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Association of ibuprofen at the polar/apolar interface of lipid membranes

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ABSTRACT

Ibuprofen is a non-steroidal anti-inflammatory drug widely used to treat inflammatory diseases, and for its analgesic and antipyretic activity. Although operating as a protein inhibitor, it is also known to interact with lipid membranes. We combined calorimetry, electron spin resonance, attenuated total reflectance-Fourier transform infrared and molecular docking to characterize the interaction of ibuprofen with dimyristoylphosphatidylcholine (DMPC) bilayers, as a function of temperature and drug concentration. At increasing concentration, ibuprofen first perturbs and then suppresses the DMPC pre-transition, stabilizes the fluid state, and favours gel-fluid phase coexistence. The drug decreases the molecular packing of the polar heads and of the first methylene segments of lipid membranes in the gel phase, whereas it leaves unperturbed the chain flexibility in the liquid-crystalline phase. The action of ibuprofen also leads to a higher degree of hydration of the bilayer polar heads and favours hydrogen bond formation with solvent molecules. The overall results reveal that ibuprofen affects a number of key molecular properties of DMPC bilayers by binding through non-specific interactions at the polar/apolar interface.

1. Introduction

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic activity [1]. It is extensively used worldwide for pain treatment of numerous diseases, including arthritis, osteoarthritis and musculoskeletal disorders. Additional benefits have also been attributed to ibuprofen, because it inhibits the growth of certain types of cancer [2,3], reduces amyloid pathology in Alzheimer's disease [4,5], and has a mild effect as an antiplatelet agent [6,7]. The therapeutic action of ibuprofen, which is common to all NSAIDs, is due to the non-selective inhibition of cyclooxygenase enzymes pathway, blocking the production of prostaglandins that are mediators of the inflammatory processes [8]. In spite of its beneficial effects on the human health, and similarly to other NSAIDs, ibuprofen produces serious side effects, such as ulcers, stomach bleeding and additional gastrointestinal complications, and it is cytotoxic after prolonged treatment [9].

From a chemical standpoint, ibuprofen is a propionic acid derivative that is poorly water-soluble, with a pH-dependent charge state ($pK_a \approx 4.6$) [1]. It has an amphiphilic character due to the presence of a carboxylic –COOH group, which is deprotonated (i.e., negatively charged) at physiological pH, and of a hydrophobic region that includes a phenyl ring and an isobutyl tail (see Fig. 1A).

Besides acting directly on specific protein targets, there is evidence that ibuprofen also interacts with phospholipid membranes, and this step might be significant for the adverse effects exerted by the drug, as well as for its other biological activities [10–14]. The interaction of ibuprofen with various types of membrane model systems has attracted the interest of researchers and is a subject of current investigations [10,15–24]. The ibuprofen/membrane interaction depends upon different experimental conditions such as membrane composition and pH and ionic strength of the dispersion medium. Several issues are still a matter of discussion, including details in the localisation of the drug into the lipid bilayers, which plays a key role in affecting the membrane structural and dynamical properties.

In the present work we combined different biophysical techniques, both experimental and computational, to investigate the association of ibuprofen with model membranes consisting of fully hydrated multilamellar vesicles of dimyristoylphosphatidylcholine (DMPC) (see Fig. 1B), a zwitterionic ester-linked glycerophosphocholine lipid that is

Abbreviations: ATR-FTIR, attenuated total reflectance-Fourier transform infrared; DMPC, dimyristoylphosphatidylcholine; DSC, differential scanning calorimetry; DPBS, Dulbecco's phosphate buffer solution; DTBN, di-*tert*-butyl nitroxide; ESR, electron spin resonance; NSAID, non-steroidal anti-inflammatory drug; PCSL, spin-labelled phosphatidylcholine; PC, phosphatidylcholine; PCSL, spin-labelled phosphatidylcholine; TEMPO-stearate, 2,2,6,6-tetramethyl-piperidin-1-oxyl-4-yl.octadecanoate

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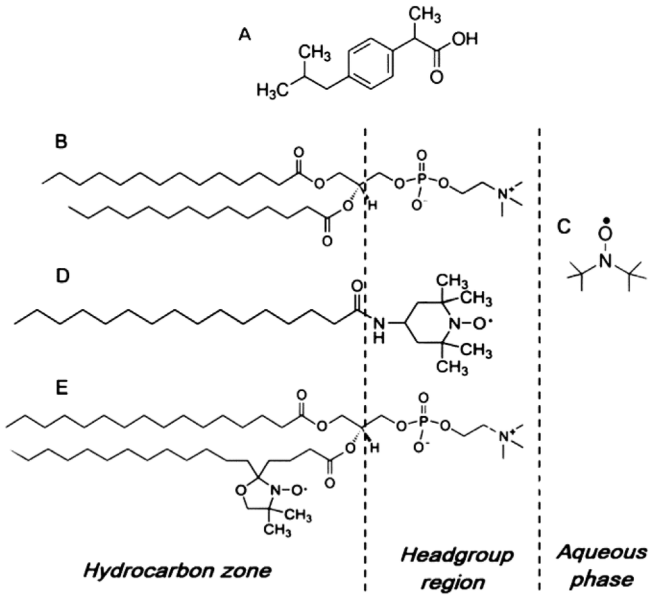

Fig. 1. Chemical structure of the molecules used in this study: (A) ibuprofen, (B) zwitterionic lipid DMPC, (C) hydrophobic spin-label DTBN, (D) spin-label TEMPO-stearate; (E) spin-labelled phosphatidylcholine: example at C_5 position in the *sn*-2 chain, 5-PCSL. The different membrane regions are also indicated.

found in eukaryotic cell membranes. The lipid bilayers were dispersed in buffer at physiological conditions (10 mM, pH 7.4) and the study was carried out as a function of ibuprofen concentration (0–60 mol%) and in a physiologically relevant temperature range (5–45 °C) that encompasses the gel-to-fluid phase transitions of DMPC membranes. Experiments were performed by using complementary techniques including differential scanning calorimetry (DSC), attenuated total reflectance-Fourier transform infrared (ATR-FTIR), and spin-label electron spin resonance (ESR) spectroscopy. ESR of the small spin-label DTBN (see Fig. 1C) added in solution allowed us to investigate the membrane fluidity, while ESR of lipids bearing the nitroxide reporter group either on the polar head or at selected positions along the chain (see Fig. 1D and E) allowed us to probe different membrane regions and to delineate the influence of the drug on transmembrane order and flexibility profiles. Finally, molecular docking was used to get insight into the atomic details of the interaction ibuprofen/DMPC bilayers and about the preferred anchoring modes of the drug in the bilayers.

The overall results indicate that ibuprofen binds at the polar/apolar interfacial region without penetrating into the inner hydrocarbon region of the DMPC bilayers, and highlight a number of significant modifications induced on the membrane molecular properties.

2. Materials and methods

2.1. Chemicals

α-methyl-4-(isobutyl)phenylacetic acid (ibuprofen, purity ≥ 98%), the synthetic lipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), the spin-labels di-*tert*-butyl nitroxide (DTBN), 2,2,6,6-tetramethyl-piperidin-1-oxyl-4-yl octadecanoate (TEMPO-stearate), and Dulbecco's phosphate buffered saline (DPBS) solution (10 mM, pH 7.4) were from Sigma/Aldrich (St. Louis, MO). Spin-labelled 1-palmitoyl-2-(*n*-(4,4-dimethyl-oxazolidine-*N*-oxyl)stearoyl)-*sn*-glycero-3-phosphocholine lipids (*n*-PCSL with $n = 5$, 7, 10, 12, and 14) were obtained from Avanti Polar Lipids (Alabaster, Birmingham, AL). All materials were used as purchased without further purification.

2.2. Sample preparation

Samples used in this work were multilamellar vesicles of DMPC prepared by thin film hydration method. The required amounts of DMPC and ibuprofen were codissolved at different ibuprofen/lipid molar ratio in chloroform. The solvent was first evaporated in a nitrogen gas stream and then under vacuum overnight. The dried lipid films were finally fully hydrated with DPBS, by heating at 30 °C and periodically vortexing.

2.3. Differential scanning calorimetry

DMPC multilamellar vesicles were prepared for calorimetry at the final concentration of 1.5 mM. DSC measurements were carried out with a VP-DSC micro calorimeter (MicroCal, Northampton, USA) with a cell volume of 0.52 mL and a temperature resolution of 0.1 °C. Both lipid and reference solutions (DPBS) were degassed before loading the cells, and the thermograms were recorded while heating at a scan rate of 5 °C/h. The scans are reported in terms of excess heat capacity as a function of temperature, and the data were analysed by using the MicroCal Origin software package. The solvent baseline was subtracted from the sample scan and the data were normalized by the lipid concentration. Finally, both the pre- and post-transition baselines were fitted by a linear function to obtain the excess heat capacity, Cp_{exc}. Phase transition temperature was determined as the value corresponding to the maximum heat capacity. Enthalpy was determined by integration of the area under the transition peak in the thermal profile.

2.4. Spin-label electron spin resonance

Multibilayers of DMPC for ESR experiments with TEMPO-stearate and *n*-PCSL were prepared as described above in 2.2 at the concentration of 50 mM, except that the lipid and ibuprofen were codissolved together with 0.5 mol% of the spin-label. For measurements with DTBN, the dried lipids were dispersed in aqueous spin-label solution at label/lipid molar ratio 1:200. In any preparation, the liposomal suspensions were concentrated by pelleting in a bench-top centrifuge and the excess water was removed. Samples were sealed off in 100 µL glass capillaries (i.d. 1 mm) for ESR measurements.

ESR spectra were acquired on an ESP-300 spectrometer (Bruker, Karlsruhe, Germany) operating at 9-GHz with 100-kHz field modulation and equipped with a Bruker ER 4111 VT temperature controller. Sample-containing capillaries were inserted in a standard 4 mm ESR quartz tube containing light silicon oil to avoid a thermal gradient and centred in a Bruker ER 4201 TE_{102} rectangular cavity.

2.5. Attenuated total reflectance Fourier-transform infrared

Fully hydrated DMPC multilayers for ATR-FTIR measurements were prepared at the concentration of 15 mM and the spectra were acquired on heating by using a Tensor II spectrometer (Bruker Optics, Germany), equipped with a liquid nitrogen cooled mercury cadmium telluride detector, a potassium bromide beam splitter and a BioATR II cell with a silicon crystal. The temperature of the cell was stabilized by a refrigerated circulator bath (Huber, Ministat 125 Pilot One). Spectra of both background (solvent) and samples were collected as a function of temperature in steps of 2 °C with an equilibration time of 2 min. After scanning the background, the cell was washed and filled with 20 µL of the sample solution. For each measurement, 120 scans were averaged in an overall scanning time of about 1 min. Spectra were recorded in the 4000–900 cm^{-1} range at a resolution of 4 cm^{-1}. Data acquisition and processing were done with the OPUS 7.5 software package.

In any given experiment, two (for ESR) or three (for ATR-FTIR and DSC) independent measurements were executed. Representative spectra are shown in the figures, whereas the data points are averages ± standard errors, corresponding to the maximum error and