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Antioxidant activity of daidzein, a natural antioxidant, and its spectroscopic properties in organic solvents and phosphatidylcholine liposomes

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ABSTRACT

Daidzein, one of major isoflavones found in soybeans, has a wide spectrum of physiological and pharmacological functions. The observed biological effects involve its interactions with lipid bilayers, usually detected by indirect methods. In this study we use the native fluorescence of daidzein to report changes observed during its interactions with organic solvents and in a phosphatidylcholine membrane.

We have investigated interactions of daidzein with lipid bilayers of egg phosphatidylcholine (PC) by absorption and fluorescence methods. The data obtained indicate emission arises from the conjugate anion in excited singlet state. The fluorescence is found to increase with the basicity of the solution and the polarity of the solvent. An increase in fluorescence anisotropy in the presence of membranes suggests partial incorporation of daidzein molecules into the bilayer. Two fluorescence lifetime components, 1.5 ns and 3.5 ns, reflects the partition of daidzein between aqueous and membrane environments, respectively. On the basis of the obtained spectroscopic data we conclude that up to 15% of daidzein is located in hydrophilic region of the membrane whereas the rest is distributed in aqueous bulk and aqueous/membrane interface.

For studying the antioxidant activity of daidzein against lipid peroxidation initiated by AAPH the molecule of C11-BODIPY581/591 has been used as a fluorescent oxidation indicator. The results show that the presence of daidzein anions in the membrane interface increases the inhibitory effect on lipid peroxidation compared to the neutral form of daidzein.

1. Introduction

Isoflavones, including genistein and daidzein (DZ), are polyphenolic compounds commonly found in legumes. Both of these isoflavones have a wide spectrum of physiological and pharmacological functions and are known to act as antioxidants in vivo and in vitro; their activity includes antiestrogenic [1–4], anticancer [5–8], anti-inflammatory [9–11], cardioprotective [12,13] and enzyme-inhibitory effects [14–16]. Due to their resemblance to estrogen-like molecules, naturally occurring isoflavones are also thought to play an important role in the prevention of osteoporosis [17,18].

The observed biological effects may involve interactions with lipid bilayers. An insight into the location of an antioxidant in a membrane and its impact on the membrane properties is necessary for understanding the mechanism of protecting the membrane against peroxidation. In the case of α-tocopherol, the antioxidant effect is known to be partly due to membrane stabilization by fluidity reduction [19–22]. Cholesterol is reported to produce a similar effect [23,24]. Lehtonen [25] showed that the presence of daidzein in phospholipids led to membrane aggregation, the extent of which depended on the phospholipid type and phase. Other effects include a decrease of the main phase transition temperature T_m as well as changes in enthalpy ΔH_m. The differences in aggregation are attributed to the changeable balance between a number of repulsive and attractive forces involved in the aggregation of vesicles and the hydration forces.

All the above-cited papers report changes in membrane properties, such as the fluidity or the main phase transition temperature of the lipid, in the presence of daidzein. Changes in the spectral properties of daidzein in the reported processes have not been investigated so far. However, the observed impact on the membrane structure can be expected to modify the spectroscopic parameters of daidzein in the heterogeneous membrane environment. Absorption and fluorescence spectra of daidzein in different solvents have been reported [26,27], demonstrating the effect of the proticity and dielectric properties of the solvent on the intensity and position of the bands. However, there are no available data on daidzein fluorescence properties during its interactions with

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membrane, whereas there is a number of reports regarding other fluorescent probes interacting with membranes, such as DPH, ANS or PRODAN [28–32].

The objective of the investigations presented in this paper was to correlate the spectroscopic data of daidzein with the physicochemical properties of the solvents. The results obtained for daidzein in solvents by absorption and fluorescence spectroscopic studies, fluorescence lifetime measurements and fluorescence anisotropy methods were used for deducing the location of DZ in the PC membrane. The results suggest limited affinity of daidzein to phosphatidylcholine bilayers what is observed as the partition between the bulk/ lipid interface and hydrophilic membrane interior. DZ antioxidant efficiency was shown by the inhibitory effect on phospholipid oxidation in the PC membrane.

The results presented in this work gave an insight into the mechanisms of interactions between phosphatidylcholine membrane and daidzein, as determined by its changing spectroscopic properties. This approach enhances the understanding of the mechanisms involved in the biological activity of this isoflavone.

2. Materials and methods

2.1. Chemicals

L-α-Phosphatidylcholine (PC) from egg yolk, Type XVI-E, ⩾99% (TLC), lyophilized powder (Sigma Aldrich Germany). It is composed of approximately 33% palmitic acid (16:0), 13% stearic acid (18:0), 31% oleic acid (18:1), and 15% linoleic acid (18:2), with other fatty acids being minor contributors, and has an average molecular weight of 768. Dimethyl sulfoxide (DMSO), sodium dodecyl sulfate (SDS), tetra dodecyl trimethylammonium bromide (TTABr) and glycerol were purchased from Sigma-Aldrich (Germany), AAPH [2,2′-azobis(2-amidinopropane) dihydrochloride] from Fluka (Germany). Daidzein (7,4′-dihydroxyisoflavone) was acquired from LCl-abs (USA). Organic solvents: ethanol, acetonitrile, ethyl acetate and 1,4-dioxane of pure grade (p.a.) were purchased from POCh (Poland), and methanol (spectral grade) was acquired from Merck (Germany). The water used came from a MicroPure Water System manufactured by TKA (Germany).

2.2. Liposome preparation

Dry PC and daidzein were dissolved in chloroform and methanol, respectively, and mixed in required proportions. The solvent was removed under reduced pressure (−0.8 bar) for 1 h by a rotary evaporator run at a water-bath temperature of 30 °C. An oil-free vacuum pump was used to maintain the flask vacuum. Then the sample was kept overnight under a vacuum to remove traces of solvent. Subsequently, the solvent was evaporated and the obtained film hydrated with 0.1 M phosphate buffer (pH 5.5–8.5) or citric buffer (pH 3.0), vortexed for 10 min at 20 °C until the solution became clear. The resultant liposomal suspension was extruded by means of a LiposoFast-Basic LF-1 extruder from Avestin Europe GmbH (Germany) with 100 nm diameter membrane. The final lipid concentration in samples used for spectroscopic measurements was 104 μM. Cosolubilization of DZ in the membranes refers to the initial concentrations of 2 mol%, 5 mol%, 10 mol% and 15 mol% of DZ in the membrane. The scattering from the sample was estimated by the turbidometric method in the emission spectrum at 600 nm.

To evaluate the antioxidant activity of daidzein in liposomes a methanol solution of C11-BODIPY581/591 [4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-*s*-indacene-3-undecanoic acid], used as a fluorescent marker. The final concentration of 0.8 μM. BODIPY/phospholipid mole ratio was 1:130.

2.3. Steady-state spectroscopic measurements

The absorption spectra were measured in a 1 cm quartz cuvette with an appropriate solvent as the reference sample with a S1000 spectrophotometer from OceanOptics (Netherlands). The steady-state fluorescence of daidzein was measured in the range from 380 nm to 600 nm with excitation wavelengths of 260 nm, 305 nm and 335 nm. The measurements were performed with an LS-55 Perkin–Elmer spectrofluorometer in a 1 cm quartz cuvette at 22 °C. The emission anisotropy was measured at 465 nm, with excitation wavelength 330 nm at 22 °C. The background anisotropy values were subtracted from those obtained for daidzein in liposomes. The final anisotropy was the average of at least five consecutive measurements of fresh samples. All the spectroscopic measurements were performed in a 1 × 1 cm quartz cuvette; concentrated solutions were measured in a 0.3 × 0.3 cm cuvette (HELMA).

2.4. Fluorescence lifetimes

Fluorescence lifetimes were measured with a TimeHarp 100 PC-board (PicoQuant) for time-correlated single photon counting with 72 ps per channel resolution. A 5000F coaxial sub-nanosecond flashlamp (IBH, England)) filled with nitrogen with maximum emission centered at 337 nm and pulse FWHM of 1.3 ns was used as excitation source. The emission was measured with a PMA 182 detector head (PicoQuant). A 337 nm XL30 interference filter from Laser Components (Germany) was used as excitation window to avoid leaking from the nitrogen spectrum. A 450 nm interference filter was placed in front of the detector on the emission side. The data were analyzed by an exponential reconvolution method using a non-linear least square fitting program. The time-resolved data in organic solvents and micelles were fitted with a single exponential decay function, whereas a biexponential decay function was used for the data obtained in PC liposomes. The goodness of fit was estimated by using χ^2 values.

2.5. Antioxidant activity of daidzein in PC membrane

AAPH is an azo-compound that generates peroxide radicals after thermal homolysis in both the aqueous and the lipid phase. We used this radical generator for lipid peroxidation. A suspension consisting of 104 μM PC liposome, 200 μM AAPH, 0.8 μM C11-BODIPY581/591 and 5 μM, 50 μM or 200 μM of DZ was incubated in dark at 50 °C. The peroxidation progress of the membrane was monitored by the increase in fluorescence intensity of C11-BODIPY581/591, measured every 30 min. The emission was measured at 520 nm with excitation at 505 nm at 22 °C in a 0.3 × 0.3 cm cuvette.

3. Results

3.1. Absorption, emission and fluorescence lifetimes in organic solvents

The absorption and emission spectra and fluorescence lifetimes of daidzein in organic solvents were measured to correlate the spectroscopic data with physical properties of the solvents. The results, with physical parameters of the solvents, are presented in Table 1. The positions of the two lowest absorption maxima are seen to change depending on the solvent used. The lowest energy transition in all the organic solvents is shifted to a shorter wavelength compared to its position in water, with the largest shift in acetonitrile. In all the solvents the second transition is shifted to lower energy compared to the data obtained in water. In this case the largest shift is seen in DMSO.