Mechanism of energy transduction by bacteriorhodopsin

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Country

USA

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Mechanism of energy transduction by bacteriorhodopsin

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The project/programme is most relevant to:

Alzheimer's disease & other dementias

Keywords

Acquired Cognitive Impairment... Aging... Alzheimer's Disease... Alzheimer's Disease including Alzheimer's Disease Related Dementias (AD/ADRD)... Bioengineering... Biotechnology... Brain Disorders... Dementia... Networking and Information Technology R&D... Networking and Information Technology R&D - Software design and productivity... Neurodegenerative... Neurosciences

Research Abstract

Summary: A.) Development of instrumentation and procedures for comparing visible and IR

kinetics of the BR photocycle in membrane protein crystals to that of in situ tiny purple membrane fragments (PM). In our last report, we described that crystals grown at pH 5.9 showed sufficient X-ray diffraction resolution to allow atomic structures to be determined, in contrast to crystals at pH 7, which do not. To isolate the structures of all individual photocycle intermediates, the precise kinetic model must be known. Available evidence suggests that the kinetic models at the two pHs are the same. But, it must be proven to be the case before time-resolved Laue X-yay diffraction is attempted. To do this, requires the fitting of 7 to 9 exponentials to raw data. The number of photons passing through the sample and the signal to noise ratio are low. Therefore, an image intensifier (ii) must be used to increase the signal. To protect the detector of the ii, a built-in circuit (IPC) automatically lowers the set gain value, which produces distortions of the collected kinetics from their true values. Data collection is initiated by a strong, 532 nm laser pulse. We have found that this actinic laser flash strongly activates the IPC. The logical solution of this problem is to employ a 532 nm notch, blocking filter in a linear position between the light passing through the sample and the entry to the spectrophotometer, via the ii. The units are connected with optic fibers. The blocking filter is placed into a special inline filter holder for the optic cables. There are many variables to optimize in order to achieve the required maximum blocking power of this kind of filter. When we have optimized these parameters, we can proceed to the analysis of the kinetics of the photocycle at pH 5.9.
B.)Studies on amyloidosis of amyloid beta (Abeta) protein in Alzheimer's disease (AD). As described in our last two reports, our focus is on identifying the small soluble oligomer that is currently thought to initiate deterioration of brain function in AD. In the 2014 report we presented reasons for our belief that the pathogenic oligomer may be a small alpha-helical peptide that can open hydrophilic channels in neurons that dissipate membrane potential. We suspect that this structure appears at the end of the initial lag phase of Abeta polymerization, which is also the beginning of the logarithmic phase. In the 2015 report, we tested the idea of using Singular Value Decomposition (SVD) to determine the number of kinetic constants involved in the early polymerization steps during fiber growth. The raw data matrix for SVD requires spectra in columns and time points in the rows. Because, different size polymers do not have unique spectra, we assigned visibly, quantifiable characteristics, revealed by atomic force microscopy (AFM), which changed during polymer growth, (viz.) height, and width of the growth origin, maximum length, width of total structure, and maximum length. We simulated a raw data matrix using the spectra defined above and four first order kinetic constants as exponentials. Based on a sequential, linear model, we isolated unique 'spectra' for each separate state of polymerization. We ended the report with the idea that the next step would be to do the same, using experimentally obtained data. But, instead, we took an entirely new perspective on a more direct way to proceed in the identification of the early soluble polymer with the suspected alpha-helical structure that can destroy neuronal action potentials. Our first drastic change in methodology is to perform wet AFM instead of the dry approach, where the polymers are dried on a mica surface for scanning. This allows continuous monitoring of the live process. Using circular dichroism (CD), we developed a method which obtains the time course for growth and dissipation of alpha-helix during Abeta polymerization. With this approach, we can quantify different sized polymers, both in solution and on the mica surface during the entire incubation using the quartz crystal microbalance method (QCM). This method can be used with and without liposomes (made with brain phospholipids) to see how polymerization may be altered by a membrane surface. We have established a collaboration with Donald DeVoe, Director, Microfluidics Laboratory, Univ. of MD. His group can make 100 nm size liposomes, with and without an internal charge of K+. Without any mathematical modeling, we can test our

hypothesis in the following way. The observation cuvette will be fitted with QCM and a K+ selective, as well as a pH electrode. Using K+ loaded liposomes, and our ability to know when the alpha-helix is present, we can see if the internal K+ is spilled out of the liposomes into the external medium, as our theory predicts. C.) Making powerful analytical computer programs developed by Richard Shrager and me over many years available to the computing community by translating them into open source R language. These programs belong to NIH and the tax-paying public, and should be freely available. No software company is allowed to profit by their use. I have recently formed a working group of experts at CIT in the Helix Division to carry out the translation of our code, written in Matlab to R. The group consists of Rick Troxel, David Godlove (Matlab programmer), Wolfgang Resch (R programmer), Richard Shrager (retired from CIT) and me, It has the blessing of Benes Truss (Scientific Director of CIT). When the final package is complete, it should be very unique. I think it should be called something like “”The NIH Computational Least Squares Regression Protocols””. D.) Characterization of NIST standard monoclonal antibody. NIST has produced a monoclonal antibody (mAb) to be used as a standard for pharmaceutical laboratories to test their accuracy and reproducibility. This standard is currently being characterized by many laboratories at NIST using a variety of different analytical instrumentation. My main NIST collaborator, Curtis Meuse, an expert in vibrational spectroscopy, has asked me to apply singular value decomposition to see what happens when concentrated mAb is injected in and diluted by the blood stream. In preliminary analyses it appears to be a complicated process. The initial state (S) appears to be a dimer, which quickly converts to a monomer intermediate (I) in a reversible step. “”I”” then converts to the final state (F) very slowly. If this situation is confirmed by further experimentation, it means that for most of the time after injection, a mixture of diminishing S and I will be converting to F. Any characterization of the standard mAb will vary according to time-dependent changes in composition of the mixture. The standardized results to be obtained in pharmaceutical laboratories would have to reproduce the precise time-dependent results we hope to establish.

Lay Summary
Further information available at:

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Member States:
United States of America

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Alzheimer's disease & other dementias

Years:
2016

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