Vibrational Spectroscopy 62 (2012) 64–69

Contents lists available at SciVerse ScienceDirect

Vibrational Spectroscopy

journal homepage: www.elsevier.com/locate/vibspec

Interaction of quercetin, genistein and its derivatives with lipid bilayers – An ATR IR-spectroscopic study

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ARTICLE INFO

Article history:
Received 12 October 2011
Received in revised form 10 May 2012
Accepted 14 May 2012
Available online 24 May 2012

Keywords:
Flavonoids
Genistein
IR spectroscopy
Lipid bilayers
Antioxidant

ABSTRACT

Genistein, a main soy isoflavone, is well known as phytoestrogen and antioxidant but details of its interactions with lipid membranes are poorly understood. The aim of this work was to elucidate the interaction of genistein, its derivatives and quercetin with lipid bilayers using attenuated total reflection infrared spectroscopy technique. Measurements performed mainly on liposomes and assistantly on dehydrated lipid films enabled us to find that studied flavonoids intercalate into phospholipid bilayers. The temperature of chain-melting phase transition for isoflavone-mixed DPPC liposomes was determined. The changes of population of the *trans*/*gauche* conformers of lipid chains were studied. They exert rigidifying effect in hydrophobic core of bilayer and due to the formation of hydrogen bonds induce a new spatial arrangement of lipid molecules.

1. Introduction

Genistein is a main soy isoflavone and therefore in humans consuming soy-rich diet its concentration in plasma reaches up to 2.4 μM [1]. Due to its diverse biological activities and beneficial health effects genistein substantially attracts the interest of many laboratories and becomes the subject of the numerous studies [2]. Most of the published papers concentrate on the effects exerted by genistein on the intracellular targets and only few deal with the problems related to interaction of this isoflavone with biological membranes. This aspect of genistein activity, however, seems to be also important for at least three independent reasons: (i) to reach its intracellular targets isoflavone must cross plasma and/or nuclear membranes, (ii) alteration of the membrane biophysical properties contributes to the modulation of integral proteins and finally (iii) lipid peroxidation is inhibited by genistein molecules already incorporated into the membrane.

Antioxidant properties of genistein in model liposomal systems were studied by Arora et al. [3]. Comparison with other isoflavones revealed that genistein is more potent antioxidant than daidzein, formononetin and biochanin A. The inhibition of lipid peroxidation by genistein, and also other isoflavones, was attributed by some research groups to the preferential partition of these compounds into the hydrophobic core of lipid bilayers [4,5] but also the interactions with polar head groups were considered [6]. The results of calorimetric and fluorescence spectroscopic experiments performed by our group [7] pointed also to more superficial interactions of genistein with lipid bilayers. According to these data genistein after its incorporation into membrane is located close to the polar-heads region of the bilayer. Almost the same location of daidzein, an isoflavone structurally similar to genistein, was proposed on the basis of calorimetric measurements by Lehtonen et al. [8]. Apart from the studies on the location of genistein in lipid bilayers also positions of other flavonoids within membranes were determined. Since those flavonoids differ significantly in their structure (i.e. number and positions of hydroxyl groups, presence of additional methyl groups or sugar substitutions) from genistein the results of these studies could not be directly used in the discussion on the genistein–lipid interactions. It is worth to mention only one study in which ^{1}H magic angle spinning NMR spectroscopy was used to demonstrate that structurally different flavonoids (flavone,

Abbreviations: DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; ATR-IR, attenuated total reflection infrared spectroscopy.

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http://dx.doi.org/10.1016/j.vibspec.2012.05.010

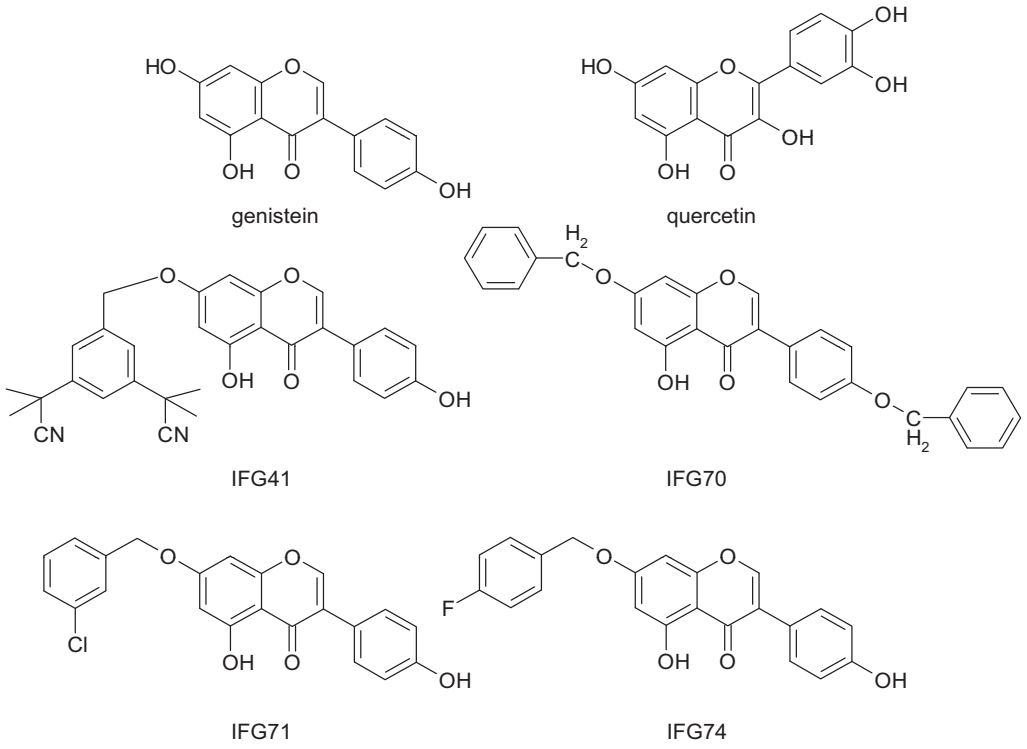

Fig. 1. Chemical structures of studied flavonoids.

chrysin, luteolin, myricetin, and luteolin-7-glucoside) do not adopt a single position within the membrane but are distributed along the bilayer normal [9].

Since the calorimetric measurements, which were used in our previous study [7], provide only rough information about the isoflavone–lipid bilayer interactions in present work we used ATR-IR spectroscopy to follow the influence of genistein and its derivatives on the different parts of lipid molecules organized in a bilayer structure. Infrared spectroscopy offers a possibility to observe the alterations of the vibration frequencies characteristic for different parts of lipid molecules, and therefore the different regions of lipid bilayer could be monitored simultaneously in one experiment. This paper will focus mainly on the effect of isoflavones on the hydrophobic part of the DPPC membrane (e.g. chain-melting phase transition) and point out that studied compounds also have an influence on the hydrophilic region of the lipid bilayer. The study of the dehydrated lipid film provides the information about the direct interactions between the lipid flavonoid molecules. Apart of genistein itself we also studied few 7-O-derivatives possessing additional benzene ring modified by different substitutions (see Fig. 1 for details). According to Arora et al. [3], a replacement of the hydroxyl group in position 7 of the flavonoid molecule by other substitutions does not significantly affect the antioxidant activity of molecules modified in this way. Such a modification alters, however, the dipolar moment and hydrophobicity of molecules and changes the liposome membrane integrity [10]. Presence (or not) of the 7-OH group might also influence several membrane-related processes like the uncoupling efficiency of the membrane potential in cytochrome c oxidase vesicles [11].

2. Materials and methods

2.1. Chemicals

Genistein and its derivatives were synthesized in the Department of Organic, Bioorganic Chemistry and Biotechnology of Silesian Technical University; their chemical structures are shown in Fig. 1. Quercetin was purchased from Sigma (Poznań, Poland). Since those flavonoids were almost not soluble in water, their ethanol or chloroform solutions were used in the experiments.

1,2-Dipalmitoyl-n-glycero-3-phosphatidylcholine (DPPC) was purchased from Sigma (Poznań, Poland). The lipid was used as delivered, without further purification. All other chemicals used in experiments were of analytical grade.

2.2. Preparation of liposomes

For each sample 12.5 mg DPPC was dissolved in the appropriate amount of ethanol or chloroform stock solution (5 mM) of the studied compound. The flavonoid/lipid molar ratio in the samples was 0.05. Then the organic solvent was evaporated by a stream of nitrogen and the residual solvent was removed under vacuum for 2 h. Samples were hydrated by 1 ml of Tris–EDTA–NaCl (20 mM Tris, 0.5 mM EDTA, 150 mM NaCl) buffer (pH = 7.4) and sonicated to obtain unilamelar DPPC liposomes, using an UP 200 s sonicator (Dr Hilscher GmbH, Berlin, Germany).

2.3. ATR-IR measurements

The ATR-IR measurements were performed using a Nicolet Avatar 360 FT-IR spectrometer equipped with a ZnSe-ATR crystal (face angle: 45°, 6 reflections, Specac). For each measurement 256 scans were collected at a resolution of 2 cm^{-1}. Interferograms were processed with Happ-Genzel apodization. The correction of the data for the wavelength dependent penetration depth was done by applying a ramp function to the uncorrected ATR spectra. The liposome suspensions were prepared according to procedure described above and spread on the surface of the ZnSe-ATR crystal. The dehydrated flavonoid-doped DPPC films were prepared by spreading 200 μl of chloroform solution of the flavonoid-doped DPPC mixture on the surface of the ZnSe-ATR crystal and evaporating the solvent under a stream of nitrogen. The concentration of DPPC in