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Influence of membrane galactolipids and surface pressure on plastoquinone behaviour

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ABSTRACT

In this work biomimetic monolayers of a MGDG, monogalactosyldiacylglycerol, and DGDG, digalactosyldiacylglycerol mixture (MD), in a ratio close to that of the thylakoid membranes of oxygenic photosynthetic organisms, have been prepared. The lipid mixture incorporates plastoquinone-9 (PQ), that is the electron and proton shuttle of the photosynthetic reaction centres. The MD:PQ mixtures have been firstly studied using surface pressure-area isotherms. Langmuir-Blodgett (LB) films of those mixtures have been transferred onto a substrate forming a monolayer that mimics one of the bilayer sides of the thylakoid membranes. These monolayers have been characterized topographically and electrochemically. The results show the influence of PQ in the MD matrix and its partial expulsion when increasing the surface pressure, obtaining two main PQ positions in the MD matrix. The calculated apparent electron transfer rate constants indicate a different kinetic control for the reduction and the oxidation of the PQ/PQH$_2$ couple, being $k_{Rapp}(I) = 0.7 \cdot 10^{-6}$ s^{-1}, $k_{Rapp}(II) = 2.2 \cdot 10^{-9}$ s^{-1}, $k_{Oapp}(I) = 7.4 \cdot 10^{-4}$ s^{-1} and $k_{Oapp}(II) = 5.2 \cdot 10^{-5}$ s^{-1}, respectively. The comparison of the different galactolipid:PQ systems that our group has studied is also presented, concluding that the PQ position in the galactolipid matrix can be tuned according to several controlled variables.

1. Introduction

Artificial lipid bilayers have been extensively studied as membrane models to mimic natural membranes. They have shown their relevance in a broad range of chemical, biological and technological applications, one of them being the development of artificial photoelectric devices [1–5]. Photosynthesis takes place in the thylakoid membrane of oxygenic photosynthetic organisms which is constituted by a lipid matrix that maintains the fluidity of the membrane, allows an electrochemical potential difference across this membrane and harbours the protein complexes of the photosynthetic machinery [6]. In cyanobacteria, thylakoid membranes develop infoldings from the plasma membrane which in higher plants are located in the chloroplasts. Moreover, this lipid matrix embeds plastoquinone-9 (PQ) (Schematic 1A), which is the electron and proton shuttle between photosystem II and cytochrome. On the other hand, the lipid content of this thylakoid matrix depends on the species and the external conditions. However, it can be agreed that the thylakoid membrane of a typical oxygenic photosynthetic organism is composed of the following lipids: monogalactosyldiacylglycerol (MGDG) ≈ 50% (Schematic 1C), digalactosyldiacylglycerol (DGDG) ≈ 30% (Schematic 1D), and small amounts of other lipids, phosphatidylglycerol (PG) and sulfoquinovosyldiacylglycerol (SQDG) [6,7]. Crystal structures of the photosystem II obtained with resolution between 3.8 and 2.9 Å reveal that its lipid composition reflects that of the thylakoid membrane but that the distribution is asymmetric [8]. The head groups of negatively charged PG and SQDG are exclusively located on the cytoplasmic (stroma) side, those of the uncharged DGDG on the luminal side and those of MGDG on both sides.

In previous work, our group has prepared biomimetic membranes composed of one kind of lipid (MGDG, DGDG or DPPC), mixed with PQ or ubiquinone-10 (UQ) (Schematic 1B) that is similar in size and shape to PQ [9–15]. Several lipid:quinone ratios have been studied and we have determined the position in the lipid matrix of the PQ or UQ. The positions obtained are in line with the diving and swimming positions proposed by the Söderhäl and Laaksonen computer simulations [16].

In order to prepare reliable membranes that can mimic natural photosynthesis, the lipid content and the chemical nature of this membrane should be close to natural ones [6,8].

With this aim, in this work, we have prepared biomimetic monolayers of MGDG-DGDG at a ratio of 2:1, that we designate as MD in the work, and MD and PQ (MD:PQ) systems at a ratio of 5:1. The selection of the 5:1 ratio is based on our experience [9–12], and is the ratio closest to the biological one and shows the best-defined characteristics, especially when using cyclic voltammetry. The MGDG:DGDG ratio in the MD mixture is close to the natural one of thylakoid membranes and, in addition, it represents ≈ 80% of the thylakoid membrane lipid

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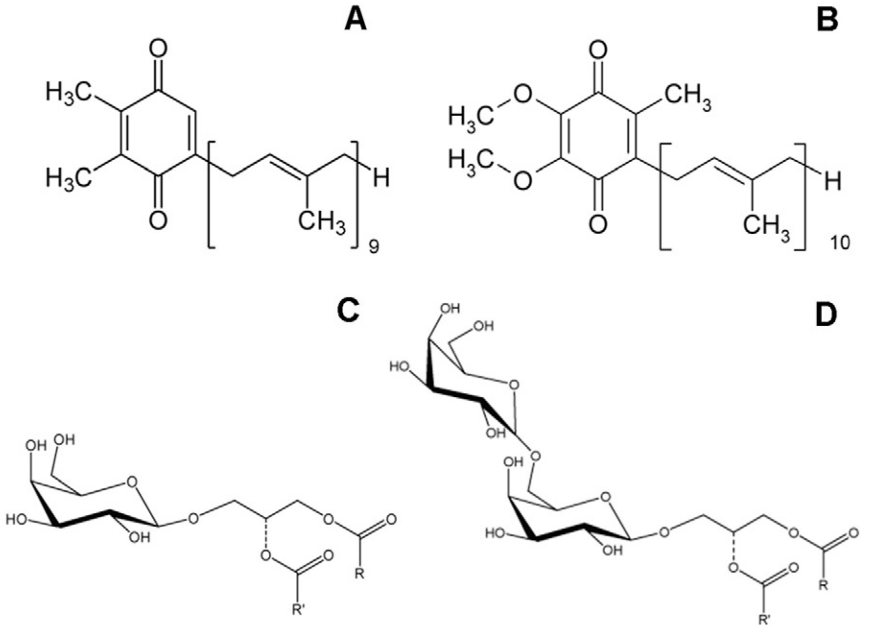

Schematic 1. Scheme of a molecule of (A) PQ-9, (B) UQ-10, (C) MGDG and (D) DGDG.

composition. The biomimetic monolayers are prepared using Langmuir and Langmuir-Blodgett (LB) techniques and are studied using surface pressure — Area (π-A) isotherms and their data are processed to discern their physical states and mixing behaviour. These monolayers, once transferred to a mica substrate at several surface pressures (including the lateral surface pressure ≈ 33 $mN \cdot m^{-1}$ of natural membranes) [17], have been topographically studied using atomic force microscopy (AFM) to observe the influence of PQ in the MD matrix. Finally, we have used the cyclic voltammetry technique for studying the electrochemical behaviour of the monolayers once transferred to indium-tin oxide (ITO), which has good optical and electrical properties, so making ITO a candidate for studying artificial photosynthesis and other energy producing devices [18].

2. Materials and methods

2.1. Materials

PQ was provided by ASM Research Chemicals. MGDG and DGDG, both with acyl = stearoyl (18:0), were purchased from Matreya (USA). KH_2PO_4, KCl and chloroform of analytical grade from Sigma-Aldrich were used in solution preparation. Water was ultrapure MilliQ® (18.2 MΩ·cm). Mica sheets were purchased from TED PELLA Inc. (CA) and ITO-deposited on glass slides was purchased to SOLEMS (France).

2.2. Methods

2.2.1. Monolayer formation

Langmuir and Langmuir-Blodgett monolayer formation were carried out in a trough model 1232D1D2 (Nima Technology, Cambridge, UK) equipped with two movable barriers. The surface pressure was measured using Whatman filter paper grade 1 held by a Wilhelmy balance connected to a microelectronic system registering the surface pressure.

The subphase used in these experiments was MilliQ® quality water. Previous to subphase addition, the trough was cleaned twice with chloroform and once with MilliQ® quality water. Residual impurities were cleaned from the air|liquid interface by surface suctioning. The good baseline in the π-A isotherms confirms the interface cleanliness. Solutions of MD (MD = MGDG:DGDG in molar ratio 2:1), PQ and MD:PQ 5:1 were prepared using chloroform and spread at the air|liquid interface using a high precision Hamilton microsyringe. LB monolayers were transferred to mica surface at defined surface pressure values. Barrier closing rates were fixed at 50 $cm^2 \cdot min^{-1}$ (8.6 $Å^2 \cdot molecule^{-1} \cdot min^{-1}$) for isotherm registration and at 25 $cm^2 \cdot min^{-1}$ (4.3 $Å^2 \cdot molecule^{-1} \cdot min^{-1}$) for LB film transfer. No noticeable influence of these compression rates was observed on the isotherm shape. Isotherm recording was carried out by adding the solution to the subphase and waiting 15 min for perfect spreading and solvent evaporation. LB film transfer was conducted by dipping the freshly cleaved mica or freshly cleaned ITO through the air|liquid interface on the subphase before adding the solution, and waiting 5 min after pressure setpoint was achieved. Transfer speed was set at 5 $mm \cdot min^{-1}$ linear velocity and the transfer ratios obtained were close to 1. Experiments were conducted at 21 ± 1 °C and repeated for reproducibility control.

2.2.2. AFM characterization

The AFM topographic images of LB films were acquired in air tapping mode using a Multimode AFM controlled by Nanoscope IV electronics (Veeco, Santa Barbara, CA) under ambient conditions. Triangular AFM probes with silicon nitride cantilevers and silicon tips were used (SNL-10, Bruker), which have a nominal spring constant ≈ 0.35 $N \cdot m^{-1}$. Images were acquired at a line frequency of 1.5 Hz and at minimum vertical force to reduce sample damage. AFM images were obtained from at least two different samples, prepared on different days, and by scanning several macroscopically separated areas on each sample.

2.2.3. Electrochemical characterization

Voltammetric measurements were performed in a conventional three-electrode cell using an Autolab Potentiostat-Galvanostat PGSTAT-12 (Ecochemie, NL). Working electrodes were freshly-cleaned ITO slides (10 mm × 25 mm) cleaned once with ethanol and three times with MilliQ® grade water. The counter electrode was a platinum wire in spiral geometry and the reference electrode was an Ag|AgCl|3-M KCl microelectrode (DRIREF-2SH, World Precision Instruments). This reference electrode was mounted in a Luggin 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 solution. All solutions were freshly prepared with MilliQ® grade water de-aerated with a flow of Ar gas for 15 min prior to the cyclic