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Tuning phospholipid bilayer permeability by flavonoid apigenin: Electrochemical and atomic force microscopy study

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ABSTRACT

The ability to tune the permeability of cell membranes might have significant implications in targeted drug delivery applications. In this work we employ electrochemical methods as well as nanolithography realized in the environment of in-situ atomic force microscopy (AFM) to demonstrate that bioactive compound apigenin is capable of altering the permeability of a model phospholipid dipalmitoylphosphatidylcholine bilayer supported by a gold surface. The electron transfer rate through the bilayer was investigated employing $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ redox couple as an electroactive probe. The value of the standard heterogeneous rate constant obtained in the absence of apigenin ($k^0 = 6.60{\cdot}10^{-5}$ cm·s^{-1}) indicates that the electron transfer through the bilayer is rather sluggish confirming its compactness. The addition of apigenin to the system leads to a considerable increase of the electron transfer rate to $k^0 = 5.74{\cdot}10^{-3}$ cm·s^{-1} being virtually identical to the value obtained at the bare electrode/electrolyte interface ($k^0 = 2.24{\cdot}10^{-3}$ cm·s^{-1}). This suggests that apigenin induces a significant rearrangement of the supported lipid bilayer increasing thus its permeability. The AFM nanolithography performed in-situ in the presence of apigenin confirmed that the supported lipid bilayer reorganizes itself to a monolayer of molecules laying parallel to the electrode surface.

1. Introduction

To operate at their respective site of action in the human body, bioactive compounds have to cross several cell membranes. The latter are complex 2D architectures based on a lipid bilayer. This implies that the bioavailability of bioactive compounds is influenced by their relative lipophilicity [1–3]. The transport of drugs across the cell membrane is a complex biological process, in which interactions between drugs and lipid molecules of the bilayer play a crucial role [4]. The ability of a lipid bilayer to realize the transport of drugs strongly depends on its permeability. The latter may be investigated by measuring electron transfer rate through the lipid bilayer. The lipid bilayer to be inspected is positioned on a conductive metallic substrate (electrode) forming what is called supported lipid bilayer (SLB) [5]. The SLB is subsequently blanketed by a conductive liquid phase (electrolyte) containing a redox couple with a reversible electrochemical behavior serving as an electroactive probe. The value of the actual electron transfer rate between the electrode and the electroactive probe reflects the magnitude of the hindrance imposed by the SLB providing the information about its physical state. The SLB may be fabricated employing two different techniques: by the Langmuir Blodgett/Schaefer method [6] and by solution spreading approach [7,8].

Dipalmitoylphosphatidylcholine (DPPC) is a zwitterionic phospholipid commonly found in eukaryotic cell membranes. Interactions of DPPC with drugs were investigated by atomic force microscopy (AFM) technique [5]. AFM is a high resolution surface imaging technique typically employing single crystal substrates such as Au(111) [9] to support examined nanostructures including adsorbed molecular layers. The tapping mode AFM (TMAFM) enables non-destructive imaging of the adsorbed molecular layer. On the contrary, the contact mode AFM (CMAFM) scanning performed at sufficiently high repulsive force induces removal of the adsorbed layer from the surface. The thickness of the layer may be determined by combining CMAFM and TMAFM, the procedure being further referred to as AFM nanolithography [10,11]. It may be realized in-situ revealing information on interactions between the adsorbed layer and species present in the bulk of the liquid phase [12].

Electrochemical techniques provide detailed information on thermodynamic as well as kinetic aspects of electron transfer phenomena and enable studying interactions in systems that involve electroactive

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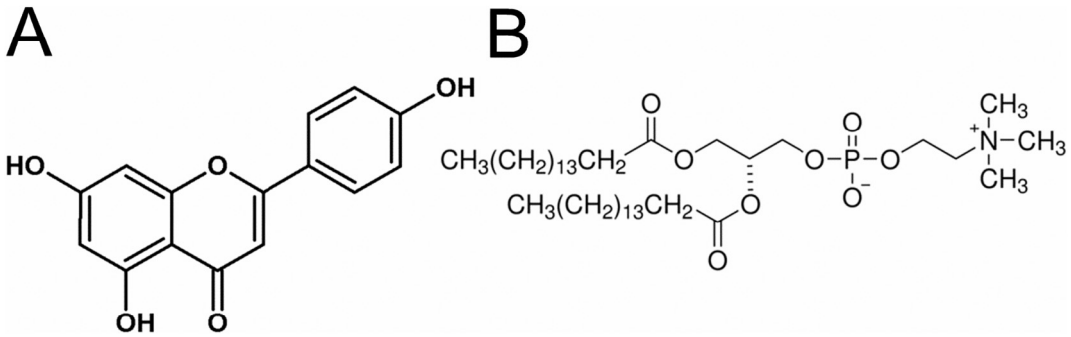


Scheme 1. Chemical structure of apigenin (A) and DPPC (B).

species [13]. Electrochemical techniques were applied to disclose the nature of interactions between bioactive compounds such as pollutants [14] and peptides [15,16] with SLBs. Rose et al. [17] employed electrochemical impedance spectroscopy (EIS) measurements to examine the interaction of ionophore valinomycin with a model bilayer. Sabo et al. [18,19] developed SLB modified Pt electrode and investigated the electron transfer through SLB modified by anthraquinone-2-sulfonic acid or tetracyano-*p*-quinodimethane by alternating current (AC) measurements. The EIS is capable of detecting subtle changes of the double layer capacitance C_{dl} and charge transfer resistance R_{ct} values. Both these quantities reflect structural variations in SLBs. The value of R_{ct} obtained at standard redox potential E^0 of the electroactive probe affords direct access to the standard heterogeneous electron transfer rate constant k^0 [20,21].

The aim of this work is to explore interactions between DPPC SLB (serving as a model of the cell membrane) with a natural product apigenin (Scheme 1). In particular, the ability of apigenin to alter the permeability of the DPPC SLB was addressed employing electrochemical measurements and in-situ AFM imaging and nanolithography. Apigenin belongs to the group of bioflavonoids and has important pharmaceutical applications. In particular, it is known as antioxidant [22] and anti-inflammatory agent [23]. Furthermore, it was found to increase the chemosensitivity of cancer cells to cytostatic drugs [24]. Its chemical structure resembles that of other important flavonols, whose membrane permeability properties were studied in the literature [25]. Recently, it was discovered that many flavonols are unstable in the solution under ambient conditions. For instance quercetin and rhamnetin decompose within minutes and several oxidation products are formed according to our findings [26,27]. Apigenin was selected because of its good stability in aqueous solution [28], particularly when studying interactions of polyphenols with biomimetic membranes.

2. Experimental

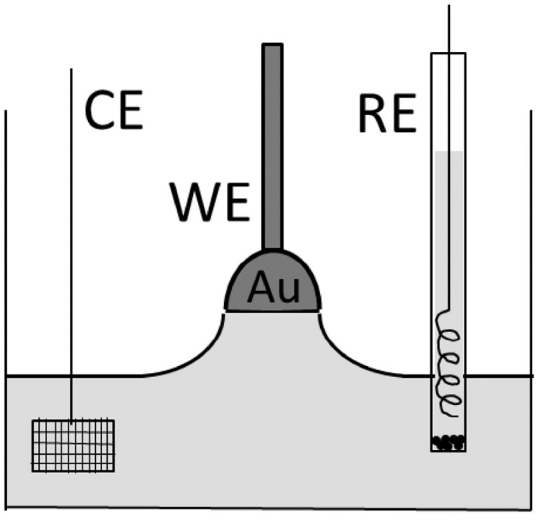
2.1. Chemicals

1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine ($C_{40}H_{80}NO_8P$, DPPC) was purchased from Avanti Polar Lipids Inc. (Alabaster, USA). Absolute ethanol (99.8%) was provided by AppliChem (Germany) while n-heptane (p.a.) was purchased from Penta (Czech Republic). Apigenin (5,7-dihydroxy-2-(4-hydroxyfenyl)-4H-1-benzopyran-4-one) was obtained from Sigma Aldrich (Germany). Potassium hexacyanoferrate(III) (p.a.) was provided by Lachema (Czech Republic). Potassium chloride (p.a., 99.5%) was obtained from Penta (Czech Republic). Sodium sulphate (Suprapur, 99.99%) was purchased from Merck (Germany). Deionized ultrapure water was prepared by means of a Milli-Q RG purification system (minimum resistivity 18 MΩ·cm, maximum content of TOC 3 ppb, Millipore Corporation, USA).

2.2. Electrochemical measurements

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed using AUTOLAB PGSTAT 12 potentiostat (Metrohm, Czech Republic) equipped with FRA2 module. A three-electrode electrochemical cell was used with cylindrical platinum net serving as a counter electrode (CE) and an Ag|AgCl|aqueous 1 mol·L^{-1} LiCl as a reference electrode (RE). The latter was separated from the inspected solution by a salt bridge with a fritted junction. A hanging meniscus type polycrystalline gold electrode (geometrical area 0.036 cm^2 determined by electrochemical measurements) served as the working electrode (WE). It was fabricated by mechanical cutting and polishing of a gold bead [29] formed by melting 0.5 mm gold wire (99.999%, GoodFellow) employing a butane flame. The design of the cell dedicated to carry out electrochemical measurements employing the hanging meniscus configuration is illustrated in the Scheme 2. In all electrochemical measurements, oxygen was removed from inspected solutions by purging with argon gas (Messer, 99.998%). All CV investigations were performed at the scan-rate of 0.1 V·s^{-1}. EIS was measured in the frequency range of 0.1 Hz to 100 kHz employing AC amplitude of 5 mV. Data obtained from EIS measurements were analysed in AUTOLAB FRA software and a ZView2 software.

The DPPC SLB on the WE was prepared as follows. First, the WE was cleaned by overnight immersion in concentrated sulfuric acid, copiously rinsed with ultrapure water and flame annealed at red heat for 1 min. The SLB was prepared by drop-casting 10 µL of 1.5·10^{-2} mol·L^{-1} DPPC in n-heptan/ethanol mixture (in ratio 10:1 (v/v), kept at 60 °C) on the WE [15,30]. Afterwards, the modified WE was immersed in



Scheme 2. Scheme of the electrochemical cell dedicated for measurements employing the hanging meniscus configuration (WE - working electrode, RE - reference electrode, CE - counter electrode).