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Fluorescence spectroscopic evaluation of the interactions of quercetin, isorhamnetin, and quercetin-3′-sulfate with different albumins

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ABSTRACT

Quercetin is one of the most commonly occurring flavonoids in nature. Although, quercetin and its metabolites express negligible fluorescence, the albumin-bound form of quercetin has a strong fluorescence property. Considering the structural variance of different albumins, we hypothesized that the fluorescence of albumin complexes of quercetin and its metabolites may vary significantly. Therefore, in this study the fluorescence enhancement of quercetin and some of its major metabolites in the presence of bovine (BSA), human (HSA), porcine (PSA), and rat serum albumins (RSA) were investigated by steady-state fluorescence spectroscopy in PBS buffer (pH 7.4). Among the tested quercetin metabolites, significant fluorescence signal was shown by albumin complexes of quercetin, isorhamnetin, and quercetin-3′-sulfate, while other metabolites (tamarixetin, quercetin-3-glucuronide, and isorhamnetin-3-glucuronide) expressed negligible fluorescence. BSA was the most potent enhancer of quercetin-3′-sulfate but it showed poor effects regarding other flavonoids. The strongest enhancement of isorhamnetin was caused by HSA, while it was less effective enhancer of quercetin and quercetin-3′-sulfate. PSA showed a strong fluorescence enhancement of quercetin and quercetin-3′-sulfate but it was poorly effective regarding isorhamnetin. RSA was the most potent enhancer of quercetin but it caused only a weak enhancement of isorhamnetin and quercetin-3′-sulfate. Large changes of the pH (such as pH 5.0 and pH 10.0) almost completely abolished the fluorescence signals of the complexes. Nevertheless, slight decrease (pH 7.0) reduced and slight increase (pH 7.8) generally enhanced the fluorescence of flavonoid-albumin complexes (only exceptions were quercetin-PSA and quercetin-RSA). Complex formations were also investigated by fluorescence quenching studies. Based on our results, the formations of quercetin-BSA, quercetin-HSA, isorhamnetin-BSA, isorhamnetin-HSA, isorhamnetin-PSA, and quercetin-3′-sulfate – HSA complexes followed 1:1 stoichiometry, while the presence of a secondary binding site of flavonoids was assumed regarding other tested albumin complexes. Our study highlights that albumins can induce significantly different fluorescence enhancement of flavonoids, and even the stoichiometry of flavonoid-albumin complexes may differ.

1. Introduction

Flavonoids are ubiquitous polyphenolic compounds in the plant kingdom. They appear in several fruits and vegetables, in numerous dietary supplements, herbal medicines, and in some medications [1,2]. One of the most frequently occurring flavonoids is quercetin which is an extensively studied molecule because of its complex mechanism of action and manifold pharmacological effects in the human organism [3–5]. Beyond that quercetin is part of the diet, many commercially available dietary supplements contain extremely high doses of quercetin [6]. After its oral consumption or administration, quercetin undergoes significant presystemic elimination [7–10]. During its biotransformation, sulfate and glucuronide conjugates as well as methylated metabolites of quercetin are formed [11,12]. In the human circulation, the parent compound quercetin represents only a few percent of the total quercetin, while quercetin-3′-sulfate (Q3′S) is the dominant circulating metabolite [12]. Furthermore, 3′- and 4′-methylation of quercetin by catechol-O-methyltransferase (COMT) enzyme result in the formation of isorhamnetin (IR; 3′-*O*-methylquercetin) and tamarixetin (4′-*O*-methylquercetin), respectively [8,13].

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Albumin is the most abundant plasma protein in humans and animals. Albumin maintains the oncotic pressure of the blood, and it is able to form stable complexes with several compounds (including fatty acids, hormones, amino acids, bile acids, metals, drugs, natural compounds, and xenobiotics) which can strongly affect the distribution and elimination of these ligand molecules [14,15]. Therefore, albumin can significantly influence the pharmacokinetic properties of these substances [16–18]. Previous studies demonstrated that quercetin forms stable complexes with human serum albumin (HSA) and bovine serum albumin (BSA), occupying Sudlow's Site I (on subdomain IIA, which is the primary binding site of warfarin as well) [19–25]. However, a recent study suggest that flavonoid aglycones do not bind at warfarin high affinity site, but rather to different regions within the IIA subdomain of HSA [26]. Investigation of quercetin-albumin interaction is an important issue because the complex formation may have both therapeutic advantages and unpleasant consequences. Based on previous studies, albumin nanoparticles can promote the stability of quercetin in the intestinal fluid [27], and albumin improves the positive effect of quercetin on survival of erythrocytes in visceral leishmaniasis [28]. On the other hand, the competitive interaction of quercetin with other Sudlow's Site I ligands can result in the displacement of drugs from albumin which may responsible for the disruption of drug therapy [24,29–32]. In addition, other investigations also highlighted the strong interaction of quercetin metabolites with HSA [6,33,34] which is very important because of the extensive biotransformation of quercetin in the body [4,7,12].

Albumin-ligand interactions are commonly investigated by fluorescence spectroscopy due to the quenching of the tryptophan residue(s) of albumins can be precisely followed with this technique. Besides quenching studies, some of the fluorescence investigations of quercetin-albumin interactions highlighted that the non-fluorescent (or poorly fluorescent) quercetin shows strong fluorescence in its albumin-bound form [20–22]. Despite their similarities, different albumin species show large variance regarding their interaction with ligand molecules [35,36]. Therefore, it is plausible to hypothesize that different albumins may cause significantly different fluorescence enhancement of quercetin or its metabolites.

In this study, the influence of BSA, HSA, porcine serum albumin (PSA), and rat serum albumin (RSA) on the fluorescence properties of quercetin and its metabolites (including IR, Q3′S, tamarixetin, quercetin-3-glucuronide, and isorhamnetin-3-glucuronide) was investigated. Since among the tested compounds, only quercetin, IR, and Q3′S (Fig. 1) gave significant fluorescence signals in the presence of albumins, complex formation of these flavonoids with the four different albumins were further investigated. During the high quercetin intake (through the consumption of dietary supplements and herbal medications), high concentrations of quercetin and its metabolites can reach the circulation. Therefore, the deeper understanding of the flavonoid-albumin complex formations may give us important and pharmacologically relevant information. Furthermore, extrapolation of animal experiments to humans is commonly a controversial issue; therefore, similarities or differences between the binding abilities of flavonoid molecules to human and animal albumins are also important. In addition, the fluorescence enhancement of quercetin, IR, and Q3′S by albumins makes possible the fluorescence spectroscopic detection and investigation of these poorly fluorescent molecules.

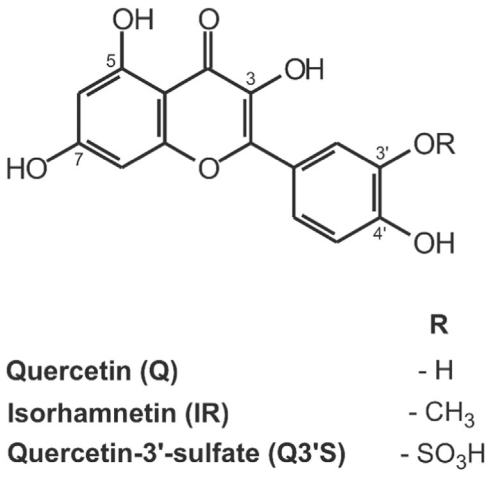

Fig. 1. Chemical structures of quercetin, isorhamnetin, and quercetin-3′-sulfate.

2. Materials and methods

2.1. Reagents

All applied chemicals were of spectroscopic or analytical grade. Quercetin dihydrate (Q; product code: 1135S, purity: ≥ 99% HPLC), isorhamnetin (IR; product code: 1120S, purity: ≥ 99%, HPLC), and tamarixetin (product code: 1140S, purity: ≥ 99%, HPLC) were from Extrasynthese. Human serum albumin (HSA; product code: A1653, purity: ≥ 96%, agarose gel electrophoresis), bovine serum albumin (BSA; product code: A2153, purity: ≥ 98%, agarose gel electrophoresis), porcine serum albumin (PSA; product code: A1830, purity: ≥ 98%, agarose gel electrophoresis), and rat serum albumin (RSA; product code: A6272, purity: ≥ 96%, agarose gel electrophoresis) were purchased from Sigma-Aldrich. Quercetin-3′-sulfate (Q3′S), quercetin-3-glucuronide, and isorhamnetin-3-glucuronide (purity of each conjugate: > 95%, HPLC) were synthetized as described elsewhere [37]. 2000 μM stock solutions of flavonoids were prepared in spectroscopic grade dimethyl sulfoxide (Fluka); thereafter these solutions were diluted (in more steps) by the appropriate buffers (for example PBS) in order to produce the samples. Most of the measurements were carried out in PBS buffer (phosphate buffered saline, pH 7.4) in order to mimic extracellular physiological environment, because this buffer contains very similar concentrations of sodium, potassium, and chloride ions which are typical of the extracellular space (including blood). To examine the influence of pH on the fluorescence enhancement of flavonoids by albumins, 0.05 M sodium acetate (pH 5.0), 0.05 M sodium-borate (pH 10.0), and PBS (pH 7.0 and 7.8) buffers were applied as well.

2.2. Spectroscopic measurements

Fluorescence spectra were recorded employing a Hitachi F-4500 fluorescence spectrophotometer (Tokyo, Japan). To test the influence of different albumins on the fluorescence signal of flavonoids, increasing albumin concentrations (0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 5.0 μM) were added to 2 μM flavonoid. Then fluorescence emission spectra were recorded using 445 nm (Q3′S) or 455 nm (quercetin and IR) as excitation wavelengths.

In order to investigate the complex formations of flavonoids with albumins, fluorescence quenching method was applied. During these experiments, increasing flavonoid concentrations (0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 μM) were added to 2 μM albumin. Fluorescence emission spectra of albumins were recorded using 295 nm as excitation wavelength. Quenching experiments were evaluated based on the graphical application of the Stern-Volmer equation:

$$\frac{I_0}{I} = 1 + K_{SV}*[Q] \quad (1)$$

where I_0 and I are fluorescence emission intensities of albumin with and without flavonoids, respectively. K_{SV} is the Stern-Volmer quenching constant while $[Q]$ is the concentration of the flavonoid. Thereafter, based on the quenching data, binding constants (K) were quantified using the graphical application of double logarithm Stern-Volmer equation as well [38,39]:

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