

Phase-Transition Based Transduction in a Biosensor

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Abstract

Shifts in the main phase transition temperatures caused by a highly specific, protein-receptor interaction in a hybrid bilayer architecture are probed utilizing temperature programmed infrared vibrational spectroscopy. The specific case of cholera toxin attachment to glycolipid receptors embedded in a phospholipid outer layer at a hydrophobized silicon surface is investigated. Potential applications of the approach as a signal amplification method in biosensors will be discussed.

Keywords: Micelles and solution self-assembly, Optical absorption, Atomic force microscopy, Infrared spectroscopy

Introduction

In many biosensor designs, transduction involves detection of changes in optical properties (*e.g.*, refractive index) of a biomimetic film due to binding of a protein. While this approach is quite general, its use for complex biological samples is prone to background responses generated by non-specific attachment of interferant molecules and proteins to the biomimetic films. In order to minimize such non-specific responses, we have begun to develop transduction methods in which a physical or chemical signal amplification process is directly linked to the specific binding event whose detection is desired. Potentially, one such amplification process for index of refraction based transduction methods would be a binding-induced structural change within the biomimetic film. To explore this possibility, we have initiated studies of the phase transition behavior of biomimetic phospholipid/ganglioside membrane films in the presence and absence of a protein (cholera toxin, CTX) that demonstrates multivalent binding to the ganglioside GM1. Gangliosides are found in most vertebrate cells and are believed to be involved in many cellular functions, including cellular signaling.[1] They also serve as receptors for certain bacterial toxins, and CTX/GM1 interactions have been extensively examined.[1-4] In the present work, we report the influence of CTX binding on the phase transition behavior of dimyristoylphosphatidylcholine (DMPC)/GM1 vesicles, studied using FTIR spectroscopy. We also present initial AFM and FTIR evidences for the formation of hybrid bilayers suitable for sensor applications using a home-built flow cell by rolling an aqueous solution of DMPC vesicles across a hydrophobic octadecyltrichlorosilane (OTS) coated silicon substrate.

Experimental

DMPC and GM1 were obtained in powder form from Avanti Polar-Lipids (Alabaster, AL, USA) and from Matreya, Inc. (Pleasant Gap, PA, USA) respectively and dissolved in chloroform and chloroform/methanol (2:1, v/v) respectively. The cholera toxin B subunit was purchased from Sigma Chemical Company (St Louis, MO, USA) and was reconstituted with D₂O to give a 8-9 micromolar solution. The sonicated vesicle solutions

were prepared following the procedure previously described [5] and finally dispersed in a phosphate buffer saline at pH 7.4. The solutions were injected in a liquid cell composed of two CaF₂ windows separated by 50 μ m and placed in an evacuable variable temperature cell. The self assembled monolayer (SAM) Si/SiO₂/OTS on wedge silicon was prepared according to the procedures previously reported [6]. Infrared spectra were measured using a Bruker IFS55 Fourier transform infrared spectrometer equipped with a DTGS detector and with a cooled MCT detector with the flow cell. A Digital Instruments nanoscope IIIa AFM in tapping mode was used in situ to probe the local structure of the vesicles on the SAMs.

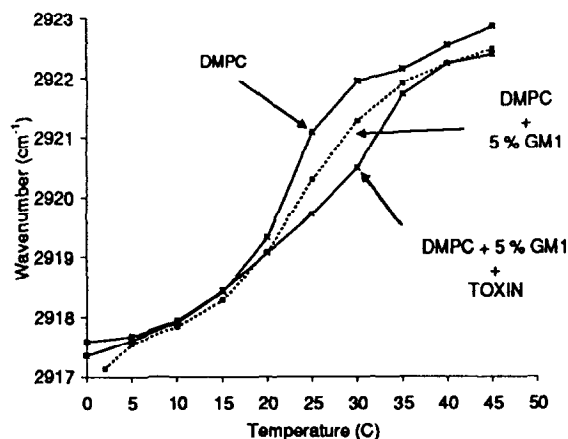


Fig 1. Frequency of the antisymmetric CH₂ stretching vibrational band as a function of temperature for DMPC, DMPC/5%GM1 and DMPC/5%GM1/toxin.

Results

The pure vesicularized DMPC, DMPC/5mol%GM1 and DMPC/5%GM1/ CTX solutions have been studied in the range 0-45°C by FTIR. Figure 1 shows the evolution of the CH₂ stretching frequencies as a function of temperature. The presence

of GM1 and the subsequent addition of toxin both led to an increase of the phase transition temperature.

In addition, we injected a pure solution of DMPC vesicles on the Si/SiO₂/OTS system previously in contact with the buffer. The preliminary results obtained with our flow cell are presented in Figure 2. After 15 hours infrared spectra reveal the appearance of the CH₂ stretching modes characteristic of the presence of the lipids at the interface.

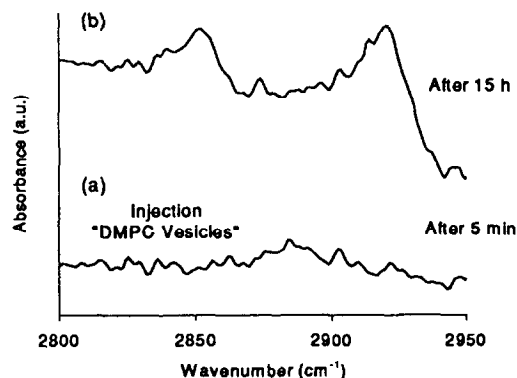


Fig. 2: The C-H stretching region of the FTIR spectra of the self assembled monolayer Si/SiO₂/OTS in contact for a) 5 min and b) 15 h with DMPC vesicles.

Discussions

The presence of 5% GM1 induces perturbations in the organization of the vesicles, providing a modification of the behavior of the phase transition. The interaction between the gangliosides and the toxin provides a measurable shift of the CH₂ stretches. The examination of the derivative of the FTIR melting curves reveals an inflection at about 22°C for the pure DMPC solution and a broader transition at about 32°C for the mixture with CTX. The variation of ~10°C and the broadening of the transition for the DMPC/GM1 mixture are consistent and in good agreement with the DSC and FTIR investigations reported recently.[7,8] It is well known that the examination of IR spectra, for instance the frequencies of the CH₂ bands, can provide information about the structure and spatial arrangement of the chains, such as the number of gauche defects.[9] Hence, the decrease in the C-H stretching frequencies in the vicinity of the transition temperature for the 5% GM1/DMPC mixture may indicate an increase in the ordering of lipids with a concomitant decrease of the gauche defect population. This is consistent with the effect of GM1 incorporated in bilayer membranes; stiffening of the membrane architecture upon GM1 incorporation is suggested.[7] The additional shift of the lipid melting curve observed after introduction of CTX suggests that the multivalent interaction of CTX with GM1 leads to further lipid ordering. Such ordering will also lead to a change in the refractive index of the lipid film. This preliminary result thus provides an indication that under appropriate conditions, multivalent binding could induce signal amplification, provided that an appropriate membrane architecture can be formed on a suitable transduction surface, such as an oxide overlayer on a high index material. The

additional studies reported here probe one specific strategy for creating such films.

The observation of the CH₂ stretching modes in the FTIR spectra after 15 h (Figure 2) suggests lipid deposition on the surface. Similar SAM substrates were imaged by AFM before and after injection of vesicles to check for the evolution of the surface. First, we systematically examined and characterized the coverage of the OTS film on the wedged Si/SiO₂ surfaces.[10] Then the same substrates are immersed in a flow cell in which vesicles are injected. The AFM images confirm the presence of adsorbed vesicles on an inhomogeneously formed hybrid bilayer. This bilayer structure was found very stable in solution (even in a flow of phosphate buffer). FTIR and AFM results obtained here can be compared with previous studies of hybrid bilayers on other hydrophobic surfaces. [11,12]

Conclusion

In situ FTIR investigations in solution demonstrate the sensitivity and the feasibility of this nonperturbative technique to monitor changes in the conformation of lipids resulting from protein-receptor interactions. Also, the preliminary results with our flow cell indicate that useful spectral data can be obtained in solution phase, and that the coupling of FTIR and AFM measurements allows consistent and complementary characterizations of the formation of the hybrid bilayer, and hence may probe CTX interactions in such of architecture. On these bases, further investigations with employing biologically relevant lipid-receptor mixtures will be carried out to advance the spectroscopic diagnosis of protein-receptor interactions in biomimetic films and explore its potential used as a signal amplification method in the biosensor design.

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