Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 155 (2016) 88–94

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

The increased binding affinity of curcumin with human serum albumin in the presence of rutin and baicalin: A potential for drug delivery system

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ARTICLE INFO

Article history:
Received 1 July 2015
Received in revised form 4 November 2015
Accepted 6 November 2015
Available online 7 November 2015

Keywords:
Rutin
Baicalin
Curcumin
Human serum albumin
Interaction
Drug delivery

ABSTRACT

The impacts of rutin and baicalin on the interaction of curcumin (CU) with human serum albumin (HSA) were investigated by fluorescence and circular dichroism (CD) spectroscopies under imitated physiological conditions. The results showed that the fluorescence quenching of HSA by CU was a simultaneous static and dynamic quenching process, irrespective of the presence or absence of flavonoids. The binding constants between CU and HSA in the absence and presence of rutin and baicalin were $2.268 \times 10^5\ M^{-1}$, $3.062 \times 10^5\ M^{-1}$, and $3.271 \times 10^5\ M^{-1}$, indicating that the binding affinity was increased in the case of two flavonoids. Furthermore, the binding distance determined according to Förster's theory was decreased in the presence of flavonoids. Combined with the fact that flavonoids and CU have the same binding site (site I), it can be concluded that they may simultaneously bind in different regions in site I, and formed a ternary complex of flavonoid–HSA–CU. Meanwhile, the results of fluorescence quenching, CD and three-dimensional fluorescence spectra revealed that flavonoids further strengthened the microenvironmental and conformational changes of HSA induced by CU binding. Therefore, it is possible to develop a novel complex involving CU, flavonoid and HSA for CU delivery. The work may provide some valuable information in terms of improving the poor bioavailabiliy of CU.

1. Introduction

Curcumin ((E,E)-1,7-bis(4-hydroxy-3-methoxy-phenyl)-1,6-heptadiene-3,5-ione, CU) (Fig. 1) is a hydrophobic polyphenolic compound extracted from turmeric (*Curcuma longa* Linn). It exhibits a broad range of biological properties, including antioxidant and anticancer activities, with high safety profile to the human body [1,2]. However, the low aqueous solubility, instability at physiological pH, rapid metabolism and fast elimination of CU limit its bioavailability [3,4]. In recent yeas, it has been reported that serum albumins have the ability to solubilize and stabilize CU in aqueous medium and can be used as carriers in CU delivery [5–7]. Albumin-based drug delivery has been considered as a promising therapeutic approach to improve unfavorable pharmacokinetic properties and enhance the therapeutic index of drugs [8,9]. In fact, albumin-bound paclitaxel (ABI007, Abraxane) was approved for the treatment of metastatic breast cancer [10]. On the other hand, the in vitro study showed that the synergistic inhibitory effects of CU and genistein on the growth of human breast cancer MCF-7 cells induced by estrogenic pesticides, and the inhibitory effects were superior to the individual effects of either CU or genistein [11]. Besides, Cruz-Correa et al. [12] found that the combination therapy with quercetin and curcumin effectively reduced the number and size of ileal and rectal adenomas in patients without appreciable toxicity. A recent study also suggested that quercetin could enhance cellular uptake of CU, which could be related to albumin-binding interaction [13]. Incidentally, Kudva et al. [14] reported that CU, human serum albumin (HSA) and amphotericin B could form the water-soluble ternary complex of CU-HSA-amphotericin B, and the presence of CU in the complex significantly delayed the red cell lysis by amphotericin B. This report, combined with the synergistic effect of CU and flavonoid compounds and the significant advantages of HSA as a drug delivery system, prompted us to investigate the possibility of formation of the ternary flavonoid-HSA-CU complex.

Flavonoids consist of a large group of polyphenolic compounds, and usually occur as aglycones, glycosides, and methylated derivatives in plants. Rutin and baicalin (Fig. 1) are the glycoside of quercetin and the glucuronide of baicalein, respectively. They appear as the most abundant flavonoids in a wide variety of plants and possess widely pharmacological properties, such as antioxidant, anti-inflammatory, and anticancer activities [15,16]. A few reports have showed that rutin and baicalin can form complexes with HSA individually [17,18], and their bindings mainly occur in the subdomain IIA (site I) [19–21]. It has been demonstrated that besides binding to bovine serum albumin and β-lactoglobulin [22,23] CU can also bind to HSA via hydrophobic interactions [24–26], and the primary binding site is located in site I [25–27]. Therefore, the CU binding region in site I on the HSA molecule is different from, or has overlap with that of the two flavonoids, which implied that it may exist the co-binding mode, or competitive binding mode between flavonoids and CU in site I upon their simultaneous binding to HSA. Consequently, in this study we investigated the binding characteristics of CU with HSA in the

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http://dx.doi.org/10.1016/j.saa.2015.11.010
1386-1425/

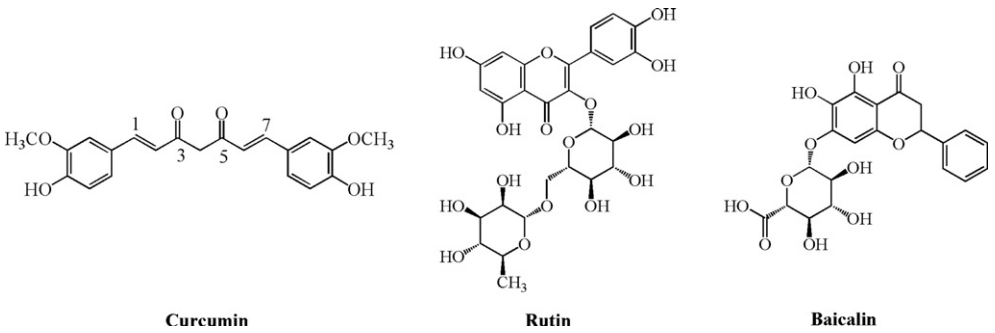

Fig. 1. The chemical structures of curcumin (CU), rutin and baicalin.

presence of rutin and baicalin, including the quenching mechanism, quenching and binding constants, by using the fluorescence quenching method. In addition, the changes in HSA conformation induced by CU before and after the addition of flavonoids were compared by means of circular dichroism (CD) and three-dimensional fluorescence measurements. Our main aim is to elucidate the molecular mechanism of the interaction between flavonoids and CU with HSA in anticipation of providing valuable information for the improvement of poor pharmacokinetics of curcumin.

2. Experimental

2.1. Materials

CU was purchased from Shunbo Biotechnology Co. Ltd., Shanghai, China. HSA was obtained from Sigma-Aldrich Co., USA. The HSA stock solution (1.0×10^{-5} M) was prepared in the 50 mM Tris–HCl buffer solution containing NaCl (50 mM, pH 7.4), and stored in the dark at 4 °C. Rutin and baicalin were obtained from Chengdu Superman Plant & Chemical Development Co., Ltd., China. The stock solutions of CU, rutin and baicalin (1.25×10^{-3} M) were prepared by dissolving them in a small amount of ethanol, and then diluting with the buffer solution. Doubly distilled water was used to prepare the buffers.

2.2. Methods

2.2.1. Fluorescence spectra

All fluorescence emission spectra and the three-dimensional fluorescence spectra were carried out on an F-7000 fluorescence spectrophotometer (Hitachi High-Technologies Co., Japan) equipped with a thermostat bath. The binding of CU with HSA in the absence and presence of flavonoids was studied by the fluorescence quenching titration method. To a 1.0 cm quartz cell, 2.5 mL of 1.0×10^{-5} M HSA solution (or the mixed solution of HSA and flavonoid at a 1:5 M ratio.) was added, and then a certain amount of CU solution was gradually titrated manually into the cell (the final volume of CU was 40 μL) using a microinjector for the binary and ternary systems, the final concentrations of CU were from 0 to 20.0×10^{-6} M at 2.5×10^{-6} M intervals. The fluorescence spectra were recorded in the range of 300–450 nm with the excitation wavelength of 295 nm. The spectra bandwidths of excitation and emission slits were both kept at 5.0 nm and the scanning speed of 1200 nm min^{-1}. The fluorescence intensity was corrected for absorption of exciting light and re-absorption of emitted light using the following equation [28]:

$$F_{corr} = F_{obs} \times exp((A_{ex} + A_{em})/2). \quad (1)$$

F_{corr} and F_{obs} are the fluorescence intensity corrected and observed, respectively, and A_{ex} and A_{em} are the absorbance of the system at the excitation and emission wavelengths, respectively. The intensities of fluorescence were corrected in this work.

The three-dimensional fluorescence spectra were performed under the following conditions: the emission wavelength was between 200 nm and 800 nm, the initial excitation wavelength was set at 200 nm with an increment of 5 nm and the scanning speed is 2400 nm/min, and other parameters are just the same as those of the fluorescence emission spectra.

2.2.2. Circular dichroism studies

CD measurements were carried out with a J-810 automatic recording spectropolarimeter (Jasco Co., Tokyo, Japan), equipped with a 1.0 mm path length quartz cuvette. All the CD spectra were recorded in the range of 200–240 nm at 298 K under constant nitrogen flush. Each spectrum was the average of three successive scans and was corrected by Tris–HCl buffer solution. The results were expressed as ellipticity in millidegree.

3. Results and discussion

3.1. Fluorescence quenching of HSA by CU in the absence and presence of flavonoids

The intrinsic fluorescence of HSA originates almost exclusively from the tryptophan residue (Trp) in the hydrophobic cavity of subdomain IIA (site I) when the 295 nm excitation wavelength is used [29,30]. To investigate the binding of CU to HSA, the fluorescence emission spectra of HSA in the presence of various concentrations of CU were recorded upon excitation at 295 nm. It can be seen in Fig. 2A that HSA had a strong fluorescence emission at 343 nm after excitation at 295 nm. The addition of varying concentrations of CU caused a noticeable decrease in the fluorescence intensity of HSA with a concomitant blue shift (7 nm). The strong quenching of the Trp residue fluorescence and the blue shift of the fluorescence emission peak indicated that the HSA conformation may have become changed and that an inter-molecular energy transfer occurred between CU and HSA. It was moreover an indication of the chromophore of HSA transferred to a more hydrophobic environment and the conformation of the protein becoming altered [31]. This suggested that CU bound to site I. Fig. 2B and C presents the effects of rutin and baicalin on the fluorescence quenching spectra of CU to HSA at the excitation wavelength 295 nm, respectively. As can be seen, when CU was respectively added to HSA solution (1.0×10^{-5} M) containing flavonoid (5.0×10^{-5} M), further attenuations in the fluorescence of HSA with blue shifts were observed, which indicated that the presence of flavonoids further affected the microenvironment around the Trp residues.

In order to compare the changes in fluorescence of HSA bound with CU in the absence and presence of flavonoids, one can examine the quenching curves ($F/F_0 \sim [CU]$) for the binary and ternary systems. The plot is depicted in Fig. 3. It can be calculated that when the concentration of CU reached 20.0×10^{-6} M, the fluorescence intensities of HSA