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Interaction of prenylated chalcones and flavanones from common hop with phosphatidylcholine model membranes

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ABSTRACT

Common hop (*Humulus lupulus*) constitutes a source of numerous prenylated chalcones such as xanthohumol (XH) and flavanones such as 8-prenylnaringenin (8-PN) and isoxanthohumol (IXH). Range of their biological activities includes estrogenic, anti-inflammatory, anti-infective, anti-cancer, and antioxidant activities. The aim of the present work was to characterize the influence of prenylated polyphenols on model 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) membranes by means of differential scanning calorimetry (DSC), fluorescence and attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopies. All studied compounds intercalated into DPPC bilayers and decreased its melting temperature as recorded by DSC, Laurdan and Prodan fluorescence, and ATR-FTIR. Polyphenols interacted mainly with glycerol backbone and acyl chain region of membrane. Magnitude of the induced effect correlated both with lipophilicity and molecular shape of the studied compounds. Elbow-shaped 8-PN and IXH were locked at polar–apolar region with their prenyl chains penetrating into hydrophobic part of the bilayer, while relatively planar XH molecule adopted linear shape that resulted in its deeper insertion into hydrophobic region. Additionally, by means of DSC and Laurdan fluorescence IXH was demonstrated to induce lateral phase separation in DPPC bilayers in gel-like state. It was assumed that IXH-rich and IXH-poor microdomains appeared within membrane. Present work constitutes the first experimental report describing interactions of prenylated hop polyphenols with phospholipid model membranes.

1. Introduction

Common hop (*Humulus lupulus*) constitutes a source of numerous prenylated chalcones such as xanthohumol (2′,4′,4-trihydroxy-6′-methoxy-3′-prenylchalcone; XH) and flavanones such as 8-prenylnaringenin (8-PN) and its 5-O-methyl derivative, isoxanthohumol (IXH) (for chemical structures see Fig. 1). The total daily intake of hop prenylflavonoids may reach 0.14 mg, and their main source in human diet is beer since hop female flowers are used as flavoring agent and preservative in production of this beverage [1]. The range of recognized biological activities of these compounds is very wide. 8-PN, apart from being a potent phytoestrogen [2], has been also found to be an inhibitor of aromatase, a key enzyme in estrogen biosynthesis [3]. Inhibitory activities of XH and IXH in this respect were much weaker. Additionally, 8-PN can inhibit angiogenesis [4] and act as a vascular-protective agent [5,6]. All three compounds have been reported to possess anti-inflammatory activity [5,7], XH has also been identified as an anti-infective agent [8]. Prenylated flavonoids from hops have been found to inhibit proliferation of lung, ovarian, melanoma, and colon cancer cells [9] as well as breast [10,11] and prostate cancer cells [12]. Additionally, XH induced apoptosis in prostate cancer [13,14] and in acute lymphocytic leukemia cells [15]. Moreover, XH and 8-PN have been observed to modulate expression [9] and activity [16] of transporters associated with multidrug resistance of cancer cells. Prenylated hop flavonoids, especially XH, possess also a significant antioxidant activity and are able to inhibit LDL [17,18] and liver microsomal lipid peroxidation [19].

The aim of the present work was to characterize the influence of main prenylated chalcone and flavanones from hops on model lipid membranes formed from phosphatidylcholine. Until now, the interaction of 8-PN, XH and IXH with lipid bilayers has been hardly studied. According to our best knowledge, only one report exists notifying

Abbreviations: ATR-FTIR, attenuated total reflection Fourier transform infrared spectroscopy; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DSC, differential scanning calorimetry; ΔH, transition enthalpy; GP, generalized polarization; IXH, isoxanthohumol; λ_{ex}, excitation wavelength; PCA, principal component analysis; PC1, first principal component; 8-PN, 8-prenylnaringenin; $T_{1/2}$, calorimetric peak half-height width determined from thermograms; ΔT_M^{IR}, width of the transition determined from infrared spectra; T_M, main phase transition temperature determined from thermograms; T_M^{IR}, main phase transition temperature determined from infrared spectra; XH, xanthohumol

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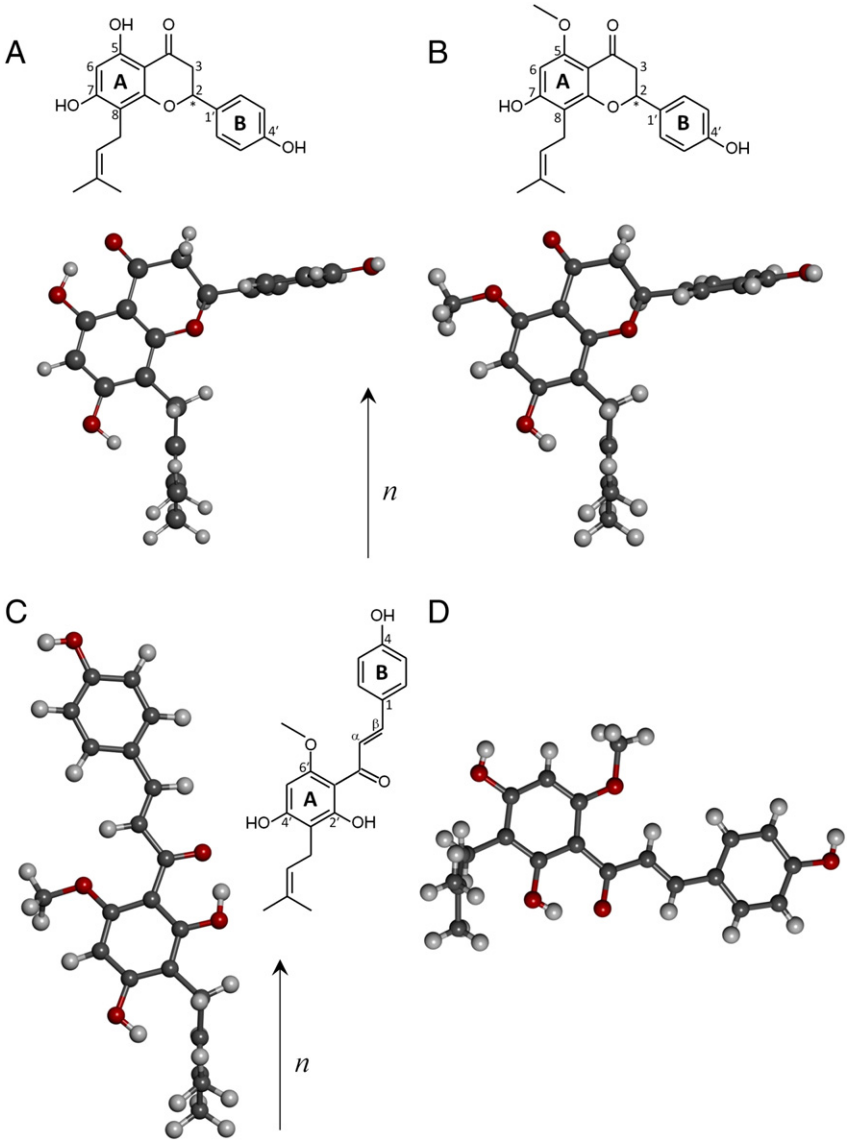

Fig. 1. The 2D presentations and the ball and stick presentations of the optimized structures for 8-PN (A), IXH (B), XH (C) and structure of XH according to the X-ray results [23] (D). The molecules have common orientation of the prenyl chain, i.e. parallel to the bilayer normal (*n*). For racemic 8-PN and IXH the chiral C2 atoms are marked by * on the 2D presentation, their 3D presentations correspond to the 2S(−) enantiomers.

interaction of XH with model lipid systems [20], and virtually none for 8-PN and IXH. Arczewska et al. [20] have used X-ray diffraction and FTIR spectroscopy to characterize the interaction of XH with dry DPPC multibilayers. The obtained results allowed the authors to conclude that XH affected molecular organization and structural properties of the polar part of the bilayer. In the present work we have employed differential scanning calorimetry (DSC), fluorescence spectroscopy and attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) to characterize the interaction of three main prenylated hop flavonoids: 8-PN, XH and IXH with fully hydrated phospatidylcholine membranes. We have shown that all of the studied compounds intercalated into lipid bilayer and affected its biophysical properties. The polyphenols interacted mainly with glycerol backbone and acyl chain region of the membrane. The magnitude of the induced effect was correlated both with the lipophilicity and the molecular shape of these compounds. Additionally, IXH was demonstrated to induce lateral lipid phase separation in phosphatidylcholine bilayers. According to our best knowledge, the present work constitutes the first experimental report aiming to characterize the interactions of main prenylated hop polyphenols with phospholipid model membranes.

2. Materials and methods

2.1. Materials

Racemic mixtures of prenylated flavanones: 8-prenylnaringenin (8-isopentenylnaringenin), isoxanthohumol (5-O-methyl-8-prenylnaringenin) and its chalcone analog, xanthohumol were purchased from Alexis Biochemicals (Lausen, Switzerland). DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine; L-α-dipalmitoylphosphatidylcholine) and DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine; L-α-dimyristoyl-phosphatidylcholine) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Laurdan (2-(dimethylamino)-6-dodecanoylnaphthalene) and Prodan (2-(dimethylamino)-6-propionylnaphthalene) were from Molecular Probes (Eugene, OR, USA).