Usage Report for Fiscal Year 2020

Project Title:

Protein-Ligand and Protein-Lipid Interaction

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1. Background and purpose

The most important border in the biological world, biological membranes, provide an essential barrier towards the outside world. Membranes protect the function of life within its boundaries and separate different areas within a cell to give rise to specialized cellular compartments. While biological membranes are crowded with proteins, their main constituents are a wide variety of lipids.

To allow the exchange of nutrients and information between the interior and the surrounding environment, a barrier or wall requires windows and doors. In a cellular context, this task is conducted by proteins, embedded in the lipid membranes of cells, such as receptors to convey signals or transporters, capable to transport nutrients or ions across this barrier.

2. Usage status and calculation methods

Quantum mechanics (QM) simulations utilizing the Gaussian 09 software package have been performed. Molecular dynamics simulations were performed utilizing the NAMD software package and results were visualized with VMD.

2.1 Results & Conclusion – Lipid-Lipid Interaction

Cholesteryl-β-D-glucoside (ChoGlc) is a mammalian glycolipid that is expressed in brain tissue. The effects of glucosylation on the ordering and lipid interactions of cholesterol (Cho) were examined in membranes composed of N-stearoyl sphingomyelin (SSM), which is abundant in the brain, and to investigate the possible molecular mechanism involved in these interactions. Differential scanning calorimetry revealed that ChoGlc was miscible with SSM in a similar extent of Cho. Solid-state 2H NMR of deuterated SSM and fluorescent anisotropy using 1,6-diphenylhexatriene demonstrated that the glucosylation of Cho significantly reduced the effect of the sterol tetracyclic core on the ordering of SSM chains. The orientation of the sterol core was further examined by solid-state NMR analysis of deuterated and fluorinated ChoGlc analogues. ChoGlc had a smaller tilt angle between the long molecular axis (C3-C17) and the membrane normal than Cho in SSM bilayers, and the alteration of the mean tilt angle was restricted even at high temperatures. This orientation of the sterol core of ChoGlc lead to reduce sterol-SSM interactions. The MD simulation results suggested that the Glc moiety perturbs the SSM-sterol interactions, which reduces the umbrella effect of the phosphocholine headgroup because the hydrophilic glucose moiety resides at the same depth as an SSM amide group. These differences between ChoGlc and Cho also weaken the SSM-ChoGlc interactions. Thus, the distribution and localization of Cho and ChoGlc possibly control the stability of sphingomyelin-based domains that transiently occur at specific locations in biological membranes.

2.2 Results and Conclusion – Lipid-Protein Interaction

Activation of lysolipid-sensitive G protein-coupled receptors (GPCR) does not only depend on lysolipid class, but also on the length and degree of saturation of their respective hydrophobic tails. Positive regulation of these signaling networks caused by the lipid chain length specificity of upstream phospholipases is firmly established. Non-agonistic lysolipid homologues, featuring incompatible lipid tails, have been suggested to indirectly modulate GPCR signaling by delaying agonist catabolism. Nonetheless, recent results seem inconsistent with this hypothesis. Utilizing a simplified lysolipid-GPCR signaling assay based on the established lysophosphatidyl glucoside-GPR55 signaling axis in primary sensory neurons, we demonstrate that short chain ligand homologs directly modulate receptor activation via a potent competitive antagonistic activity. Considering the well-documented tissue-specific concentration of lysolipid homologs, we propose that endogenous lysolipids with insufficient chain length for stable receptor activation exert an antagonistic activity, effectively representing a negative control mechanism for GPCR-associated lysolipid signaling.

4. Schedule and prospect for the future including aims for the next usage term

Specifics of the lipid-lipid and lipid-protein interaction at atomistic levels are still not well understood and thus remain under investigation.

For example, preferred conformation of lyso-phospholipids in solution state, micelle arrangement or solublized with methyl-β-cyclodextrine are still not well understood. To gain a more complete atomistic understanding of this lipid conformation, we will probe lyso-phospholipid conformation by MD simulation in different solution states and correlate our results with experimental observation such as nuclear magnetic resonance spectroscopy.

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Fiscal Year 2020 List of Publications Resulting from the Use of the supercomputer

[Paper accepted by a journal]

1. “Lysolipid Chain Length Switches Agonistic to Antagonistic G Protein-Coupled Receptor Modulation”; A.T. Guy, F. Ding, J. Abe, M. Inoue, Y. Hirabayashi, Y. Ito, H. Kamiguchi, P. Greimel, *ACS Chem. Neurosci.*, **2020**, 11(21):3635-3645.
2. “Systematic Synthesis of Novel Phosphoglycolipid Analogues as Potential Agonists of GPR55”; J. Abe, A.T. Guy, F. Ding, P. Greimel, Y. Hirabayashi, H. Kamiguchi and Y. Ito, *Org. Biomol. Chem.*, **2020**, 18(41):8467-8473.
3. “β-Glucosylation of Cholesterol Reduces Sterol-Sphingomyelin Interactions”; S. Hanashima, N. Fukuda, R. Malabeda, M. Murata, M. Kinoshita, P. Greimel, Y. Hirabayashi, *BBA – Biomembranes*, **2021**, 1863(2):183496.