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Structural changes in single membranes in response to an applied transmembrane electric potential revealed by time-resolved neutron/X-ray interferometry

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ABSTRACT

The profile structure of a hybrid lipid bilayer, tethered to the surface of an inorganic substrate and fully hydrated with a bulk aqueous medium in an electrochemical cell, was investigated as a function of the applied transbilayer electric potential via time-resolved neutron reflectivity, enhanced by interferometry. Significant, and fully reversible structural changes were observed in the distal half (with respect to the substrate surface) of the hybrid bilayer comprised of a zwitterionic phospholipid in response to a +100 mV potential with respect to 0 mV. These arise presumably due to reorientation of the electric dipole present in the polar headgroup of the phospholipid and its resulting effect on the thickness of the phospholipid's hydrocarbon chain layer within the hybrid bilayer's profile structure. The profile structure of the voltage-sensor domain from a voltage-gated ion channel protein within a phospholipid bilayer membrane, tethered to the surface of an inorganic substrate and fully hydrated with a bulk aqueous medium in an electrochemical cell, was also investigated as a function of the applied transmembrane electric potential via time-resolved X-ray reflectivity, enhanced by interferometry. Significant, fully-reversible, and different structural changes in the protein were detected in response to ±100 mV potentials with respect to 0 mV. The approach employed is that typical of transient spectroscopy, shown here to be applicable to both neutron and X-ray reflectivity of thin films.

1. Introduction

Intense, pulsed laser sources greatly facilitated the development of time-resolved (or transient) techniques for photon spectroscopy at longer wavelengths, e.g., IR to UV. Particle accelerator based synchrotron and free electron laser (FEL) sources have enabled the extension of these photon techniques, including scattering, into the hard X-ray regime. The time-scales accessible for the latter at shorter X-ray wavelengths are now approaching those of the former at much longer wavelengths, namely the femtosecond regime [1]. In stark contrast, the very low flux of thermal or cold neutrons available from reactor sources has generally limited any such time-resolved studies to exceedingly long time-scales. However, with the implementation of particle accelerator based spallation neutron sources, such as the Spallation Neutron Source at Oak Ridge National Laboratory in the U.S., time-resolved studies have become possible extending to the millisecond regime, potentially limited only by the duration of the neutron pulse for those systems exhibiting fully-reversible cycles of excitation–relaxation.

Time-resolved spectroscopic and scattering techniques are most easily applied to the study of systems that can be excited by an external perturbation and then relax reversibly, thereby enabling the enhancement of signal-to-noise levels by averaging over many cycles of the excitation–relaxation process. In such classic "pump–probe" studies, the duration of both the "pump" pulse of the perturbation responsible for the system's excitation and the subsequent "probe" pulse investigating the response of the system must be short relative to the kinetics of the response. In addition, the repetition rate for the excitation–relaxation cycle is then limited by the kinetics of the response to the excitation and the subsequent kinetics of the system's relaxation to the unperturbed initial state.

For the membrane proteins involved in neurological signal transmission in biological systems, namely ligand-gated and voltage-gated ion channel proteins, the kinetics of channel opening and closing are relatively slow in the sub-millisecond to millisec-

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onds regime, depending on how individual channels contribute to the ensemble average [2]. Here, the perturbing excitation is the change in the transmembrane electric potential for the case of voltage-gated ion channel proteins. Herein we report the development of time-resolved neutron and X-ray reflectivity techniques, enhanced by interferometry, for the investigation of the structural response of the proteins to changes in the transmembrane electric potential. The first "test case" for this approach is provided by a hybrid lipid bilayer, its proximal side comprised of a self-assembled monolayer chemisorbed onto the surface of an inorganic substrate and the distal side comprised of a subsequently physisorbed phospholipid monolayer, with proximal/distal defined with respect to the substrate's surface. For the neutron case, the design of the electrochemical cell required for the application of a transmembrane potential to the fully-hydrated hybrid bilayer is simplified by the extraordinary penetration of most solid or liquid materials by thermal or cold neutrons. For the X-ray case, this is more complicated by the far more limited penetration of these materials by the photons. Significant, and fully reversible structural changes were observed in the distal half (with respect to the substrate surface) of the hybrid bilayer comprised of a zwitterionic phospholipid in response to a transbilayer potential of +100 mV with respect to 0 mV, using time-resolved neutron interferometry. These arise presumably due to reorientation of the electric dipole present in the polar headgroup of the phospholipid and its resulting effect on the thickness of the phospholipid's hydrocarbon chain layer within the hybrid bilayer's profile structure. Appropriate reconstituted membrane specimens for similar time-resolved investigation of the voltage-gated ion channel proteins have already been prepared and extensively characterized. Significant, fully-reversible, and different changes in the profile structure of the voltage-sensor domain (VSD) of a voltage-gated potassium channel, vectorially-oriented within a reconstituted phospholipid bilayer membrane, in response to transmembrane potentials of ±100 mV with respect to 0 mV, have been detected using time-resolved X-ray interferometry.

2. Experimental methods

2.1. Hybrid bilayer and neutron interferometry

The hybrid bilayer was formed via the chemisorption of OTS (octadecyltrichlorosilane) onto the silicon oxide surface of a Si–Ge–Si multilayer substrate fabricated on a commercial silicon wafer by dc magnetron sputtering. The thickness for each of the sputtered layers was 20 Å. A monolayer of POPC (palmitoyloleoylphosphatidylcholine) was physisorbed onto the hydrophobic surface of the substrate alkylated with the OTS via the rapid-solvent exchange technique [3].

The electrochemical cell [4a] and the Magnetism Reflectometer [4b] on beamline 4A at the SNS have been described previously. The heavily-doped, sputtered silicon layers in the multilayer served as the working electrode while a platinum wire in the aqueous phase hydrating the hybrid bilayer on the substrate's surface served as the counter electrode in the electrochemical cell. The transbilayer electric potential was controlled with a commercially available potentiostat (CH Instruments, Inc., Model CHI660D). With the pulsed, polychromatic spallation source, neutron reflectivity data is collected via time-of-flight, the incident beam comprised of a defined band of wavelengths, at a fixed angle of incidence equal to the angle of reflection with respect to the plane of the surface of the substrate. Several values of the angle of incidence/reflection are utilized in order to effectively span a range of momentum transfer perpendicular to the substrate surface, Q_z. In order to obtain sufficient counting statistics for each angle, the potential on the working electrode was cycled periodically between two values, 0 mV and +100 mV, and neutron reflectivity data was collected at each potential value for 20 s and stored. The total time for data collection at each potential, necessary to provide reasonable statistics for the range of Q_z accessed via each value of the angle of incidence/reflection, ranged from 5 min for Q_z nearer the critical angle where the specular reflectivity count-rate is high to 5 h for larger Q_z where the specular reflectivity is diminished by several orders of magnitude. The data collected at each value of the potential was then combined additively for each region of Q_z and, using a standard data reduction procedure, spliced together using the overlapping portions of adjacent regions of Q_z to thereby provide the average reflectivity data over the full range of Q_z accessed for each value of the transbilayer electric potential.

The extracted specular neutron reflectivity data for each transbilayer potential was then normalized by the Fresnel function for an ideal interface, using a value of $Q_c = 0.014$ Å^{-1} corresponding to the critical angle for a silicon/90%D_2O:10%H_2O interface. The resulting Fresnel-normalized specular reflectivity data were then analyzed by three methods. The first was via the autocorrelation of the gradient of the neutron scattering-length density (nSLD) profile obtained by an inverse Fourier transform of the Fresnel-normalized data. The second utilized the so-called "slab-models" comprised of error functions for the nSLD profile of the Si–Ge–Si multilayer with the hybrid OTS:POPC bilayer on its surface refined against the Fresnel-normalized data. The third utilized a "constrained refinement" approach [5], employing the known profile structure of the multilayer substrate as a reference structure to solve the phase problem and thereby derive the nSLD profile of the hybrid bilayer directly.

Two measures of the errors in the data were utilized. The first was the standard error in the specular reflectivity $R(Q_z)$ at a particular value of Q_z, where $R(Q_z)$ was simply the sum over repeated measurements at one value of the applied transmembrane potential, with the standard error expressed as that for the sum. The second was the point-to-point variation in $R(Q_z)$ along Q_z, since these fluctuations are not physical in the sense that they would arise from correlations in the *bounded* gradient profile $d\rho_b(z)/dz$ over distances greatly exceeding its boundaries. Conversion of $R(Q_z)$ to the Fresnel-normalized $R(Q_z)/R_F(Q_z)$ introduces no additional error given that $R_F(Q_z)$ is an analytic function. Both measures are shown for the Fresnel-normalized $R(Q_z)/R_F(Q_z)$ in Figs. 1c and 3a, since the latter data were the input for subsequent analysis. Further discussion is provided in the Supporting Information.

2.2. Reconstituted VSD:POPC membrane and X-ray interferometry

Two methods were recently described for the preparation of single membranes tethered to the surface of an inorganic substrate, the membrane comprised of a phospholipid bilayer (POPC) containing an ion channel protein (VSD) vectorially-oriented with respect to the normal to the membrane plane at high in-plane surface density [5]. The latter aspects (vectorial orientation and in-plane density) greatly facilitate the structural characterization of the membrane protein's profile structure utilizing X-ray and neutron reflectivity techniques.

The usual method for the collection of specular X-ray reflectivity from such a substrate:bio-organic overlayer system, utlilizing a flat substrate and monochromatic X-rays, is to perform a $\theta - 2\theta$ scan, where θ is the angle of photon incidence with respect to the plane of the substrate's surface possessing the overlayer and 2θ the angle of reflection with respect to the incident beam direction. High energy X-rays (e.g., >20 keV) are required to penetrate the aqueous medium fully hydrating the membrane within an electrochemical cell. The method described in (a)-above for the collection of reflectivity data at different values of the transmem-