to an increasing number of lipid membrane/protein systems. Although the movements of molecules in aMD are artificial and not natural, aMD may help analyses when one needs to analyze quickly what types of lipid (e.g., cholesterol or unsaturated phospholipids, etc...) bind to the protein. Miao et al applied aMD to conformational changes of a G-protein coupled receptor (GPCR) and free energy analysis in lipid bilayer system [33]. One may envisage the potential usefulness of this method in obtaining the free energy landscape covering various conformations of the peptide as well as in examining the types of lipid that associate the peptide, for each peptide conformer. The combination of conventional MD and aMD will also give insights in such analyses. The study by Wang and Miao is another example of aMD application, in which the potential energy for a protein-protein interaction was boosted so as to facilitate the slow dissociation process between protein partners [34].

Clearly, aMD can benefit the sampling of various conformers. However, we surmise that aMD-based free energy computation of membrane protein/peptide conformations as well as of peptide-induced membrane perturbation needs further checking of the accuracy of this technique. It is important to ensure that the free energy landscape covering different conformers derived by aMD is correct to the degree comparable to the analyses with metd and other methods. This is especially pertinent to the membrane systems, given that slow processes, such as diffusion of peptide and lipid molecules, have to be covered for each conformer, so rapid conformational changes may lead to insufficient relaxation in terms of interaction between peptide and surrounding lipids.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Received date: February 09, 2025; **Accepted date:** February 10, 2025; **Published date:** February 21, 2025

Citation: Nishizawa K (2025) Cholesterol in Virus-Cell Membrane Fusion – A Perspective from Computational Chemistry. *Ann Biomed Res* 6(1): 131.