Project Title:

Biomembranes - 10N-Nonyl Acridine Orange Inhibits Cardiolipin Polymorphism

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1. Background and purpose

Cardiolipin (CL) is a unique, four acyl-chain anionic phospholipid. The interesting features of this lipid include its structural uniformity and molecular symmetry. In eukaryotic cells, CL is restricted to mitochondria, the powerhouse of the cell. The formation of local non-bilayer structures has been proposed to be crucial for the mitochondrial membrane dynamics and is thought to control the function of mitochondria membrane proteins.

10*N*-nonyl acridine orange (NAO) is a fluorescent dye, which preferentially binds to CL. Consequently, NAO is commonly used as a mitochondria specific dye in fluorescence microscopy. On the other hand, it is known, that micromolar concentrations of NAO inhibit cristae formation in mitochondria, leading to cell death.

We determined the effect of NAO on the morphology of CL membranes by means of electron microscopy, small angle X-ray scattering, scanning transmission x-ray microscope and ^{31}P-NMR. Our results indicate that NAO inhibits Ca^{2+} induced CL polymorphism. We demonstrated the ability of NAO to rescue CL from non-bilayer structures, for the first time.

2. Usage status and calculation methods

Primarily quantum mechanics (QM) simulations utilizing the Gaussian 03 and 09 software package have been performed. Additionally the NAMD software package has been employed for molecular dynamics (MD) simulations.

3. Results

Suitable force field parameters (CHARMM) for CL analogues featuring mono negatively charged as well as double negatively charged headgroups were developed. E. Coli type cardiolipin (ECCL) models, with a single and a double negative charge, were created to extend the reach of the project to cardiolipin of bacterial origin. This enabled the creation of hydrated membrane patches consisting of 72 TOCL, TLCL or ECCL molecules. Furthermore, membrane patches featuring additional 144 molecules of NAO were created and are in production runs. QM simulations of doxorubicine (DOX) as well as pirarubicine (PIR) fragments is currently ongoing. The QM data will be used to establish optimized parameters for molecular mechanics simulations of the interaction of CL with DOX as well as PIR.

4. Conclusion

Based on our previously established experimental data in combination with the preliminary MD simulations we are currently developing a new model describing the interaction of NAO and CL interaction in lamellar phase from a molecular point of view.

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

The focus of the next usage period will be set on QM simulations (Gaussian software package) of doxorubicine (DOX) as well as pirarubicine (PIR) to establish suitable CHRAMM force field parameters.

In combination with our experimental data the interaction of DOX as well as PIR with CL membranes will be studied utilizing MD simulations. This will significantly contribute to a better understanding of CL-drug interaction, a prerequisite to clarify some of the underlying molecular mechanism of the side effects of anthracycline chemotherapeutics.

6. Concerning research achievements

Preliminary results have been presented at national and international meetings. Currently, preparations of a manuscript for a peer reviewed journal are underway to facilitate a timely submission once the MD simulations and final data evaluation have been completed.

Fiscal Year 2013 List of Publications Resulting from the Use of RICC

[Publication]

[Proceedings, etc.]

[Oral presentation at an international symposium]

1. "Cellular Membrane"
   P. Greimel, T. Kobayashi
   3rd Austria/Japan Seminar on Comparative and Developmental Glycobiology

[Others]

Invited lectures

1. "Lipids – Movement and Phase Behavior"
   P. Greimel
   Seminar, McGill University, Canada, January 17th, **2014**.

Poster/Scientific exhibit presentation

1. “Cardiolipin Polymorphism: Molecular Mechanism 2. of Inhibition by 10-*N*-Nonyl Acridine Orange”
   P. Greimel, M. Murate, F. Hullin-Matsuda, K. Iwamoto, K. Ito, M. Takata, M. Kobayashi, H. Takahashi and T. Kobayashi
   54th International Conference on the Bioscience of Lipids, Italy, 17.-21.September **2013**.
2. “Cardiolipin Polymorphism: Molecular Mechanism 2. of Inhibition by 10-*N*-Nonyl Acridine Orange”
   P. Greimel, M. Murate, F. Hullin-Matsuda, K. Iwamoto, K. Ito, M. Takata, M. Kobayashi, H. Takahashi and T. Kobayashi
   Satellite meeting of 54th ICBL & Euro Fed Lipid meeting, Italy, 17.September **2013**.
3. “Molecular mechanism of the inhibition of cardiolipin polymorphism by 10-N-Nonyl Acridine Orange”
   P. Greimel, M. Murate, F. Hullin-Matsuda, T. Nabetani, K. Iwamoto, K. Ito, M. Takata, M. Kobayashi, H. Takahashi and T. Kobayashi
   14th International Membrane Research Forum, Japan, 15.-17.March **2013**.