APPROCHER LES DEUX INFINIS PAR LES ONDES ELECTROMAGNETIQUES

Microspectroscopie DRASC en temps réel pour suivre les changements de l'eau associée aux membranes cellulaires induits par l'électropulsation de liposomes

Real-time CARS microspectroscopy to follow changes of membrane associated water molecules induced by the electropulsation of liposomes

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Key words: CARS, pulsed electric fields, liposome suspensions, water vibrational modes.

Abstract

To deep more insight into basic phenomena occurring during and after electropulsation of biological membranes, a new experimental modality has been used. It combines a wide field Coherent Anti Stokes Raman Spectroscopy system with a coplanar wave guide able to deliver nanosecond pulsed electric fields to different in vitro samples. The experiments have been conducted on liposome suspensions. These systems well mimic phospholipid double layers. Spectra of liposome suspensions have been acquired immediately after electropulsation. Liposome suspension evidenced an increase of the vibrational modes around 3345 cm^{-1} for pulsed samples with respect to the non-pulsed ones. These vibrational signatures seem associated to the so called lipid associated water molecules. These molecules represent a water structure in which the intermolecular OH bonds become weak leading to the possibility that single water molecules can interact with the liposome lipidis making the pulsed membranes more permeable. Membranes assumed a less organized structure due to the persistence of these water defects.

Résumé

Pour mieux comprendre les phénomènes basiques qui se produisent pendant et après l'électroperméabilisation des membranes biologiques, une nouvelle configuration expérimentale a été mise en place. Le système combine un microscope DRASC (Diffraction Raman Anti Stokes Cohérente) et un guide coplanaire pour l'application des impulsions électriques et électromagnétiques. Les expériences ont été conduites avec des suspensions de liposomes qui sont des vésicules de phospholipides. Ces liposomes sont très similaires aux parties lipidiques des membranes des cellules biologiques. Des spectres DRASC des suspensions de liposomes ont été acquis immédiatement après l'application d'impulsions électriques de 10 nanosecondes de durée. Ces spectres montrent une augmentation des modes vibrationnels de l'eau associée aux lipides à 3345 cm^{-1} en comparaison avec les suspensions liposomales non exposées aux impulsions électriques. Les signatures observées peuvent être associées à des molécules d'eau avec des liaison OH intermoléculaires très faibles comme celles engagées dans l'interaction des molécules d'eau isolées avec les lipides. Cette restructuration rend la membrane des vésicules plus perméable grâce à l'installation de cette eau désorganisée qui permet hydratation des lipides.

1 Introduction

To deep more insight into basic phenomena occurring during and after electropulsation of biological membranes, a new experimental modality has been used combining a wide field Coherent Anti Stokes Raman Spectroscopy system [1] with a coplanar wave guide able to deliver nanosecond pulsed electric fields to different *in vitro* samples [2]. This setup allows to acquire CARS hyper-spectra at specific Raman bands from 2900 to 3500 cm^{-1} (into the so called water vibration region) as well as to acquire in real time the CARS signature at specific

wavelengths with a spectral resolution of few ns. This time scale is comparable to the duration of the electrical stimulation synchronised to the laser emission. As the biophysical and chemical bases of cells electropermeabilization are still debated, our setup will allow the experimental assessment of the role of water molecules and phospholipid bilayers during the occurrence of this phenomenon which is used in various biotechnological, biological and medical applications.

2 Experimental Setup

The experiments have been conducted on liposome suspensions placed between the central and lateral (ground) electrodes a grounded closed coplanar waveguide (GCCPW) [2], assuring the transmission of short pulses (10 ns, and even shorter) to the biological samples without distortions. Liposomes, that is lipid spherical unilamelar vesicles, where chosen as a suitable synthetic system to mimic phospholipid double layers as they are similar to the structure of real cell membranes. The GCCPW inter electrodes gap was fixed to 0.5 µm in order to allow the pump and Stokes laser beams (diameters of about 100 µm) alignment and focusing into the liposomes suspension in a controlled way. The liposome suspension was contained in a PDMS holder with a total volume of 30 µL. The illumination scheme of the CARS microscope followed a non-phase-matched geometry as suggested in [1]. The pump/probe laser axis was kept parallel to the microscope objective one, while the Stokes beam was tilted by 7° to efficiently attenuate the non-resonant signal. An inverted microscope (Zeiss Axiovert 200) was used and the sample was imaged by means of a 50×, NA=0.55 objective. Image of the observation plane was formed on an intensified CCD camera (PIMAX 3 Camera, Roper Technologies, Sarasota, Florida). The bandpass filters (F1=CVI-CP-AG-540 and Semrock, F2=FF01-534/42 respectively, Fig. 1) were placed in order to reflect the pump/probe and Stokes beams right after interacting with the sample of interest. Additionally, a further bandpass filter (Semrock, F3=FF01-534/42, Fig. 1) was placed at the exit of the objective in front of the CCD camera to block residual light. The camera was triggered by the laser pulse using a delay generator (DG 545, Princeton instruments) with a temporal gate of 40 ns. A scheme of the experimental setup is reported in Fig. 1, together with the laser beams focusing into the GCCPW electrodes is also shown.

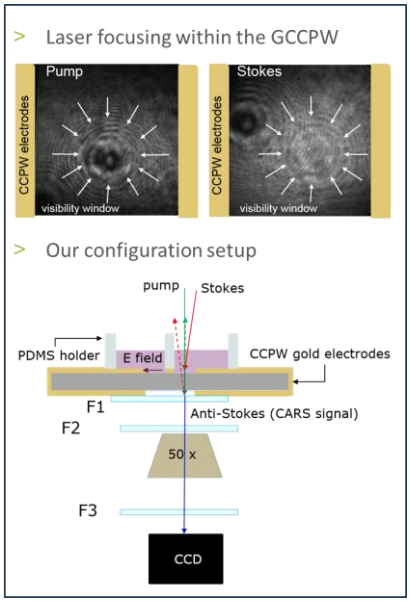

Fig. 1: Scheme of the experimental setup: combination of a ground closed coplanar waveguide (GCCPW) and a coherent anti-Stokes Raman microscope. The laser beams focused into the GCCPW electrodes are also sketched.

3 Results

Spectra of liposomes suspensions were acquired immediately after electropulsation evidencing an increase of the vibrational modes around 3345 cm-1 in the pulsed samples with respect to the non-pulsed one as shown in Fig. 2. Pulsed samples received 2000 pulses consecutively at 1 Hz and at an amplitude of 9 MV/m. This vibrational signature (3345 cm-1) seems to be related to the so called lipid associated water molecules, representing a water structure in which the intermolecular OH bonds become weak (asymmetric OH stretch modes) leading to the interaction of single water molecules with lipids. This association makes the pulsed membrane more permeable

due to this less organized and persistent structure of the water molecules. The appearance of this vibrational mode has been also verified during the exposure, in real-time specific experiments.

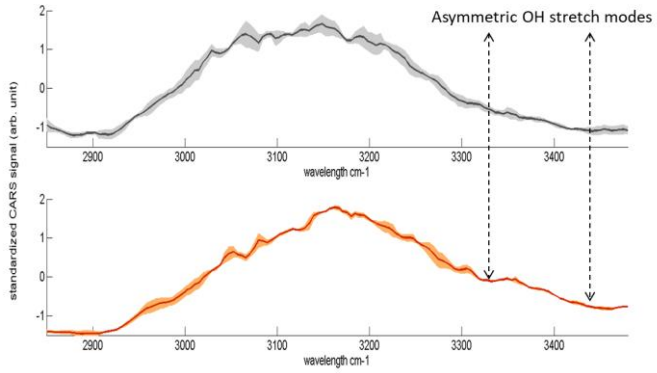

Fig. 2: Spectra of the liposomes suspension in exposed and unexposed conditions. Asymmetric vibrational bands modification is highlighted.

Finally, the effective permeabilization of liposome suspensions after the electric pulses delivery was verified looking at the release of a fluorescent dye (5-6-carboxfluorescein) included into the liposomes' core as presented in Fig. 3 (panel A). Dynamic light scattering measurements (performed before and after the exposure) demonstrated the maintenance of the vesicles integrity (Fig. 3 panel B) supporting the permanent hydration of the liposome membranes after electropulsation.

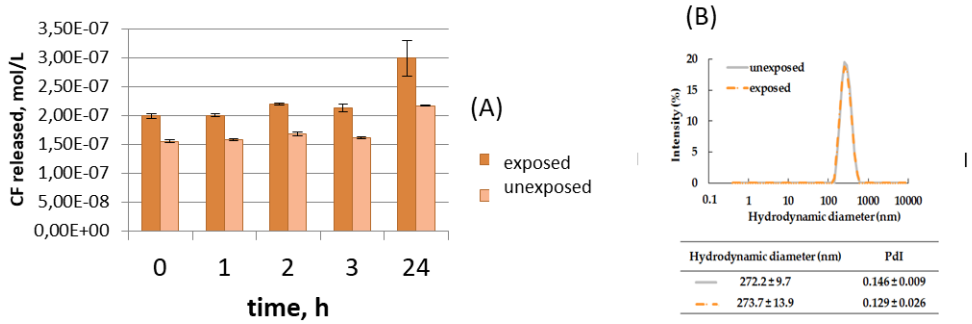

Fig. 3: 5-6CF release demonstrating the permeabilization of the liposomes under the action of the electric pulses. Dynamic light scattering performed before and after the liposomes exposure does not shown modification of the liposomal structure.

4 Conclusions

In summary, CARS, employing nanosecond lasers pulses and the properties of our wide field microscope and its intrinsic ability to sense complex interferences, has provided us with an appropriate diagnostic tool. Thanks to this setup, we were able to observe the spectra arising from interfacial and interstitial water molecules in liposome suspensions. The liposomes permeabilization was also confirmed by 5-(6) CF release after the exposure evidencing the interest of our results not only for the basic understanding of electropulsation mechanisms but also for their possible exploitation to smart drug delivery applications mediated by electric pulses. In a future, the underlined mechanism will be investigated on cells, hence taking into account recovery processes as well as the different interactions elicited by the application of longer μs electric pulses.

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