Usage Report for Fiscal Year 2018

Project Title:

Protein-Ligand and Protein-Lipid Interaction

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1. Background and purpose

Biological membranes, the most important borders in the biological world, provide an essential barrier towards the outside world and protect the function of life inside each cell. Additionally, they separate different areas within a cell and thus give rise to specialized cellular compartments. While biological membranes are crowded with proteins, their main constituents are a wide variety of lipids.

Naturally each barrier or wall requires windows and doors to allow the exchange of nutrients and information between the two sides. 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.

Recently, we have demonstrated that phosphatidyl-ß-d-glucoside derived lyso-phosphatidyl-ß-d-glucoside (lyso-PtdGlc) is the first lipid derived regulator of central projections in nociceptive (pain) sensory afferents during embryonic development. Lyso-PtdGlc associated patterning of nociceptive sensory neurons in the developing spinal cord is associated with G-protein coupled receptor 55 (GPR55). GPR55, is highly expressed in the brain and peripheral nervous system, including dorsal root ganglion (DRG) sensory neurons, and immune system. It was initially suggested to be the surmised third cannabinoid receptor, due to its mild activation by cannabinoid ligands. Later, its homology to lysophosphophatidic acid (LPA) receptors was recognized and lyso-phosphatidylinositol was reported as a potent ligand. More recently, we demonstrated that lyso-PtdGlc is an endogenous ligand of GPR55 that regulates targeting of sensory neurons during development. GPR55 upregulation has been reported in a variety of cancers, while GPR55 knock out mice show abnormal responses to inflammation and mechanical stimuli, suggesting a role of GPR55 in neuropathic pain and inflammatory processes. Furthermore, GPR55 has been linked to a variety of physiological and pathological processes, such as synaptic transmission, obesity, bone development and gastrointestinal functions.

2. Usage status and calculation methods

Qquantum 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. Results

Utilizing our phosphorus(III) based approach, we have established synthetic access to lyso-PtdGlc and prepared a variety of synthetic lyso-PtdGlc analogues. The biological activity of our synthetic analogues was evaluated using our previously developed functional assay, based on primary cultured DRG sensory neurons endogenously expressing GPR55. Additionally, we investigated the biophysical properties of PtdGlc, compared it with phosphatidylinositol and validated our lyso-PtdGlc force field parameters. To overcome the lack of structural information of GPR55, we performed molecular dynamics simulations with our GPR55 homology model in the presence of natural and synthetic lysolipid ligands. The dynamics simulation were in good agreement with our assay results.

3. Conclusion

The molecular dynamics simulation were in good agreement with our biological assay results of synthetic lyso-PtdGlc analogues. This allowed us to identify the potential ligand entry port and binding pocket specifics of GPR55. Additionally, our results highlight the preference for gluco- over inositol- and galacto-configured head groups.

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

Many questions concerning lipid-lipid and lipid-protein interaction are currently not well understood and remain under investigation.

For example, cholesterol (Chol) is an essential component of mammalian cellular membranes and its de novo biosynthesis is tightly controlled by a complex set of feedback mechanisms. Early stage Chol biosynthesis is regulated via HMG-CoA reductase, while mid stage is regulated via squalene monooxygenase (SM) and final stage via sterol Δ24-reductase. Recently it has been demonstrated that sterol excess in the endoplasmic reticulum (ER) membrane is sensed by the N-terminal region of SM and induces SM degradation. To gain an atomistic understanding of the SM sterol sensing mechanism, we will probe the membrane attachment of the key amphiphilic helix of SM to model membranes at a variety of Chol levels, utilizing primarily dynamics simulations.

Fiscal Year 2018 List of Publications Resulting from the Use of the supercomputer

[Paper accepted by a journal]

1. "Preference for Glucose over Inositol Headgroup during Lysolipid Activation of G Protein-Coupled Receptor 55"
   AT. Guy, K. Kano, J. Ohyama, H. Kamiguchi, Y. Hirabayashi, Y. Ito, I. Matsuo, P. Greimel
   ACS Chem. Neurosci., **2019**, 10 (1), 716–727 – **communicating author**
2. "Squaryl group modified phosphoglycolipid analogs as potential modulators of GPR55"
   F. Ding, A.T. Guy, P. Greimel, Y. Hirabayashi, H. Kamiguchi, Y. Ito
   Chem. Comm., **2018**, 54, 8470-8473.

[Oral presentation]

1. "Phosphatidyl-ß-D-glucoside: From Biosynthesis to Biological Activity of Synthetic Analogues"
   P. Greimel, AT. Guy, HH. Hung, Y. Nagatsuka, I. Matsuo, Y. Ito, H. Kamiguchi, Y. Hirabayashi
   29th International Carbohydrate Symposium, Lisboa, Portugal, July 15th-19th, **2018**

[Poster presentation]

1. "Lyso-Phosphatidyl-ß-D-glucoside Analogues as GPR55 Agonists: Synthesis and Modality-Specific Repulsive Guidance of Nociceptive Neurons"
   AT. Guy, Y. Ito, H. Kamiguchi, Y. Hirabayashi, P. Greimel
   59th International Conference on the Bioscience of Lipids, Helsinki, Finland, Sep. 4th-7th, **2018**
2. "Synthetic Lysolipid Analogues as GPR55 Ligands"
   AT. Guy, K. Kano, J. Ohyama, H. Kamiguchi, Y. Hirabayashi, Y. Ito, I. Matsuo, P. Greimel
   37th Annual Meeting of the Japanese Society of Carbohydrate Research, Sendai, Japan, Aug. 28th-30th, **2018**