EFFECTS OF QUERCETIN ON ARTIFICIAL LIPID MEMBRANES

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Lucrarea are ca obiectiv utilizarea clorofilei a (Chla) ca marker spectral pentru monitorizarea efectelor quercetinei asupra membranelor lipidice. Două /tipuri de membrane artificiale au fost investigate: Chla/DPPC (0.5 mM) - SUVs and Chla/β-carotene/DPPC (0.5 mM) – SUVs.

Chla a suferit o degradare mai pronunțată în bistraturile fără β-caroten.

The present paper aims to use the chlorophyll a (Chla) as a spectral marker to monitor the quercetin effects on lipid membranes. Two types of artificial membranes were investigated: Chla/DPPC (0.5 mM) - SUVs and Chla/β-carotene/DPPC (0.5 mM) – SUVs.

The Chla degradation was more pronounced in the bilayers without β-carotene.

Keywords: quercetin, liposomes, chlorophyll a, β-carotene

1. Introduction

Quercetin (*3,3',4',5,7-pentahydroxyflavone*), a very common flavonoid widely distributed in the plant kingdom (apples, onions, olives, apple skin, broccoli, red wine, tea, Ginkgo biloba etc.), is known to possess a broad spectrum of biological activities: anti-inflammatory and antiviral activities, prevention of platelet aggregation, prevention of apoptosis induction, scavenging of free radicals, reduction of tumour incidence and others [1-5].

Quercetin has a three-ring structure (Fig. 1). The hydroxyl groups of quercetin are involved in hydrogen bonds between flavonoids and the polar headgroup of lipids at the water–lipid interface of membranes [6].

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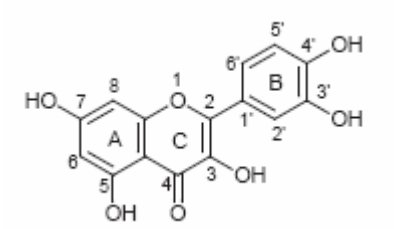

Fig. 1. The structure of quercetin [1]

Quercetin induces changes in the electrical parameters and the fluidity of the cell membranes [4, 7, 8].

In artifical membranes, quercetin causes an increase in the membrane fluidity [4]. Quercetin has the tendency to penetrate into the lipid bilayer, which results in a lower conductance of the lipid bilayer due to increasing membrane thickness [7, 8].

Due to its ability to insert in the lipid bilayers, quercetin exerts a protective role on artificial lipid membranes subjected to the action of Hofmeister anions [7].

The flavonoid-membrane interaction is a subject of interest; the studies show that interactions of polyphenols at the surface of bilayers through hydrogen bonds can act to reduce the access of deleterious molecules (i.e. oxidants), thus protecting the structure and function of membranes [6].

Unilamellar liposomes are spherical, self-closed lamellar structures composed of a phospholipidic bilayer (lipophilic compartment) which encloses an aqueous media (hydrophilic compartment) [9, 10]. Due to these structural characteristics, they are considered analogues of biomembranes [9].

The objective of this work was to study the effects of quercetin on lipid membranes using the liposomes with chlorophyll *a* (Chl*a*) as models of cell membranes.

Chl*a* embedded into lipid vesicles was used as a spectral marker to monitor the changes occurred in the membranes.

2. Experimental

Materials and method

Reagents

Dipalmitoylphosphatidylcholine, DPPC, ($C_{40}H_{80}NO_8P$; MW = 734.050 g/mol; T_c = 41 ^{0}C) and quercetin were purchased from Sigma Aldrich (Germany). β-carotene, KH_2PO_4, Na_2HPO_4, DMSO and the organic solvents of analytical

grade (chloroform, ethanol, petroleum ether, methanol, n-propanol) were supplied from Merck (Germany).

All solutions were prepared with bidistilled water.

Quercetin (Sigma) has been prepared as 10 mM stock solution in DMSO (Dimethyl sulfoxide) and added in the cuvette solution to obtain a concentration range of 0 ÷ 60 μM. The stock solution of quercetin was kept in the dark.

Methods

Chlorophyll a extraction. Chlorophyll *a* was extracted from fresh spinach leaves by a chromatographic method using a sugar powdered column with 4% starch and different organic solvents (petroleum ether, methanol, n-propanol and ethanol) as mobile phase, according to the procedure of Strain and Svec [11].

The purity of the collected Chl*a* samples was monitored by VIS absorption spectra recorded with a computer assisted PERKIN-ELMER LAMBDA 2S spectrophotometer, following the purity criteria:

- if $A^{430}/A^{662} = 1.3$, it is considered that Chl*a* sample do not contain carotenoids

and

- if $A^{662}/A^{505} > 50$ it is considered that Chl*a* sample do not contain pheophitines.

The Chl*a* used in our experiments was pure ($A^{430}/A^{662} = 1.3$ and $A^{662}/A^{505} = 54$).

The visible spectrum of Chl*a* presents two intense bands: one in the blue region of the electromagnetic spectrum (the Soret band) which is characteristic for porphyrins and another in the red region of the electromagnetic spectrum characteristic for chlorophylls [12].

The VIS spectrum of Chl*a* presents two peaks: 430 nm and 665 nm (Fig. 2).

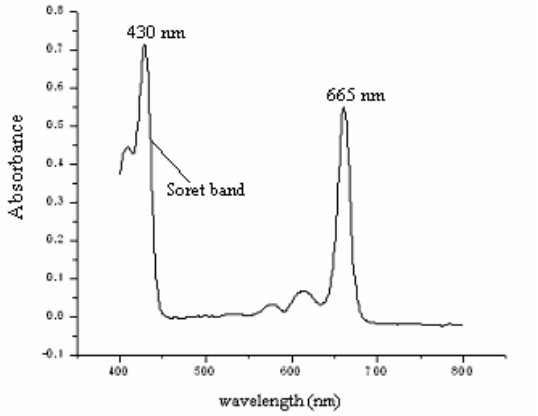

Fig. 2. The visible spectrum of Chl*a* in ethylic ether

Due to their spectral features (strong visible absorption and fluorescence emission), the Chl*a* embedded into lipid bilayers was used as a sensor to monitor the effects of quercetin on liposomes.

From our previous experience [13, 14], it is known the existence of a connection between liposome degradation and Chl*a* oxidation.

Preparation of lipid model membranes. Liposomes were prepared according to the thin-film hydration method [10] with little modifications.

The lipid DPPC was dissolved in chloroform together with Chl*a* (Chl*a*/lipid molar ratio = 1/100) and the solvent was removed in a rotary evaporator (BIOBLOCK SCIENTIFIC – Heildolph 94200, 60–90 rpm) using a vacuum pump. The lipid films were hydrated in a phosphate buffer solution (PBS) (NaH_2PO_4-K_2HPO_4), pH 7.1. The resulted suspensions of multilamellar vesicles were mechanical mixed (VIBRAX stirrer, 200 rpm) and then sonicated using a titanium probe sonicator (15 min, Hielser, UP 100 H) above the critical temperature (T_c) of phase transition of lipid. Small unilamellar vesicles (SUVs) were obtained. These suspensions were centrifuged (45 min, 20 000 g, SIGMA 2-16 K centrifuge) in order to remove the titanium traces. Only the supernatants were used in investigations.

Two different artificial membranes were obtained: Chl*a*/DPPC (0.5 mM) - SUVs and Chl*a*/β-carotene/DPPC (0.5 mM) – SUVs.

β-Carotene was incorporated into liposomal membrane during the lipid film preparation, in a final concentration of $7 \cdot 10^{-5}$ M in the lipid film.

All the absorption spectra of Chl*a* in liposomes, recorded in the UV-VIS region, were corrected against the contribution of light scattering and then normalized at 800 nm.

3. Results and discussion

The knowledge of quercetin action with lipid bilayers is very important to understand the role of flavonoid/membrane interactions on membrane protection from external and internal aggressors (i.e. oxidants) [6].

Fig. 3 presents the absorption spectra of quercetin (20 μM) in PBS pH 7.1. Two peaks are observed: one at around 375 nm (characteristic for B-ring) and another at 257 nm (characteristic for A-ring) with a shoulder at around 270 nm.

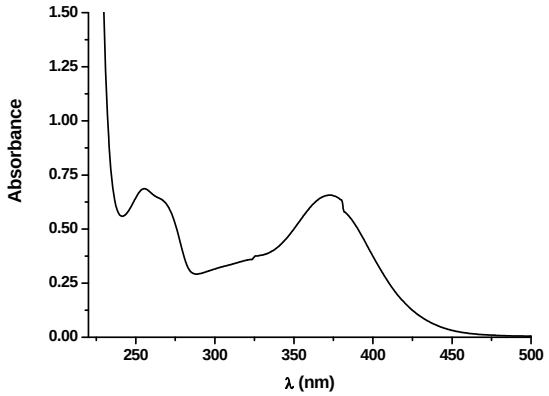

Fig. 3. The absorption spectra of quercetin (20 μM) in PBS pH 7.1

By the addition of quercetin to liposome systems, the peaks at 257 nm and 375 nm became more intense and a red shift of the B-ring peak at 375 nm (in aqueous solution) to 390 nm is observed (Figs. 4 and 5). This red shift could be explained by the location of the flavonoid into a polar medium, in the neighborhood of the lipid polar head groups, therefore the quercetin do not penetrate strongly into lipid bilayer.

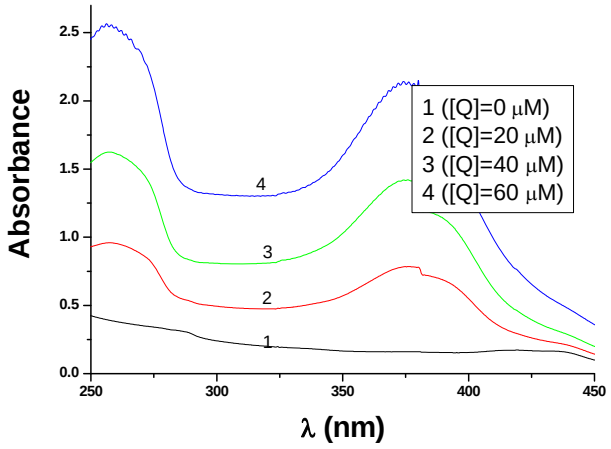

Fig.4. Effect of quercetin addition (0-60) μM upon the UV-VIS spectrum of Chl*a*/DPPC (0.5 mM) small unilamellar liposomes at pH 7.1 (PBS buffer)

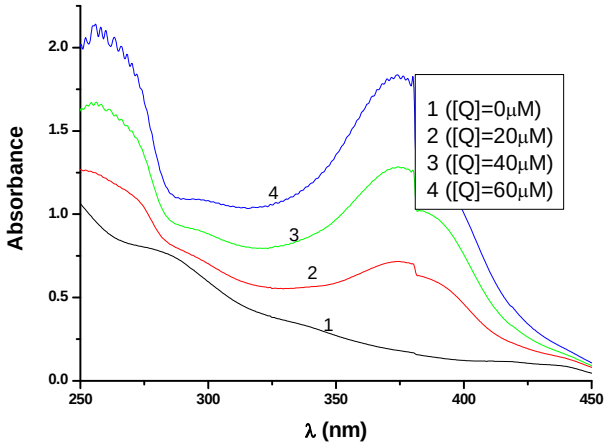

Fig. 5. Effect of quercetin addition (0-60) μM upon the UV-VIS spectrum of of Chl*a*/β-carotene/DPPC (0.5 mM) small unilamellar liposomes at pH 7.1 (PBS buffer)

Quercetin addition to lipid vesicles induced modifications in the absorption spectra of the Chl*a* in liposomes.

In order to monitor the effects of quercetin on artificial membranes, by using the spectroscopic properties of Chl*a* incorporated into lipid bilayer, the following parameters have been evaluated:

- *bleaching %: $[(A_0-A_i)/A_0]*100$*, where A_0 is the absorbance at the main red peak at [quercetin] = 0 μM, and A_i is the absorbance at the main red peak upon addition of quercetin in different concentrations.
- *the Soret band absorbance ratio: $R = A^{437nm}/A^{419\,nm}$;*

The degradation by oxidation of Chl*a*, incorporated in the lipid bilayer, is conducting to the increase of the *bleaching* value and to the decrease of *the Soret band absorbance ratio.*

The quercetin addition has no significant effect on the position of the red maximum (669-671 nm), but variations of the optical density values in the absorption spectra of the Chl*a* embedded into lipid bilayers, at the main red peak and also changes in the Soret band were observed (Figures 6 and 7).

An exponential decay of the Soret band absorbance ratio it is observed upon quercetin addition to liposomes (Figure 6). For the system Chl*a*/β-carotene/DPPC (0.5 mM) – SUVs, the decrease of this parameter is slower than

for the system Chl*a*/DPPC (0.5 mM) – SUVs, therefore the Chl*a* degradation in the β-carotene containing bilayers is less.

The kinetic models of the oxidative degradation of Chl*a* embedded into liposomes are described by the equations:

$R = 0.35029 + 0.66474 \cdot \exp(-[Q]/22.08337)$, for the Chl*a*/DPPC (0.5 mM) small unilamellar liposomes

and

$R = 0.35029 + 0.66474 \cdot \exp(-[Q]/22.08337)$, for the Chl*a*/β-carotene/DPPC (0.5 mM) small unilamellar liposomes,

where R is the Soret band absorbance ratio and [Q] is the concentration of quercetin.

β-Carotene, a photopigment with antioxidant activity that protects Chl*a*, is located, entirely, in the hydrophobic region of the lipid bilayers, without a preferred well-defined orientation, reducing significantly the membrane mobility [15].

The addition of quercetin to liposome suspensions results in an increase of the lipid mobility and thus Chl*a* located in the liposome lipid bilayer with the macrocycle at the interface with the water phase, becomes more mobile in the lipid bilayer. Therefore, within the liposomes Chl*a*/DPPC (0.5 mM) – SUVs, Chl*a* is more mobile and thus more damaged than in the system Chl*a*/β-carotene/DPPC (0.5 mM) – SUVs. This fact is suggested also in the Figure 7: the *bleaching* values for the Chl*a* embedded into the β-carotene containing bilayers are lesser than in the case of the membranes without β-carotene, therefore the Chl*a* degradation is more pronounced in the last case.

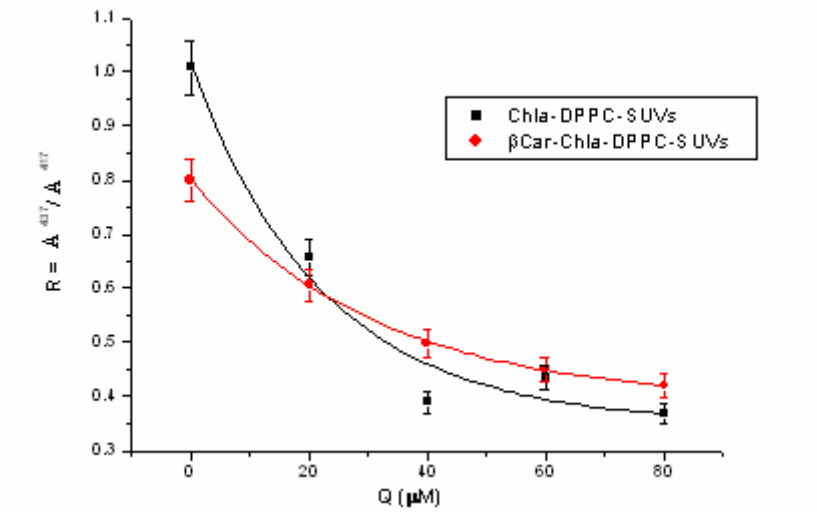

Fig. 6. The Soret band absorbance ratio of Chl*a*/DPPC (0.5 mM) - SUVs (correlation coefficient $r^2 = 0.97304$) and of Chl*a*/β-carotene/DPPC (0.5 mM) - SUVs (correlation coefficient $r^2 = 0.9996$) versus quercetin concentration

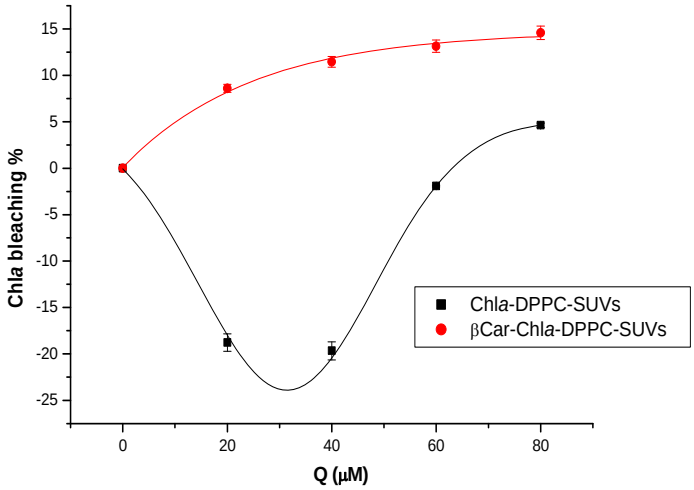

Fig. 7. The bleaching values of Chl*a*/DPPC (0.5 mM) - SUVs (correlation coefficient $r^2 = 0.999$) and of Chl*a*/β-carotene/DPPC (0.5 mM) - SUVs (correlation coefficient $r^2 = 0.99565$) versus quercetin concentration

4. Conclusions

The addition of the quercetin on artificial membranes results in significant spectral changes of the liposomes.

The studies were performed on liposomes with chlorophyll *a* incorporated into lipid bilayers as models of cell membranes.

The effects of quercetin on liposomal membranes were monitored by exploiting the spectral properties of chlorophyll *a* (Chl*a*) embedded into liposome bilayer (Chl*a*/lipid molar ratio = 1/100). Several spectral criteria (the Chl*a* *bleaching* and the *Soret band absorbance ratio*) were evaluated to monitor the oxidative degradation of these membrane models.

The addition of quercetin to liposome suspensions results in a Chl*a* degradation (i.e liposome damage) observed by an exponential decay of the Soret band absorbance ratio and by an increase in the Chl*a* bleaching values. The quercetin greatly affects the lipid membranes without β-carotene.

A future task is to study other types of liposomes with Chl*a*, using a combination of techniques to investigate the effects of different flavonoids on lipid bilayers.

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