Role of L-Proline in Stabilization of Biological Membranes

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Abstract

L-proline, a natural α-amino acid, has been found useful as an osmoprotectant and antioxidant. It can prevent the denaturation of peptides and increase the survival rate of freeze-dried fungi by inhibiting the generation of intracellular reactive oxygen species. L-proline was shown to effectively preserve the structure and function of the frozen vesicles. But there is evidence showing that L-proline was able to destabilize the lamellar liquid-crystalline phases in both fully hydrated and freeze-dried lipids, which is undesirable. Given the complex role of L-proline in the stabilization of biologics, the current study conducted molecule dynamics simulations of hydrated 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) bilayers in the presence of L-proline to elucidate the interactions between L-proline and lipid bilayers. The results show that the proline molecules can slightly perturb lipid headgroups with occasional insert of proline molecules between lipid headgroups. In the fully hydrated state, proline molecules prefer to hydrogen-bond with water molecules rather than lipids. The MD simulation results do not support the 'water replacement' hypothesis for the mechanism of membrane protection by proline.

Keywords: Lipids; Osmoprotectant; Antioxidant; Molecular dynamics simulation; Biopreservation

Introduction

L-proline is a natural α-amino acid that is used in the biosynthesis of proteins. In the aqueous solution of L-proline, an H^+ ion is transferred from the -COOH group to the $-NH_2$ group to form a zwitterion as illustrated in Figure 1. L-proline has multiple advantages such as high solubility (up to 6 M at 25 °C), neutral pH, and low toxicity even at high concentrations. In plants, proline is accumulated intracellularly at high levels as an osmoprotectant to cope with stresses such as drought and salinity. L-proline is also synthesized in overwintering insects to stabilize membranes at subzero temperatures. Rudolph and Crowe[1] demonstrated that L-proline could effectively preserve the structure and function of the frozen vesicles as assessed by the freeze fracture and Ca^+-transport ability of membranes. Also, Jas et al.[2] showed that L-proline could prevent the denaturation of N-acetyl-tryptophan-amide by strongly stabilizing both water−water and water−peptide hydrogen bonds. L-proline was also found to increase the survival rate of freeze-dried fungi *M. rouxii* by inhibiting apoptosis-inducing stresses such as the generation of intracellular reactive oxygen species (ROS).[3] However, the study by Tsvetkova et al.[4] proposed that L-proline was able to destabilize the lamellar liquid-crystalline phases in both fully hydrated and freeze-dried lipids. So far, the molecular mechanisms underlying either the stabilizing or destabilizing effect of L-proline on biomolecules and living organisms have rarely been explored. This study conducts molecule dynamics (MD) simulations of hydrated 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) bilayers in the presence

of L-proline to investigate the interactions between L-proline and lipids.

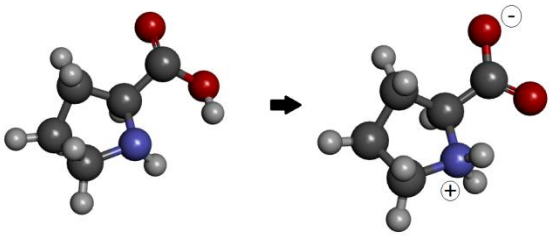

Figure 1. L-proline molecule (left) and its zwitterionic structure in aqueous solutions (right). (Blue: nitrogen, red: oxygen, grey: carbon, and white: hydrogen)

Computational Methods

The simulation system consists of a DPPC bilayer (128 lipid molecules in each leaflet) hydrated by 10240 water molecules as shown in Figure 2. A total of 128 L-proline molecules are dissolved in the aqueous phase, yielding a concentration of ~695 mM. The simulations in this study were conducted by using the MD simulation package NAMD.[5] The CHARMM36 force field for lipids and the modified TIP3P water model was employed. The L-proline molecule was parameterized by the MATCH webserver.[6] The simulation system was equilibrated with the isothermal-isobaric ensemble for 1 ns and then run for another 10 ns for data collection. The isothermal-isobaric ensemble kept the number of molecules constant and maintained the pressure and temperature at 1 atm and 323 K, respectively. Other parameters related to the simulation procedures are the same as those reported in the previous studies.[7, 8]

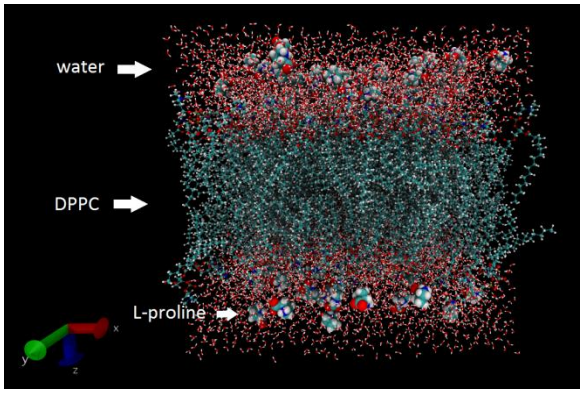

Figure 2. The simulation system of hydrated DPPC bilayers in the presence of L-proline.

Results and Discussion

The position of L-proline molecules with respect to the x-y plane of lipid bilayer was evaluated from the density profiles of representative atoms including the nitrogen ($N_{proline}$) and oxygen ($O_{proline}$) atoms of proline molecules and the nitrogen (N_{DPPC}) and phosphorus (P_{DPPC}) atoms of lipid headgroups as shown in Figure 3. Proline molecules were shown to slightly perturb lipid headgroups because the density profiles of $N_{proline}$ and $O_{proline}$ overlap with those of N_{DPPC} and P_{DPPC}. But the L-proline molecules do not penetrate into the lipid bilayer as far as the water molecules do (data not shown).

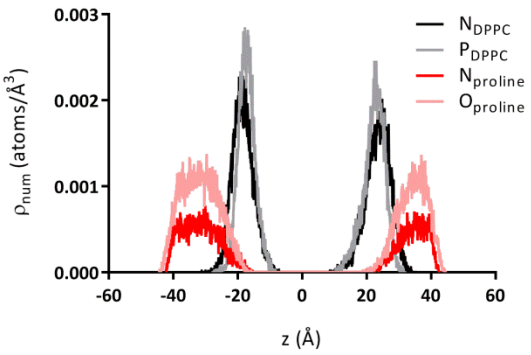

Figure 3. The density profiles of various atoms representative of the DPPC lipid headgroups and the L-proline molecule.

Nevertheless, a couple of L-proline molecules were observed to occasionally insert deeply between the lipid headgroups as shown in Figure 4. One may speculate that the further increase in the proline concentration can increase the incidence of such phenomenon. As seen in Figure 4, a proline molecule is surrounded by three lipid molecules. It is suggested that proline molecules are capable of staying between lipid headgroups and keeping the spacing, which may prevent the structural disruption of lipid bilayers when biomembranes are dehydrated. But given the radial distribution profiles that will be shown in Figure 5, the insert of proline molecules cannot be frequent.

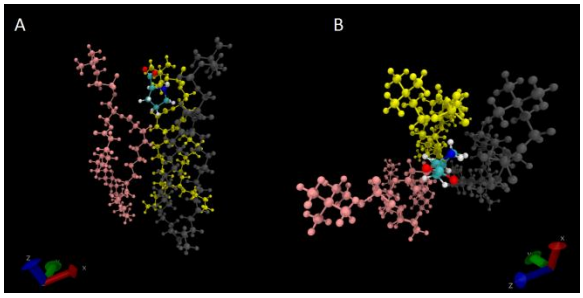

Figure 4. An L-proline molecule interacting with DPPC headgroups. (A: side view; B: top view. The three lipid molecules are indicated by yellow, grey and pink, respectively. The proline molecule is represented in atom-specific colors)

The radial distribution function (g(r)) is a pair correlation function that describes how the atoms in a system are radially packed around the reference atoms. The g(r) of the pairs of $O_{proline}$-O_w (O_w represents the oxygen atom of water) and $O_{proline}$-O_{DPPC} were shown in Figure 5. It is evident that the proline molecules prefer to interact with water molecules through hydrogen-bonding. The interaction preference can be demonstrated by the g(r) peak at r=~2.7 Å which is the first hydration shell of proline molecules. Therefore, in fully hydrated state, the interaction between proline and DPPC molecules are unnoticeable as shown in Figure 5.

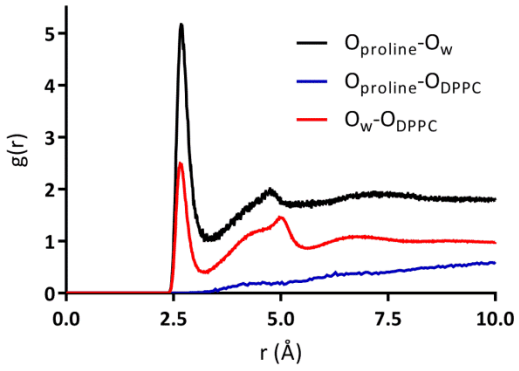

Figure 5. The radial distribution function g(r) of the pairs of $O_{proline}$-O_w, $O_{proline}$-O_{DPPC}, and O_w-O_{DPPC}.

This study investigated the interactions between L-proline and lipid bilayers by MD simulations of hydrated DPPC bilayers in the presence of L-proline. Even though proline molecules were observed to insert between lipid headgroups occasionally, they can only slightly perturb lipid headgroups. Moreover, proline prefer to hydrogen-bond with water rather than lipids. The MD simulation results do not support the 'water replacement' hypothesis for the mechanism of membrane protection by proline, at least in the fully hydrated state. According to the 'water replacement' hypothesis, sugar molecules such as sucrose and trehalose can stabilize the dry lipid membranes by keeping the spacing between lipid headgroups under dehydration. In contrast, the previous study showed that proline molecules may act as water-structure making reagents by stabilizing the structure of the bulky water.[4] It is speculated here that, due to the increased interfacial tension, the presence of proline molecules in the aqueous phase may

contribute to the preferential formation of more closely packed lipid phases, with less interface between aqueous and lipid phases. More details have to be elucidated in future studies.

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