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Electrochemical behaviour of mixed LB films of ubiquinone – DPPC

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ABSTRACT

The structure and the electrochemical behaviour of Langmuir and Langmuir–Blodgett (LB) films of the biological ubiquinone-10 (UQ) and a mixture of dipalmytoilphosphatidylcholine (DPPC) and UQ at the molar ratios DPPC:UQ 5:1 and 10:1 have been investigated. The surface pressure-area isotherms of the Langmuir films and the AFM images of the LB films show the formation of a monolayer in the DPPC:UQ mixture till a certain surface pressure is attained, and then at higher surface pressures the UQ is progressively expelled. The cyclic voltammograms of DPPC:UQ LB films formed on indium tin oxide, ITO, at different surface pressures show one reduction and one oxidation peak at low surface pressures, but two or even more reduction and oxidations peaks at medium and high surface pressures. The electrochemical behaviour is correlated with the film structure.

1. Introduction

The understanding of electron transport processes in biological membranes is one of the current challenges to mimic natural processes. Focusing the attention in bioenergetics processes, ubiquinone-10 (UQ) and plastoquinone-9 are known to be an important kind of natural quinones involved in the respiratory and photosynthetic pathways of the mitochondrial and thylakoid membranes, respectively [1–3]. In particular, the UQ molecule contains a quinone redox centre (head) and a long isoprenoid chain (tail) of ten isoprenoid units (Scheme 1). This tail confers a high hydrophobic character to the molecule.

In order to study the electrochemical behaviour of the ubiquinone-10 (UQ)/ubiquinol-10 (UQH_2) redox couple in an environment that mimic the biological membranes, some important aspects must be understood like reproducing the structure of the lipid bilayer, inserting the UQ molecules in the lipid membrane and controlling the redox state of the UQ/UQH_2 couple. One of the first attempts to study the electrochemistry of UQ in this kind of environments used lipid monolayer and bilayer systems supported on Hg as biological membrane models [4]. Using such systems Guidelli et al. [5,6] and Gordillo et al. [7,8] studied the electrochemistry of UQ on a phospholipid monolayer by chronocoulometry and cyclic voltammetry. On solid electrodes, different methods have been applied to modify the surface of the electrode with a thin layer simulating the structure of the lipid layer [9], where the quinone molecules can be embedded. The Langmuir–Blodgett (LB) and the vesicle fusion methods have been the most often applied to generate supported lipid bilayers [10–18]. Bilayers so obtained have been used to study the electron transport properties of UQ [19,20], duroquinone [21] and naphtoquinone derivatives [22] by voltammetry. Other techniques like cast coating [23,24], black lipid membrane (BLM) [25], and other thin layer approaches [26–29] have also been used to study the voltammetric behaviour of UQ and other quinone derivatives.

As a result of all these studies, the thermodynamic properties of the UQ/UQH_2 redox couple in different model systems have been reported, but one important factor as the location of the ubiquinone molecules in the membrane during the redox process is still unclear. As the electron and proton transfers involving the quinone moiety depend on the availability of water molecules for the redox centre whereas a lipid environment should be available for the tail, the Langmuir technique offers a unique way to control these factors through the lipid film formation and compression. Moreover the orientation of the phospholipid molecules will depend on the hydrophobic/hydrophilic character of the surface. Therefore when the LB film is transferred on an indium tin oxide (ITO) electrode, which is hydrophilic, the obtained lipid film structure, with the polar phosphatidyl groups towards the electrode surface [10], is very different to that obtained on a mercury electrode [9], which is hydrophobic having the hydrocarbon chains towards the electrode surface.

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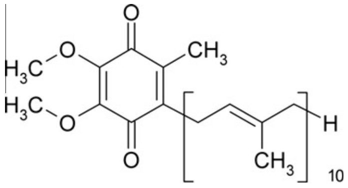

Scheme 1. Ubiquinone-10.

The surface-active properties of ubiquinone monolayers, and mixed ubiquinone-phospholipid monolayers were investigated previously by Quinn and Esfahani [30] by obtaining the surface pressure isotherms of the Langmuir films. These authors assessed the amphipathic character of ubiquinone molecules and that their ability to penetrate the phospholipid monolayers increased as the isoprenoid chain length increases. This former study also reported that the characteristics of the mixed monolayers depend markedly on the surface pressure of the monolayer.

In the present work, mixed films of the phospholipid dipalmytoilphosphatidylcholine (DPPC) with the biologically significant quinone UQ have been studied at the molar ratios DPPC:UQ 5:1 and 10:1. These ratios, a bit higher but not too far from the biological ones, provides enough UQ concentration for a good analysis and for observing the influence of the lateral pressure on the UQ insertion in the DPPC matrix. In order to study the influence of the UQ in the DPPC film at different surface pressures, the Langmuir and LB techniques have been used [31,32]. These techniques also allow us to control the film structure and the monolayer or multilayer formation, and surprisingly they have not been applied rigorously to these systems despite the fact that better quality layers can be assembled using the LB technique than by fusion of vesicles [15]. The use of several surface pressures allows us studying the influence of this parameter on the UQ location. Atomic Force Microscopy (AFM) has been used to characterize the organization at the nanometric/micrometric level of the LB films of DPPC, UQ and the DPPC:UQ mixtures. The redox behaviour of the LB films has been characterized by cyclic voltammetry (CV) using ITO as working electrode, due to its optical and electrical properties and its hydrophilic character, being these properties more suitable for solar cells and artificial photosynthesis applications. Finally, we try to correlate the film formation and film structure with the redox response.

2. Experimental

2.1. Materials

Dipalmitoylphospatidylcholine (DPPC) was purchased from Avanti Polar Lipids and ubiquinone-10 (UQ) HPLC grade was from Sigma–Aldrich. KH_2PO_4, KCl and chloroform of analytical grade were used in solutions preparation. Water was ultrapure MilliQ® (18.2 MΩ cm). Indium tin oxide deposited glass slides (ITO) were purchased to SOLEMS (France) and mica sheets to TED PELLA Inc. (CA).

2.2. Monolayer formation

Langmuir and Langmuir–Blodgett (LB) monolayer formation were carried on a Nima model 1232D1D2 trough using MilliQ® quality water as subphase. Solutions of DPPC, UQ and DPPC:UQ were prepared using chloroform. LB monolayers were transferred to the corresponding substrate surface at defined surface pressures values (π). Barrier closing rates were fixed at 50 $cm^2\ min^{-1}$ (6.3 $Å^2\ molec^{-1}\ min^{-1}$) for isotherm registration and at 25 $cm^2\ min^{-1}$ (3.1 $Å^2\ molec^{-1}\ min^{-1}$) for LB film transfer. No noticeable influence of these compression rates was observed on the isotherm shape. Isotherm recording was carried out adding the solution to the subphase and waiting 15 min for perfect spreading. LB film transfer was conducted dipping the substrate (ITO slide or mica sheet) on the subphase before adding the solution and 5 min were lagged after pressure setpoint was achieved. Transfer speed was set at 5 mm/min linear velocity. Experiments were conducted at 22 ± 1 °C.

2.3. AFM characterization

The AFM topographic images of LB films were acquired in tapping mode using a Multimode AFM controlled by a Nanoscope IV electronics (Veeco, Santa Barbara, CA). Silicon tips with a nominal spring constant of 40 $nN\ nm^{-1}$ were used (ACT-W, Applied Nanostructures, Santa Clara, CA). Images were acquired at 1.5 Hz and at minimum vertical force so as to reduce sample damage.

2.4. Electrochemical characterization

The voltammetric measurements were performed in a conventional three-electrode cell using an Autolab Potentiostat–Galvanostat PGSTAT-12 (Ecochemie, NL). Working electrodes used were ITO slides (10mm × 25 mm) cleaned once with ethanol and three times with MilliQ® grade water. Counter electrode was a platinum wire in spiral geometry and the reference electrode was an Ag/AgCl/3 M KCl microelectrode model DRIREF-2SH from World Precision Instruments (WPI). This reference electrode was mounted in a Lugging capillary containing KCl solution at the same cell concentration. All reported potentials were referred to this electrode. The electrochemical cell contained 0.150 M KCl as supporting electrolyte at pH = 7.4 adjusted with the KH_2PO_4/K_2HPO_4 buffer. All solutions were freshly prepared with MilliQ® grade water and deaerated with flow of Argon gas for 15 min prior the CV experiments, which were conducted at 22 ± 1 °C. Voltammetric experiments were carried out at different scan rates, scanning towards negative potentials in a home-made glass cell with a reaction area of 33 mm^2.

3. Results

3.1. Surface pressure-area isotherms and AFM characterization

Fig. 1 shows the surface pressure-area (π-A) isotherms for Langmuir films of DPPC, UQ and the DPPC:UQ 5:1 and 10:1 mixtures. The DPPC isotherm shows the known phase transition from liquid expanded (LE) to liquid condensed (LC) at low surface pressures (around 6 $mN\ m^{-1}$), and a collapse at ca. 55 $mN\ m^{-1}$. The UQ isotherm presents only a LE phase followed by a multilayer formation at a surface pressure of 11 $mN\ m^{-1}$. In the isotherms of the DPPC:UQ mixtures the influence of UQ addition on the DPPC isotherm is strong compared with that of duroquinone [21] indicating a considerable incorporation of UQ in the DPPC matrix. In the isotherm we can distinguish three regions delimited by two inflexion points done at surface pressures of ca. 18 and 26 $mN\ m^{-1}$ for the 5:1 mixture and ca. 9 and 20 $mN\ m^{-1}$ for the 10:1 mixture. In order to investigate deeply the film structure in correspondence with the isotherm characteristics, LB films were transferred at selected surface pressure values onto mica and the corresponding AFM images where obtained. The first region, from 0 to 18 $mN\ m^{-1}$ for the 5:1 mixture and from 0 to 9 $mN\ m^{-1}$ for the 10:1 mixture, corresponds to a DPPC:UQ mixed LE phase. Fig. 2 shows AFM images of DPPC, UQ and the DPPC:UQ 5:1 and 10:1 mixtures, extracted at π = 6 $mN\ m^{-1}$. The figure shows the strong influence of UQ mole-