Biochimica et Biophysica Acta 1818 (2012) 695–702

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Calcium-dependent aggregation and fusion of phosphatidylcholine liposomes induced by complexes of flavonoids with divalent iron

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ARTICLE INFO

Article history:
Received 18 August 2011
Received in revised form 30 November 2011
Accepted 1 December 2011
Available online 8 December 2011

Keywords:
Flavonoid
Liposome
Iron
Calcium
Membrane adhesion
Membrane fusion

ABSTRACT

It was found that complexes of the flavonoids quercetin, taxifolin, catechin and morin with divalent iron initiated an increase in light scattering in a suspension of unilamellar 100 nm liposomes. The concentration of divalent iron in the suspension was 10 μM. Liposomes were prepared from 1-palmitoyl-2-oleoylglycero-3-phoshpatidylcholine. The fluorescent resonance energy transfer (FRET) analysis of liposomes labeled with NBD-PE and lissamine rhodamine B dyes detected a slow lipid exchange in liposomes treated with flavonoid-iron complexes and calcium, while photon correlation spectroscopy and freeze-fracture electron microscopy revealed the aggregation and fusion of liposomes to yield gigantic vesicles. Such processes were not found in liposomes treated with phloretin because this flavonoid is unable to interact with iron. Rutin was also unable to initiate any marked changes because this water-soluble flavonoid cannot interact with the lipid bilayer. The experimental data and computer calculations of lipophilicity (cLogP) as well as the charge distribution on flavonoid-iron complexes indicate that the adhesion of liposomes is provided by an iron link between flavonoid molecules integrated in adjacent bilayers. It is supposed that calcium cations facilitate the aggregation and fusion of liposomes because they interact with the phosphate moieties of lipids.

1. Introduction

Flavonoids are polyphenolic compounds abundantly constituent in dietary fruits and vegetables. Their daily consumption can reach tens and hundreds milligrams [1]. Depending of diet the concentration of these compounds in blood considerably changes and can reach a few micromoles [2]. The presence of flavonoids in diet is very important for human health because they reveal antiinflammatory [3], antibacterial [4], antiviral [5] and antifungal [6] activity. Flavonoids are also known to prevent cardiovascular [7], cancer [8], and neurodegenerative [9] diseases.

The mechanisms of flavonoid functioning are still not sufficiently studied. It is known that they are able to influence the activity of some enzymes [10,11], or cell signaling processes [12,13] though the main attention is attracted to their antioxidant properties. Flavonoids can protect cells from oxidative stress owing to their radical scavenging activity and also to the ability to helate transient metals including cations of iron and copper [14,15]. Moreover, it was found that flavonoid-metal complexes reveal an elevated radical scavenging activity similar to superoxide dismutase [16].

Lipids of biological membranes are the main targets of oxidative stress. The biological functioning and antioxidative activity of flavonoids correlate well with their lipophilicity and ability to interact with biological membranes [17–19]. The present study is concerned to the interactions of flavonoid-iron complexes with PC liposomes because this subject is scarcely studied. To exclude the influence of lipid oxidative processes on our measurements, we used divalent iron which is less oxidative compared to trivalent iron or copper [15].

2. Materials and methods

2.1. Preparation of liposomes

2-Oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine (Sigma-Aldrich) was dissolved in chloroform, dried under argon stream, and vacuumed overnight to remove the traces of organic solvent. The dried lipid film was hydrated in 10 mM Tris–HCl (pH 7.0) to a lipid concentration of

Abbreviations: PC, phosphatidylcholine; POPC, 2-Oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine; DMSO, dimethyl sulfoxide; NBD-PE, [1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl)]; Rhodamine-PE, [1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl)]; PCS, Photon correlation spectroscopy

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doi:10.1016/j.bbamem.2011.12.001

15 mg/ml which approximately corresponded to 20 mM. A Mini Extruder (Avanti Polar Lipids, USA) was used for preparation of 100 nm liposomes in accordance with a standard procedure.

2.2. Light scattering measurements

Light scattering was measured at 90° on an MPF-44B spectrofluorometer (Perkin Elmer, USA) at $\lambda = 650$ nm. 20 μL of 20 mM liposomes were added at continuous stirring to 10 mM Tris–HCl buffer (pH 7.0) in a 2 ml cuvette. To prepare stock solutions, rutin was dissolved in water, while taxifolin, catechin, morin, and phloretin were dissolved in 70% ethanol, and quercetin in 50% DMSO. The concentration of all flavonoids was 1 mM. For measurements, 20 μL of flavonoids, 20 μL of 1 mM $FeSO_4$ and 20 μL of 10 mM $CaCl_2$ were added to the cuvette, so that the final concentration of lipids in the cuvette was 2×10^{-4} M. The concentrations of flavonoids and iron were 1×10^{-5} M, of calcium 1×10^{-4} M, of DMSO (present only in samples with quercetin) 5×10^{-6} M. All chemicals were from Sigma-Aldrich (USA). In the control measurements, we did not reveal any influence of DMSO or ethanol amounts used on the samples in all experiments presented in this study.

2.3. Liposome size measurements

The size of liposomes was determined with Photon Correlation Spectroscopy (PCS) on an N4 PLUS Submicron Particle Size Analyzer (Beckman Coulter, USA) equipped with a size distribution processor (SDP) that we used for detailed analysis of particle size distribution. We used the same cuvette and the same concentrations of chemicals as in the light scattering experiments. After addition of chemicals to cuvette, we evaluated the light scattering on a spectrofluorometer as described above, to reach the equilibrium, and after that we transferred the cuvette to the Particle Size Analyzer. The whole time of measurements was 30–40 min.

2.4. Fluorescent assay of lipid exchange

For the fluorescent assay, we prepared POPC liposomes containing 0.5% of NBD-PE and 0.5% of rhodamine-PE (Avanti Polar Lipids, USA). Liposomes were prepared according to the procedure described above, except that the concentration of lipid in the suspension was 5 times lower than that of our standard nonfluorescent liposomes. The equal volumes of fluorescent and nonfluorescent liposomes were added to the cuvette so that the final concentration of lipids was equal to that used in light scattering experiments (2×10^{-4} M). The ratio of labeled to unlabeled liposomes was 1:5. Accordingly, the concentrations of flavonoids, iron, and calcium were also equal to those described in our light scattering experiments. Measurements were performed on an MPF-44B spectrofluorometer (Perkin Elmer, USA) at continuous stirring. The excitation wavelength was $\lambda = 500$ nm. The fluorescence of NBD-PE was registered at $\lambda = 537$ nm, while rhodamine-PE fluorescence at $\lambda = 590$ nm. To determine the extent of fusion as a percentage of the total fluorescence change, we introduced 20 to 40 μL of 1 mM triton X100 to the cuvette at the end of measurements.

2.5. Freeze-fracture electron microscopy

For electron microscopy, we mixed the same volumes of all chemicals as it was described for light scattering experiments, except that they were not diluted in 2 ml of buffer. After 10–20 min incubation at room temperature, the suspension of liposomes was placed between 100 μ thick copper sheets and plunged into a container with liquid propane stored in liquid nitrogen. Sample fracturing and shadowing with platinum and carbon were performed at −150 °C and in vacuum (10^{-6} Torr) on a JEE-4B vacuum station (JEOL, Japan) we had modified for freeze-fracture experiments [20]. The platinum-carbon replicas were examined on a JEM-100B transmission electron microscope (JEOL, Japan).

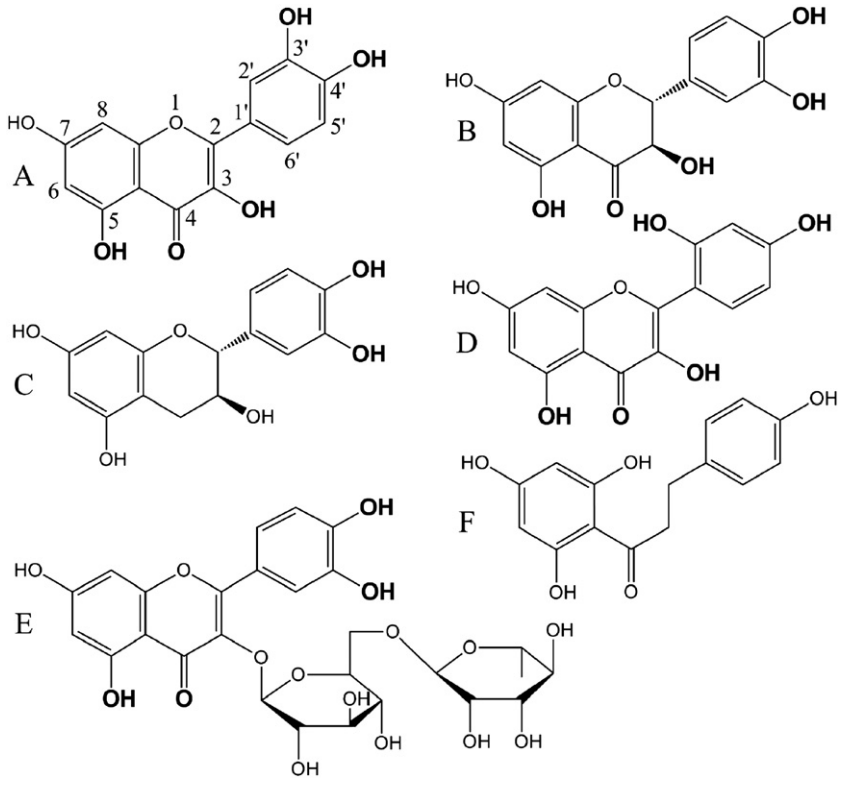

Fig. 1. Flavonoids used in our study. (A) quercetin. The numeration of carbon atoms is presented. (B) taxifolin. (C) catechin. (D) morin. (E) rutin. (F) phloretin. The moieties able to be involved in the interaction with iron are highlighted with bold letters.