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PROTECTIVE EFFECT OF QUERCETIN ON AMYLOID-INDUCED ALTERATIONS IN LIPID BILAYER INTEGRITY

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The present study employs molecular dynamics simulations to investigate the interactions between quercetin, amyloid fibrils, and POPC lipid bilayers. The results demonstrate that quercetin does not significantly affect the molecular organization of the bilayer, while IAPP fibrils induce substantial structural changes, particularly in the outer monolayer. Quercetin mitigates these effects by reducing the impact on headgroup and glycerol regions and causing a more superficial positioning of IAPP. Additionally, quercetin slightly decreases the order of sn-2 acyl chains, indicating a disordering effect. In a ternary system with POPC, quercetin, and IAPP, the reduction in the deuterium order parameter of sn-2 acyl chains is less pronounced, underscoring quercetin's protective role. Unlike IAPP, ApoAI and insulin fibrils undergo significant structural reorganization in the membrane-bound state. Quercetin attenuative effects are observed only with ApoAI, highlighting its potential as a protective agent against amyloid-induced membrane disruption. These findings provide valuable insights into the interactions between polyphenols, amyloid fibrils, and lipid membranes, contributing to the understanding of membrane-associated amyloid pathologies.
Key words: *Amyloid fibrils; Lipid membranes; Polyphenols; Molecular dynamics*
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The interaction between amyloid fibrils and lipid bilayers is a critical factor in the pathogenesis of several neurodegenerative diseases, including Alzheimer's and Parkinson's diseases. These fibrils, formed by the misfolding and aggregation of proteins, possess a unique structural arrangement characterized by cross-β-sheet conformations, which confer them with significant mechanical stability and resistance to proteolytic degradation [1,2]. The cytotoxicity associated with amyloid fibrils is primarily attributed to their ability to disrupt cellular membranes, thereby compromising cellular integrity and function. This membrane disruption is facilitated by the interaction of amyloid fibrils with lipid bilayers, leading to increased membrane permeability and potential cell death. Quercetin, a naturally occurring flavonoid, has garnered attention for its potential protective effects against oxidative stress and its ability to modulate the interactions of lipid membranes with biomacromolecules [3]. Quercetin is known for its antioxidative properties, which include the inhibition of lipid peroxidation and the scavenging of reactive oxygen species (ROS) [4,5]. These properties are crucial in maintaining the structural integrity of lipid bilayers, which are susceptible to oxidative damage. Moreover, quercetin has been shown to interact with lipid membranes, potentially altering their physical properties and influencing their interactions with amyloid fibrils [6,7]. Recent studies have demonstrated that quercetin can disaggregate amyloid fibrils, such as those formed by Aβ-peptide, and reduce their cytotoxic effects [8]. This disaggregation ability suggests that quercetin may alter the morphology of amyloid fibrils, leading to the formation of less toxic aggregates that are less capable of disrupting lipid bilayers. Additionally, the binding of quercetin to amyloid fibrils has been shown to decrease the fibril-induced cytotoxicity, further supporting its protective role [9]. The protective effect of quercetin on lipid bilayers against the modulatory action of amyloid fibrils is thus extremely important since understanding the mechanisms by which quercetin exerts its protective effects could lead to the development of novel therapeutic strategies aimed at mitigating the deleterious effects of amyloid fibrils in neurodegenerative diseases. In the present work, using the molecular dynamics simulations, we aimed at elucidating the molecular-level interactions between quercetin, amyloid fibrils, and lipid bilayers to provide insights into the potential of quercetin as a protective agent in the context of amyloid-related cytotoxicity.

METHODS

To perform molecular dynamics (MD) simulations of the model membrane systems the input files were prepared using the web-based graphical interface CHARMM-GUI. The topologies of polyphenols were generated using the CHARMM-GUI Ligand Reader and Modeler. The obtained files were further used to generate the quercetin-lipid systems using the Membrane Builder option. The model lipid bilayer was composed of 72 of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC) in each monolayer. The initial distance of quercetin translation from the membrane midplane along the bilayer normal was 10 Å. To obtain a neutral total charge of the system a necessary number of counterions was added. The molecular dynamics simulations of the model membrane systems and analysis of the trajectories were carried out using the GROMACS 2024.2 software with the CHARMM36m force field at a temperature of 310 K in the NPT ensemble with the time step for MD simulations 2 fs. The Particle Mesh Ewald method was utilized for correct treatment of the long-range electrostatic interactions. The bond lengths were constrained using the LINC algorithm. The pressure

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and temperature controls were performed using the V-rescale thermostat. The MD simulations were performed with minimization of 50000 steps and equilibration of 12500000 steps. The whole-time interval for MD calculations was 10 ns. The GROMACS command gmx density was used to calculate the mass density distribution for various components of the lipid bilayer and density distribution of quercetin across a lipid bilayer. The molecular graphics and visualization of the simulation evolution over time were performed using the Visual Molecular Dynamics (VMD) software.

RESULTS AND DISCUSSION

Molecular dynamics simulations of the systems POPC + quercetin, POPC + IAPP fibril and POPC + quercetin + IAPP fibril revealed that quercetin and IAPP reside at the lipid-water interface and do not leave the lipid bilayer throughout the simulation time. The persistence of both quercetin and IAPP fibrils at the lipid-water interface during the simulation period suggests a stable interaction with the lipid bilayer, which is crucial for understanding their modulatory effects on membrane integrity. Specifically, the localization of quercetin at the lipid-water interface aligns with its amphipathic nature, allowing it to interact favorably with both the hydrophobic core and the polar head groups of the lipid bilayer. Similarly, the retention of IAPP fibrils at the lipid-water interface underscores their propensity to interact with lipid membranes, a characteristic that is implicated in their cytotoxic effects.

The analysis of the density distributions showed that quercetin does not exert significant influence on the molecular organization of headgroup, glycerol and acyl chain regions of POPC bilayer (Fig. 1).

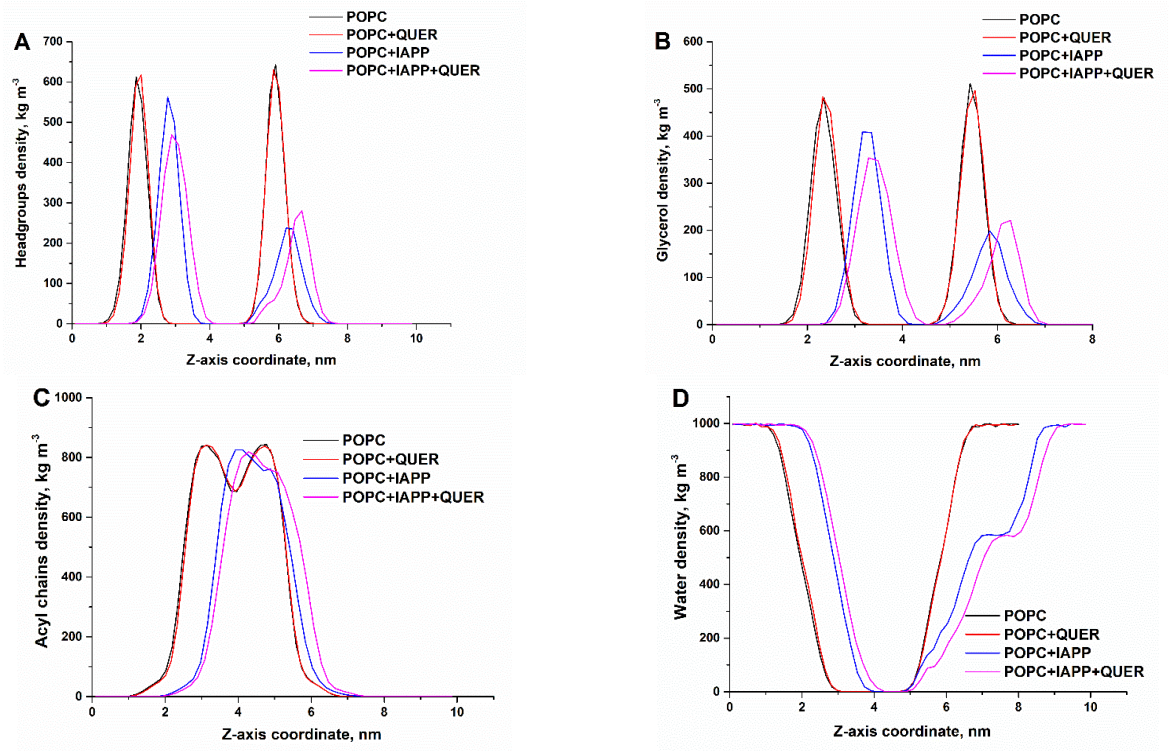

Figure 1. The density distributions of headgroups (A), glycerols (B), acyl chains (C) and water (D) in POPC bilayer in the absence and presence of quercetin, IAPP and quercetin + IAPP

Quercetin may integrate into the bilayer through hydrophobic interactions without disturbing the tightly packed acyl chains, thus maintaining the bilayer's structural integrity. Additionally, its polar nature might allow it to associate with the headgroups without causing significant rearrangement or density changes. In contrast, IAPP fibrils cause a decrease in the density of headgroups and glycerols, with the magnitude of this effect being much greater for the outer monolayer. Likewise, the density distribution of acyl chains and water become asymmetric in the presence of IAPP. These findings suggest that fibrillar IAPP is capable of inducing substantial changes in the lipid bilayer structure. This disruptive influence can be attributed to several factors. IAPP fibrils may insert into the bilayer, displacing or reorganizing lipid molecules, particularly in the outer monolayer where they are more accessible. The fibrillar structure of IAPP can disrupt the orderly arrangement of lipids, leading to decreased density and asymmetry. This disruption is more pronounced in the headgroup and glycerol regions due to their proximity to the aqueous environment where fibrils form. Additionally, IAPP fibrils may interact with both the hydrophobic core and the polar headgroups of the bilayer, causing reorganization that results in the observed density changes.

Remarkably, addition of quercetin leads to some attenuation the fibril impact on the regions of head groups and glycerol in the outer monolayer, accompanied by slight alterations in the acyl chain and water distributions. Additionally, in the presence of quercetin the position of IAPP becomes more shallow (data not shown). The polyphenolic structure of quercetin allows it to integrate into the lipid bilayer, where it can form stabilizing interactions with the polar head groups

and glycerol backbone. This integration may enhance the rigidity and stability of the bilayer, thereby reducing the susceptibility of the membrane to perturbations caused by amyloid fibril formation. By interacting with the lipid head groups, quercetin may also influence the distribution of acyl chains and water molecules, promoting a more ordered and less permeable membrane structure.

As seen in Fig. 2, the deuterium order parameter of sn-1 acyl chains was not influenced by quercetin, but showed a slight decrease in the presence of IAPP fibrils. At the same time, the deuterium order parameter of sn-2 acyl chains attained markedly less values in the presence of quercetin or IAPP relative to the neat POPC, suggesting the disordering effects of this polyphenol and amyloid fibrils on the nonpolar part of POPC bilayer. Notably, in the ternary system POPC + quercetin + IAPP the magnitude of the IAPP-induced decrease of the deuterium order parameter of sn-2 acyl chains appeared to be markedly less compared to the system POPC + IAPP. This finding provides additional arguments in favor of quercetin ability to exert protective effect against the disruption of lipid bilayer structural integrity produced by the IAPP amyloid fibrils

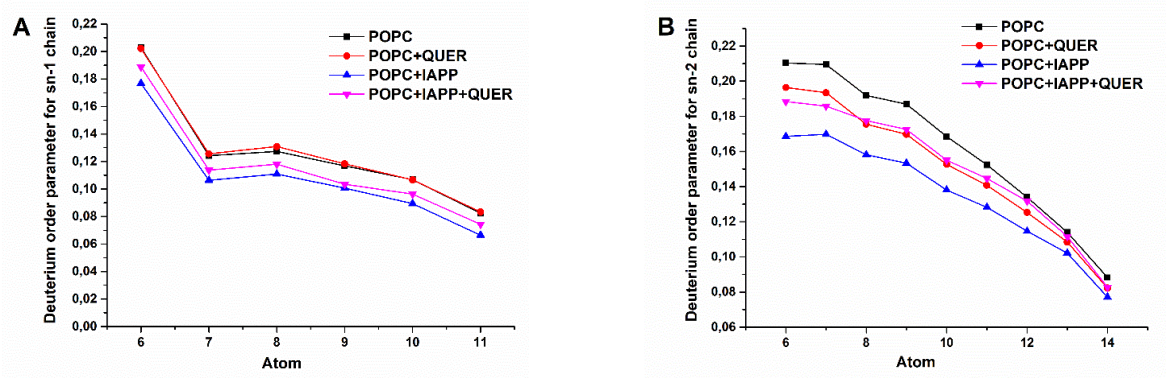

Figure 2. Deuterium order parameter for sn-1 (A) and sn-2 (B) chains calculated for the neat POPC, binary systems POPC + quercetin / IAPP and ternary system POPC + quercetin + IAPP

Contrary to IAPP, amyloid fibrils of apolipoprotein A-I (ApoAI) and insulin (InsF) have undergone significant structural reorganization in the membrane-bound state followed by essential loss of the InsF beta structure and disruption of ApoAI fibril integrity which may be indicative of a destabilization or unfolding of the fibril highly ordered conformation.

These findings highlight the dynamic nature of amyloid fibrils and their potential to alter conformation in response to environmental factors such as membrane binding, which could have implications for understanding the pathophysiology of amyloid-related diseases.

Furthermore, the analysis of the density distributions showed that ApoAI and InsF, similar to IAPP, induce considerable decrease in the density of headgroups, glycerols and acyl chains (Figs. 3, 4).

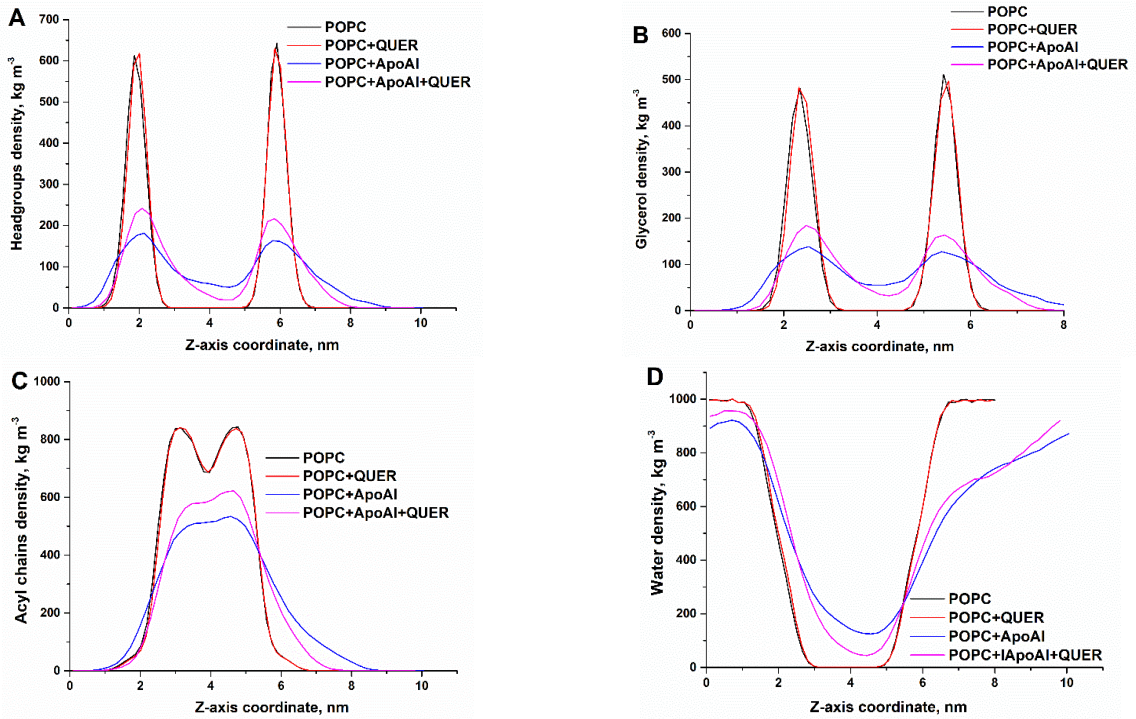

Figure 3. The density distributions of headgroups (A), glycerols (B), acyl chains and water (D) in POPC bilayer in the absence and presence of quercetin, ApoAI and quercetin + ApoAI

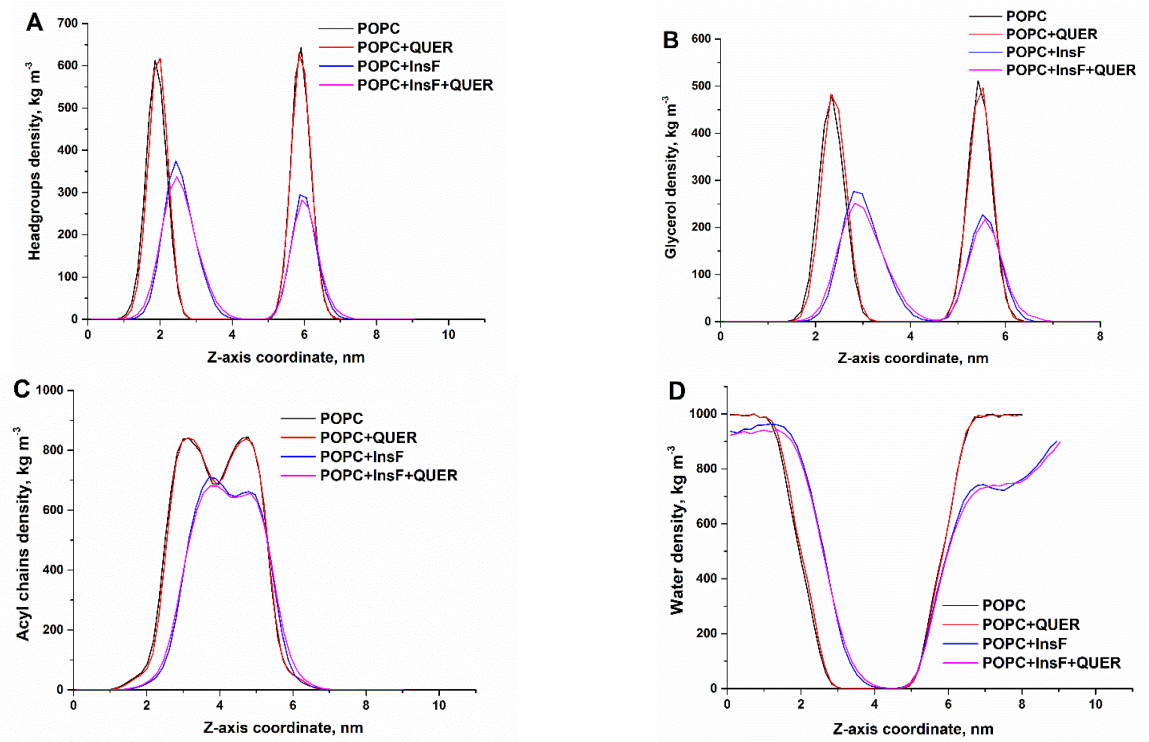

Figure 4. The density distributions of headgroups (A), glycerols (B), acyl chains and water (D) in POPC bilayer in the absence and presence of quercetin, InsF and quercetin + InsF

Remarkably, the attenuative effects of quercetin on the density of lipid molecular groups and the deuterium order parameter of sn-2 acyl chains were observed only for ApoAI (data not shown). These observations may suggest that quercetin interacts with lipid membranes in a manner that alters their structural properties, influencing membrane fluidity and dynamics when ApoAI is present. Likewise, compared to the binary systems POPC + quercetin / ApoAI / InsF, in the ternary systems containing both PF and fibrils, the quercetin tends to reside in a shallower location in the presence of ApoAI and InsF, the ApoAI center of mass shifts to bilayer center, while the position of the InsF center of mass remains unchanged (Fig. 5). This differential positioning may influence the structural and functional dynamics of the lipid bilayer, potentially affecting membrane stability and protein function.

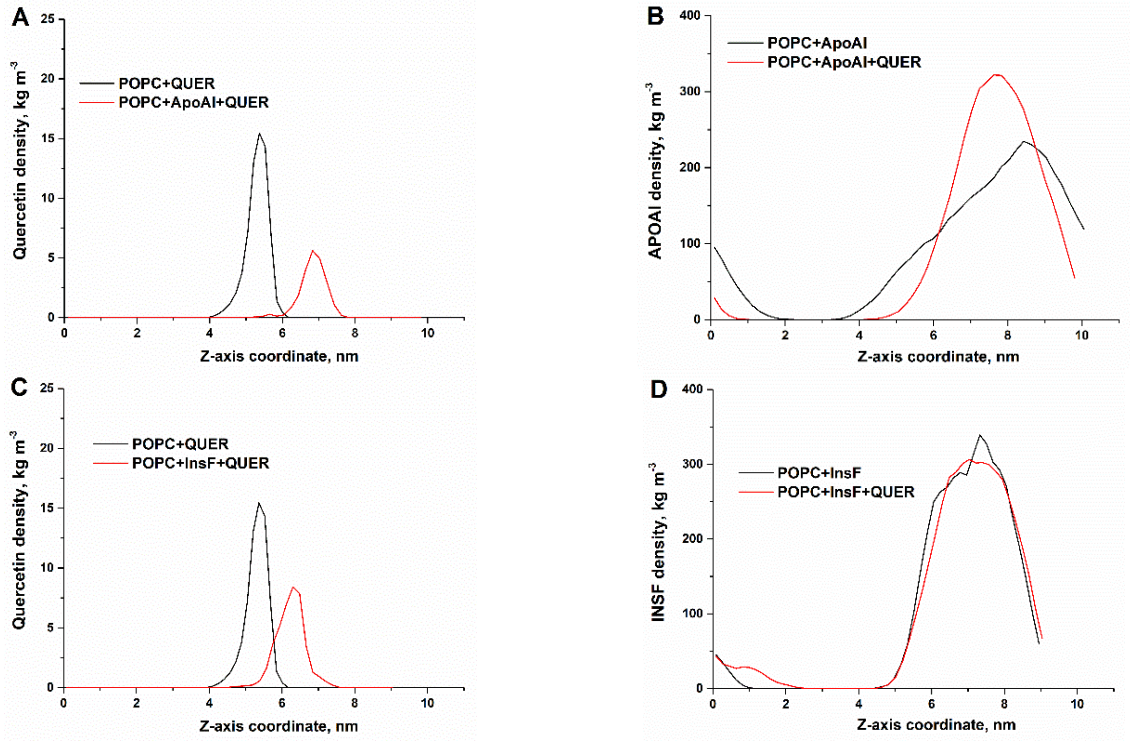

Figure 5. The mass density distributions of quercetin (A, C), ApoAI (B) and InsF (D) in POPC bilayer

Overall, the molecular dynamics simulations conducted on the model systems comprising lipid bilayers and amyloid fibrils reveal the protective mechanisms of polyphenols against amyloid fibril-induced membrane disruption. The inclusion of quercetin demonstrates a protective effect on both the polar and nonpolar regions of the lipid bilayer, mitigating the disruptive influences exerted by amyloid fibrils. Polyphenols, such as quercetin, have been extensively studied for their interactions with lipid membranes, where they exhibit the ability to penetrate and integrate into the lipid bilayer [10]. This integration can lead to alterations in the structural and physicochemical properties of the membrane. The protective role of quercetin in this context is likely attributed to its capacity to interact with both the hydrophilic and hydrophobic regions of the lipid bilayer [11]. This dual interaction is crucial as it allows quercetin to stabilize the membrane structure against perturbations caused by amyloid fibrils, which are known to disrupt membrane integrity by forming pores or altering membrane fluidity. Furthermore, the presence of quercetin in the lipid bilayer may also influence the mechanical properties of the membrane, enhancing its resilience to amyloid-induced stress. By decreasing membrane fluidity, quercetin can potentially prevent the insertion and destabilization caused by amyloid fibrils, thus maintaining membrane integrity. This protective effect is vital in biological systems where membrane disruption can lead to cellular dysfunction and contribute to the pathogenesis of amyloid-related diseases.

CONCLUSIONS

To summarize, the molecular dynamics simulations reveal that quercetin does not significantly alter the molecular organization of the POPC bilayer, while IAPP fibrils induce notable structural disruptions, particularly in the outer monolayer. Quercetin mitigates the impact of IAPP on the headgroup and glycerol regions, aligning with its protective role against polar region changes. The presence of quercetin results in a more superficial positioning of IAPP, and it slightly decreases the order of sn-2 acyl chains, suggesting a disordering effect. In a ternary system with POPC, quercetin, and IAPP, the reduction in the deuterium order parameter of sn-2 acyl chains is less pronounced, further supporting quercetin protective role. Unlike IAPP, ApoAI and insulin fibrils undergo significant structural reorganization when bound to the membrane. Quercetin's attenuative effects are observed only with ApoAI, and in ternary systems, it localizes more shallowly, with ApoAI center of mass shifting toward the bilayer center. These findings highlight quercetin potential as a protective agent against amyloid-induced membrane disruption.

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ЗАХИСНИЙ ЕФЕКТ КВЕРЦЕТИНУ ПРОТИ АМІЛОЇД-ІНДУКОВАНИХ ЗМІН ЦІЛІСНОСТІ ЛІПІДНОГО БІШАРУ

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У даному дослідженні було використано метод молекулярної динаміки для вивчення взаємодій між кверцетином, амілоїдними фібрилами та ліпідними бішарами з фосфатидилхоліну. Отримані результати показують, що кверцетин не має значного впливу на молекулярну організацію бішару, тоді як фібрили IAPP викликають суттєві структурні зміни, особливо у зовнішньому моношарі. Кверцетин зменшує ці ефекти, послаблюючи вплив фібрил на зону полярних голівок ліпідів та гліцеролові ділянки, а також спричиняючи більш поверхневе розташування IAPP. Крім того, кверцетин знижує ступінь впорядкованості sn-2 ацильних ланцюгів, що свідчить про його дестабілізуючий ефект. У трьохкомпонентній системі, що складалася з фосфатидилхоліну, кверцетину та IAPP, зниження параметра порядку sn-2 ацильних ланцюгів було менш виражене, що є додатковим аргументом на користь захисної ролі кверцетину. На відміну від IAPP, фібрили ApoAI та інсуліну зазнають значної структурної реорганізації у мембранозв'язаному стані. Ефекти ослаблення у присутності кверцетину спостерігаються лише з ApoAI, що підкреслює його потенціал як захисного агента проти амілоїд-індукованої деструкції ліпідного бішару. Отримані результати мають важливе значення у контексті взаємодії між поліфенолами, амілоїдними фібрилами та ліпідними мембранами, що сприяє розумінню мембрано-асоційованих амілоїдних патологій.

Ключові слова: амілоїдні фібрили, ліпідні мембрани, поліфеноли, молекулярна динаміка