Research

Quartz Crystal Microbalance with Dissipation Monitoring: A Method for Studying Biomimetic Membranes

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Abstract:

The human body contains a few trillions of cells of different types and functions, which are crucial for living. The study of cell membranes is important for understanding biological events and developing new medicines. As cell membranes are very complex, researchers often create and study simple models, referred to as biomimetic membranes, in order to understand their behavior and properties. In this paper, we briefly present quartz crystal microbalance with dissipation monitoring (QCMD) as a surface-sensitive technique to study biomimetic membranes. By measuring changes in the oscillation frequency and dissipation energy of quartz crystal sensors, QCMD can monitor in real time molecular events occurring at the quartz sensor-sample interface, such as formation of supported layers, changes in thickness and structural properties, as well as biomolecular interactions. We present a concise description of the basic QCMD principles, followed by a few examples on the adsorption of model membranes, namely, solid-supported lipid bilayers and vesicles, as well as on studying lipid phase transitions.

Keywords: supported lipid bilayers, supported lipid vesicles, phase transitions, quartz crystal microbalance with dissipation monitoring (QCMD)

1. Introduction

Phospholipids are amphiphilic biomolecules composed of a hydrophilic head and hydrophobic chains, thus, within an aqueous environment they assemble to create bilayers, vesicles or micelles. Phospholipid bilayers form the fundamental infrastructure of the membranes enclosing the biological cells and the contained organelles. The proteins, carbohydrates, as well as the other types of lipids which are embedded in the bilayers, make the structure and composition of the latter very complex, and modify the membrane properties (e.g., fluidity), or cell functions (e.g., transport of substances). The various parameters are thus hard to investigate independently. For that reason, model systems of lower complexity, such as solid-supported lipid bilayers (SLBs) or solid-supported lipid vesicles (SLVs) are utilized and are easy to prepare.
Quartz crystal microbalance with dissipation monitoring (QCMD) is an advantageous technique to study supported lipid systems since it can monitor in the liquid state and in real time the formation of lipid layers with a high sensitivity (mass detection of a few ng per cm^2). In addition, QCMD does not require any labelling which could influence the lipid membrane organization, contrary to other techniques such as nuclear magnetic resonance or fluorescence microscopy.

2. Quartz crystal microbalance with dissipation monitoring

QCMD is an acoustic device working on the inverse piezoelectric effect: the application of an alternating AC voltage on an AT-cut quartz crystal used as sensor leads to its oscillatory motion. A standing wave is generated when the applied voltage matches the crystal resonance frequency f (typically of 5 to 10 MHz) or its odd overtones n (generally, n = 3 to 11). When the voltage is switched off, the oscillation decays exponentially. From the cyclic deformation of the quartz sensor, two parameters are recorded upon the adsorption of molecules: the frequency changes $\Delta f/n$, related to the adsorbed hydrated mass on the surface, i.e. the mass of the molecules and water trapped ($\Delta f/n$ decrease denotes a mass increase), and the dissipation energy loss ΔD, related to the viscoelastic properties of the supported layer (ΔD increases with viscosity) (Reviakine et al., 2011). Different coatings can be formed on the sensor surface to match the particular needs of each experiment, among them SiO_2, TiO_2, Al_2O_3 and Au. Prior to experiments, all sensors are thoroughly cleaned following standard protocols (chemical cleaning, exposure to UV light), to remove any impurities.
Our QCMD setup (Q-Sense Analyser, Biolin Scientific, Sweden) uses four modules (sensor chambers) enabling simultaneous experiments, variable flow rates of liquid samples (typically 50 to 100 µl/min for SLBs and SLVs studies). The temperature can be changed in the range from 15 °C to 60 °C using a Peltier element. Performing temperature ramps is useful to detect lipid phase transitions in adsorbed lipid films (SLBs, SLVs).
After presenting the formation of a SLB and a SLV on quartz sensors, we will introduce some examples how the QCMD is used for studying biomimetic membranes. We will first show the kinetics of adsorbing unilamellar vesicles with three different sizes, as well as multilamellar ones. Afterwards, we will demonstrate how to detect phase transitions of a lipid vesicle layer.

3. Results

The formation of SLBs or SLVs can be easily followed by QCMD via the frequency or dissipation changes when lipid vesicles are injected on surfaces. Vesicles are prepared by a well-defined, standard protocol: lipid monomers are first dissolved in chloroform and dried out slowly under an inert gas flow to obtain a homogeneous lipid film. The film is then hydrated with buffer, generating multilamellar vesicles, which are in turn extruded to become unilamellar with a defined diameter.
A typical QCMD experiment is structured as follows: first, a buffer injection is performed to create a baseline, then lipid vesicles are injected until reaching a stable signal plateau,

and a buffer-rinsing step follows. The obtainment of SLV or SLB depends on several parameters, among them: the vesicle composition (transition temperature of lipids, bending modulus) and size, the sensor surface energy, and the adsorption temperature (Bar et al., 2023). In **Figure 1**, a solution of 1,2-dipalmitoylphosphatidylcholine (DPPC) vesicles of 50nm diameter was injected on two sensors with different surface coverage, namely, Au and SiO2.

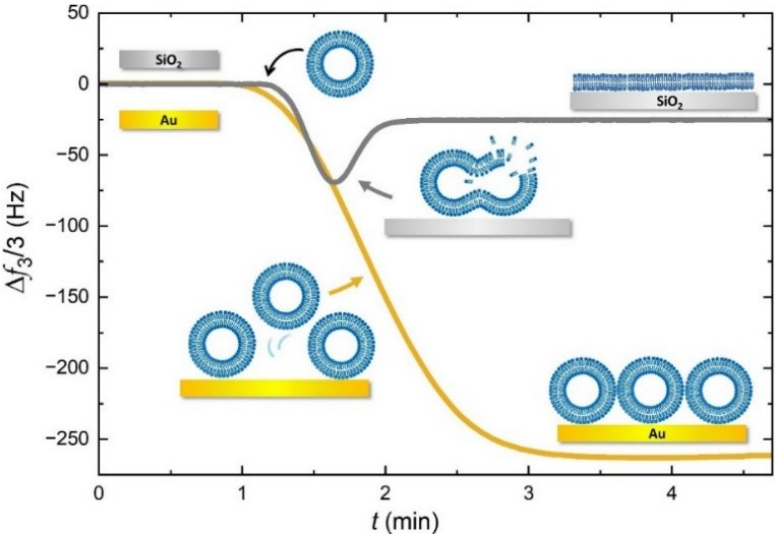

Figure 1: Frequency changes obtained while adsorbing DPPC lipid vesicles (0.5 mg/ml in Hepes buffer) at 50µl/min on Au (at 50°C) and SiO_2 (at 20°C), giving rise to the formation of a SLV and a SLB respectively.

When adsorbed on Au substrate at a temperature T below the DPPC main transition temperature ($T_m \approx 42.5$°C), the lipid is in the gel phase (quite rigid) and the adhesion energy is not strong enough to break the vesicles. They simply adsorb and form a homogeneous SLV. This is probed by a continuous drop in frequency until the sensor surface is fully covered with vesicles, i.e., when it reaches a plateau. If the adsorption is done at 50°C ($T > T_m$, DPPC being in the liquid phase) on SiO_2 substrate (strong surface energy), then vesicle breaking can occur. We observe first a $\Delta f_n/n$ decrease due to vesicle adsorption. Once a certain quantity is adsorbed (maximum negative peak value), vesicles fuse and break, releasing the trapped water, with a mass loss displayed by a $\Delta f_n/n$ increase. A final value of -25 Hz is typical for a homogeneous lipid bilayer (Reviakine et al., 2011).

The observation of the kinetics of vesicle adsorption, as well as the plateau values, provide extra information about the lipid behavior. In **Figure 2**, we compare the adsorption of DPPC vesicles on Au-coated quartz sensors as a function of their size. We have used multilamellar (in µm range), and unilamellar vesicles extruded through membrane filters with pore sizes of 200 nm, 100 nm and 50 nm. The slopes of frequency shifts reveal that the mass gain is fastest for the smallest 50 nm vesicles, providing first a full coverage of the sensor surface, i.e., the frequency shift reaching the plateau value. The bigger the vesicles (going from 50 nm, to 100 nm, 200 nm to multilamellar in µm scale), the longer they take to adsorb and reach a full coverage of the sensor surface. This can be explained by a slower diffusion onto the surface, and a longer-lasting reorganization and ordering once they are adsorbed. One has the possibility to explore the impact of various parameters on the vesicle kinetics (inclusion of new constituents, salts, etc.)

QCMD can also be used to detect phase transitions between various lipid phases in SLBs and SLVs (Cordoyiannis et al., 2021). For SLBs, the phase transitions signatures are weak and often difficult to probe, whereas in SLVs they can be nicely seen. After the SLV formation, temperature ramps can be set.

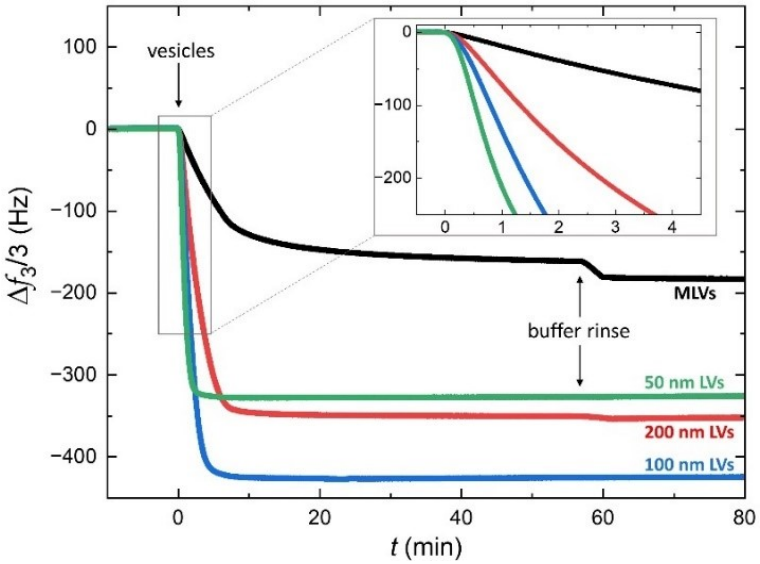

Figure 2: Kinetics of the adsorption of DPPC vesicles (0.5 mg/ml in Tris buffer) of three different sizes and their multilamellar representative, on Au sensor at 50µl/min and 20°C. The inset is a zoom in the first minutes of injection showing the slopes of frequency shifts.

During the heating or cooling runs, frequency and dissipation changes show irregularities at temperatures when a phase transition occurs. The temperature derivatives of $\Delta f_n/n$ and ΔD data make these irregularities appear as clear peaks. In **Figure 3**, an example of such lipid phase transition is presented. DPPC SLVs are heated from the low-temperature, ordered gel phase at 20 °C to the disordered liquid phase at 50 °C. Two anomalies highlight two phase transitions from gel-to-ripple (at 32.5 ± 0.5 °C) and from ripple-to-liquid phase (at 42.3 ± 0.2 °C). These two transitions are referred to as pretransition and main transition respectively. The intermediate ripple phase possesses ordered and disordered domains of few nm ranges. QCMD enables the observation of how an additional component (ex. cholesterol or some proteins) in the vesicle can affect its phase transitions between various phases and, thus, its stability (Cordoyiannis et al., 2021).

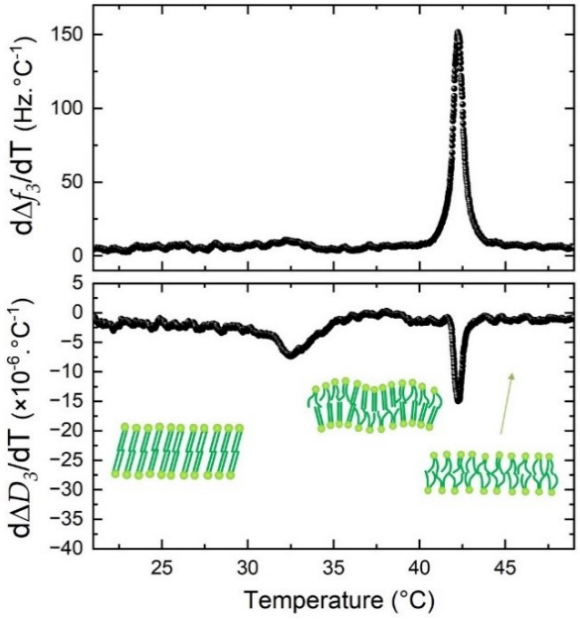

Figure 3: Frequency and dissipation temperature derivatives mark the phase transitions between three lipid phases (gel, ripple, liquid) in DPPC SLVs: (a) from the ordered gel phase to the ripple phase, and (b) from the ripple phase to the disordered liquid phase at high temperatures.

4. Conclusions

We have briefly presented the QCMD as a useful tool to study biomimetic membranes, such as SLVs and SLBs. Examples have been given about how we probe phase transitions between various lipid phases occurring upon changing temperature, as well as regarding the kinetics of lipid vesicle adsorption.

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Conflicts of Interest: The authors declare no conflict of interest.

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