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Effect of Zinc (II) on the interactions of bovine serum albumin with flavonols bearing different number of hydroxyl substituent on B-ring

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ABSTRACT

The impact of Zn^{2+} ion on interactions of flavonols galangin (Gal), kaempferol (Kae), quercetin (Que) and myricetin (Myr) with bovine serum albumin (BSA) in aqueous solution were studied by fluorescence quenching technique. The results exhibited that Zn^{2+} ion affected significantly the interactions and the effect was distinct for the flavonol bearing different number of B-ring hydroxyl. Each flavonol can quench the fluorescence of BSA, displaying a quenching extent of Myr > Que > Kae > Gal, which is in good agreement with the number variation of the B-ring hydroxyl. The presence of Zn^{2+} ion promoted the quenching for the flavonols, exhibiting an extent of Que > Myr > Kae > Gal. The values of K_a for Kae, Que and Myr decreased whereas K_{SV} and k_q for Gal, Kae and Que increased with the number of B-ring hydroxyl. The type of BSA fluorescence quenching for Gal, Kae and Que hardly changed but the preference of static quenching increased. The values of K_{SV} and k_q for Myr remarkably decreased and the fluorescence quenching of BSA alternatively occurred via both static and dynamic type instead of only one (static or dynamic). The results suggest the key role of the B-ring hydroxyl and the distinct effect of its number in the interactions. Each flavonol may capture the BSA-bound Zn^{II} in the solution, forming Zn^{II}–flavonol complex that is possibly responsible for BSA fluorescence quenching. The B-ring hydroxyl could establish hydrogen bonds with BSA in the absence of Zn^{2+} and act as donors for chelating in the presence of Zn^{2+}. The formation of dinuclear Zn^{II}–Myr complex together with the hydrogen bonds between the free B-ring hydroxyl and BSA may contribute to the exceptional behavior of Myr.

1. Introduction

Flavonols, a subgroup of flavonoids, are polyphenol compounds that distribute ubiquitously in a wide range of vascular plants [1]. Over the past years, much attention has been paid to their structure-dependent biological and pharmacological activities [2,3]. The fundamental molecular structure of a flavonol comprises two aromatic rings (A and B) linked through a heterocyclic pyran-4-one ring C. The C_2–C_3 double-bond and 4-oxo functional group of the C-ring are suggested to be important factors of flavonols for their biological activities, such as the inhibitory activity in expression of Cox-2 protein [4]. The hydroxylation at the A- and C-ring moieties is proven to be important structural requirement for a significant *in vitro* anti-oxidant [5] and myeloperoxidase inhibitory activity [6]. The presence of a dihydroxyl group (catechol-type) or three adjacent hydroxyl groups (pyrogallol-type) on the B-ring are also recognized to be very important for some of their biological activities such as anti-oxidant and antiradical activity [7]. The B-ring dihydroxyl is even proposed to be an indicator of anti-oxidant activity [8]. Galangin (3,5,7-trihydroxy-2-phenyl-4*H*-chromen-4-one, Gal), kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4*H*-chromen-4-one, Kae), quercetin (3,5,7-trihydroxy-2-(3,4-dihydroxyphenyl)-4H-chromen-4-one, Que) and myricetin (3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-4H-chromen-4-one, Myr) are the typical representative of flavonols (Fig. 1) [9,10]. They share the same substituents on the A- and C-ring but feature different number of hydroxyl on the B-ring. Specifically, Gal has no hydroxyl group on the B-ring; Kae, Que and Myr have the structure of Gal with one additional hydroxyl on the C_4', two hydroxyls on the C_4' and C_5', and three hydroxyls on the C_3', C_4' and C_5' positions on the B-ring, respectively. They all were recognized to possess a variety of biological activities and important therapeutic applications [11–18].

The biological or therapeutic activities of flavonoids could be altered to some extent by metal ions present due to the chelation of flavonoids to metal ions, forming metal–flavonoid complexes [19]. A flavonol molecule possesses three potential sites for chelating of cations [20]. These are the –OH group on the B-ring, between the 4-CO and 3-OH groups of the C-ring, and between the 4-CO of the C-ring and 5-OH of the A-ring. The 4-CO and 3-OH groups are the first sites to be involved in the complexation process

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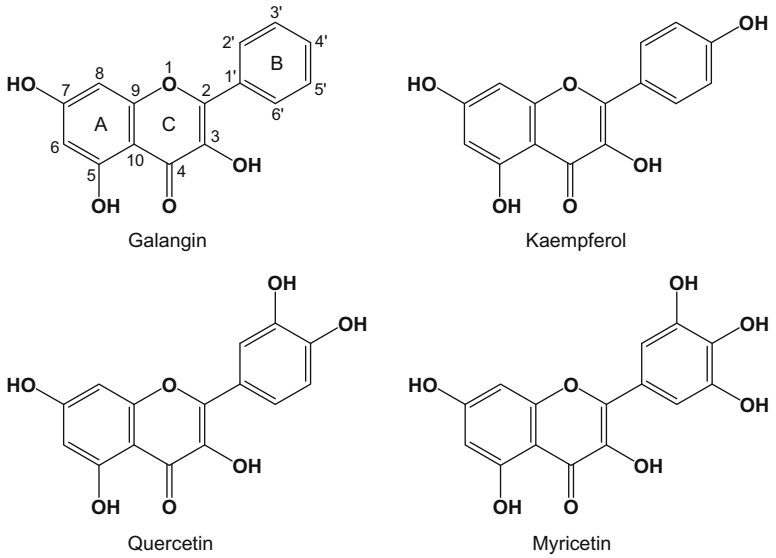

Fig. 1. Molecular structure of Gal, Kae, Que and Myr.

because the 3-OH group has a more acidic proton [21]. The –OH groups on the B-ring bind a second metal ion. The 5-OH group is usually not involved in the chelation due to its lesser proton acidity and the steric hindrance caused by the first complexation [22]. Related studies on flavonols Gal, Kae, Que and Myr have been reported previously [20–31].

Moreover, many biologically active flavonoids appear to have effects on various proteins [32]. Serum albumin is a most important depot and transport protein of the circulatory system in the body of all vertebrates [33]. Study on the interactions between serum albumin and drugs can provide general fundamental insights into drug-protein binding [34]. Molecules having higher affinity for serum albumin and showing preferential binding sites on serum albumin may find potential therapeutical applications [35]. Thus, the relevant protein bovine serum albumin (BSA) has become the best-studied model particularly because of its high structural homology and similarity to human serum albumin (HSA) in sequence and conformation [36]. By means of the fluorescence properties of the tryptophan (Trp) residues on BSA molecule, the interactions between various species and BSA have been extensively studied, including the flavonols bearing different number of hydroxyl on the B-ring, Gal, Kae, Que and Myr [5,37–41]. However, little is known about the effect of metal ions present on the interactions between each above flavanol and BSA. Zinc (II) is a second most abundant essential element that possesses many physiological functions in human body [42]. Herein, Gal, Kae, Que and Myr were selected to investigate their interactions with BSA in the presence of Zn^{2+} ion, in attempt to gain insight into the impact of Zn^{2+} ion on the interactions.

2. Experimental

2.1. Apparatus

Fluorescence spectra were recorded on a JASCO FP-6500 spectrofluorometer equipped with a thermostated compartment and a 150 W Xenon lamp. A set of 1.0 cm quartz cells was used in the fluorescence titrations. The pH measurements were carried out on a PHS-3C Exact Digital pH meter equipped with Phonix Ag–AgCl reference electrode (Cole–Paemer Instrument Co.).

2.2. Chemicals and reagents

BSA (fraction V), Gal (⩾98%), Que (⩾98%), and Myr (⩾98%) were purchased from sigma–aldrich. Kae (⩾98%) was obtained commercially from the National Institute for Control of Pharmaceutical and Biological Products (China). BSA and all flavonols were stored in refrigerator under 303 K prior to use.

A stock solution of Gal, Kae, Que and Myr (1.0×10^{-4} M) was respectively prepared by dissolving each flavonol in methanol–water solution (1:4, v/v). A stock solution of Zn^{2+} (1.0×10^{-2} M) was prepared by dissolving Zinc acetate dihydrate in water. Aqueous Tris–HCl (0.05 M)-NaCl (0.10 M) solution was used as buffer to keep pH value (pH 7.40), and to maintain the ionic strength of all solutions in experiments. A working solution of BSA (1.0×10^{-5} M) was prepared with the Tris–HCl buffer. All solutions were prepared using freshly doubly-distilled water and stored less than 303 K prior to use. All reagents and solvents are of analytical reagent grade.

2.3. Fluorescence spectrum

Appropriate aliquots of BSA working solution were transferred to eleven of 10 mL flask, respectively. To each flask were added quantitative Zn^{2+} solution and different aliquots of a flavonol solution, and then diluted to 10 mL with the Tris–HCl buffer. Each solution was mixed fully and incubated at 310 K for 1 h. The fluorescence of BSA was obtained by scanning each solution on the fluorophotometer within the range of 290–550 nm at 310 K against the same concentration of each flavonol and Tris mixture, respectively. The emission intensity was recorded at 342 nm. The excitation wavelength is 280 nm and the spectral bandwidths of excitation and emission slit were both set at 3.0 nm.

3. Results and discussion

3.1. Fluorescence quenching of BSA

3.1.1. Fluorescence quenching of BSA induced by Gal, Kae, Que and Myr or Zn^{2+} ion

The complexation of polyphenols with proteins in aqueous solution has been extensively studied by means of various tech-

niques [38]. The fluorescence quenching was proven to be a very sensitive one with potentiality to analyze the interactions between polyphenols and proteins [43]. When excited by radiation of 280 nm, BSA in aqueous Tris–HCl buffer (pH7.40) emits fluorescence with characteristic wavelength at 342 nm that is attributed mainly to the indole group of Trp residues [44]. Upon addition of Gal, Kae, Que or Myr to the BSA solution, respectively, the fluorescence quenching of BSA occurred. The fluorescence intensity attenuated gradually with increasing concentration of each flavonol. Approximately 35.9%, 55.8%, 66.3% and 74.1% of fluorescence quenching was observed when the concentration of each flavonol reached 2.00×10^{-6} M (Fig. 2). The extent of the fluorescence attenuation is in an order: Myr > Que > Kae > Gal (Fig. 3), which is just coincidental with the number variation of the substituted hydroxyl on the B-ring of corresponding flavonol. The result suggests a key role of the B-ring hydroxyl and a distinct effect of its number in the interactions. In addition, except for Gal, other three flavonols resulted in a small blue-shift of the maximum emission of BSA to 337 nm, 338 nm and 338 nm from 342 nm, respectively, which is also correlated slightly to the number of B-ring hydroxyl. Such efficient quenching was accompanied by appearance of a weak emission band in the range of 510–530 nm (figures not shown). The emission could be attributed to the formed flavonol–BSA complexes because each flavonol also absorbs the excitation light but does not fluoresce around this band. In this case, the energy transfer from the excited Trp residues to the flavonol may simultaneously occur. BSA contains two Trp residues that possess intrinsic fluorescence, namely, Trp-134 and Trp-212 [36]. Trp-134 is located on the surface and Trp-212 is located within the hydrophobic pocket of BSA molecule [44]. The results indicate that the flavonol molecules should be located on or at least close to the Trp residues when they bind to BSA. Moreover, the small blue-shift of the maximum emission of BSA resulted by Myr, Que and Kae suggests occurrence of some changes of the microenvironments around the fluorophores. Such changes may be caused by the binding of the flavonol to BSA via formation of hydrogen bonds between the hydroxyl groups of flavonol and the amino and

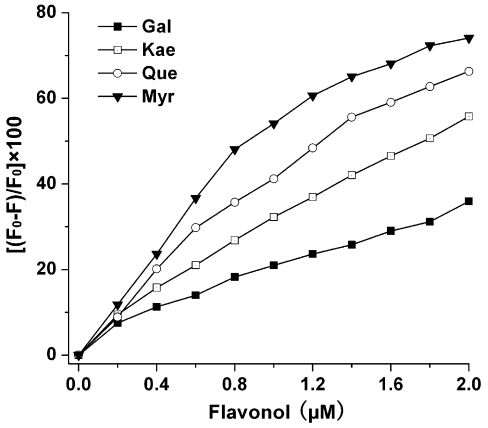

Fig. 3. The fluorescence quenching of BSA plotted as relative percent of $(F_0 - F)/F_0$ against the concentration variation of the flavonol. The fluorescence intensity was recorded at the maximum emission wavelength; the conditions are the same as those in Fig. 2.

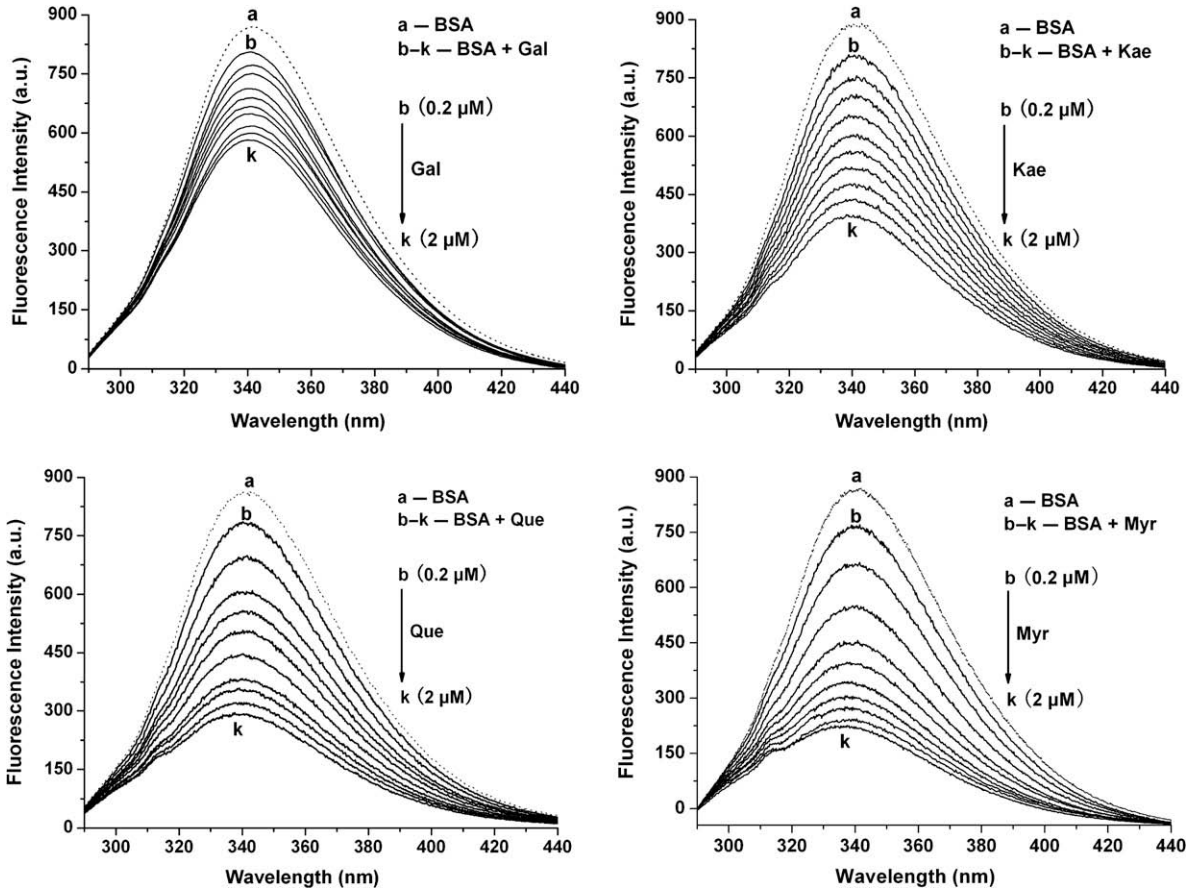

Fig. 2. The fluorescence quenching of BSA by Gal, Kae, Que or Myr. BSA, 1.00×10^{-6} M; Flavonol (from top to down), 0.20, 0.40, 0.60, 0.80, 1.00, 1.20, 1.40, 1.60, 1.80, 2.00 ($\times 10^{-6}$) M; λ_{ex} = 280 nm; T = 310 K; Tris–HCl buffer (pH 7.40).

hydroxyl, etc. of BSA. The hydrophobic interactions between the hydrophobic groups of flavonol and the hydrophobic cavity of BSA may also contribute to the changes. The binding via hydrogen bonds should be dominant in terms of the remarkable dependence of BSA fluorescence variation on the B-ring hydroxyl number of the flavonols.

A similar fluorescence quenching but stronger blue-shifts of the maximum λ_{em} of BSA were observed upon continuous addition of Zn^{2+} ion to the BSA solution (Fig. 4). When the concentration of Zn^{2+} was approaching to 10^{-4} M, the maximum emission of BSA hardly further decreased. Approximately 37% of the fluorescence was quenched and *ca* 11 nm of blue-shift occurred when 10^{-4} M of Zn^{2+} was added. The blue-shift is indicative of changes in the immediate environment of the Trp residues, typically, the polarity of Trp residues and the hydrophobicity of the hydrophobic cavity of BSA [44]. The hydrophobic groups are in the interior of the tertiary structure and the polar groups are on the surface of native protein [36]. The emission of BSA may be blue-shifted if the indole group of Trp is buried within native protein, and its emission may be red-shifted when the protein is unfolded [45]. The result suggests that a greater change in the immediate environment of the Trp residues occurred in addition that Zn^{2+} was situated close proximity to or on the surface of the Trp residues. The buried indole group of Trp could be re-deployed in a more hydrophobic environment after addition of Zn^{2+} ion [46]. Thus, the molecular conformation of the protein was accordingly affected, similar to the previous report [47]. BSA molecule can provide donors N, O and S from amino, hydroxyl, carbonyl and sulfhydryl, *etc.* for coordination of metal ions. Zinc (II) ion could be bound by these donors or accommodated into the cavity of BSA, forming Zn^{II}–BSA complex under the experimental conditions. Moreover, the solution pH 7.40 is higher than the isoelectric point of BSA (4.70) [43], which renders BSA having a negative charge that can bind to Zn^{2+} ion through electrostatic attraction. All these interactions may account for the remarkable fluorescence quenching of BSA and the prominent blue-shift of its maximum emission.

3.1.2. Fluorescence quenching of BSA induced by Gal, Kae, Que or Myr in the presence of Zn^{2+} ion

To the BSA solution (1.00×10^{-6} M) containing 10^{-4} M of Zn^{2+} ion was added continuously each of the four flavonols, further attenuation in the fluorescence of BSA was observed (Fig. 5). When each flavonol in the same final concentration (2.00×10^{-6} M) was added, the fluorescence was decreased by *ca* 38.3%, 42.8%, 55.2% and 48.1%, respectively, from the original Zn^{2+}-resulted 37%. The fluorescence quenching extent is larger than that by either Zn^{2+} ion or corresponding flavonol alone except Myr, and exhibits an order of Que > Myr > Kae > Gal (Fig. 6). Nevertheless, it is much less than that by a simple arithmetic sum of both Zn^{2+} ion and each flavonol. Obviously, these cases are not the same as those resulted by each flavonol alone (Figs. 2 and 3). The results suggest that a newly-formed species rather than Zn^{2+} ion or the flavonol alone consequently resulted in the fluorescence quenching of BSA. Given excess of Zn^{2+} ion, the synergistic effect of the species and excessive Zn^{2+} ion could not be ruled out. In addition, a small blue-shift of the maximum emission of BSA (*ca* 1–3 nm) was observed again for each flavonol. However, the shift magnitude is less than that resulted by each flavonol but is almost the same as that by Zn^{2+} alone (Fig. 4), which indicates that such blue-shift is dominantly ascribed to the effect of the formed species or/and Zn^{2+} ion.

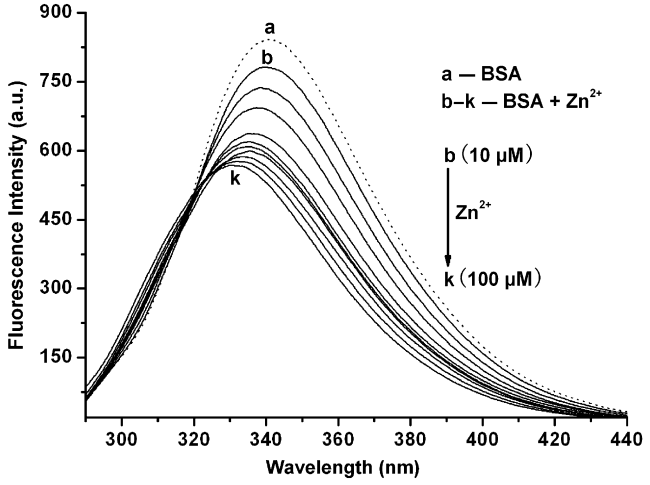

Fig. 4. The fluorescence quenching of BSA by Zn^{2+} ion. BSA, 1.00×10^{-6} M; Zn^{2+}, 10^{-5}–10^{-4} M; λ_{ex} = 280 nm; T = 310 K; Tris–HCl buffer (pH 7.40).

As mentioned above, Gal, Kae, Que and Myr are effective metal ion chelators as other flavonols. In the BSA solution containing Zn^{2+} ion, the added flavonol may compete for Zn^{2+} ion with BSA, partially catching the BSA-bound Zn^{2+} and forming Zn^{II}–flavonol complex that acted as the new species to quench the fluorescence of BSA. The four flavonols share the same substituents on the A- and C-ring whereas possess different number of B-ring hydroxyl that involves in metal chelating. So, the structural difference between them may lead to the formation of different Zn^{II}–flavonol complexes under the same conditions. Studies showed that Gal and Kae employ the 4-CO and 3-OH groups on the C-ring to chelate Zn^{2+} ion, forming mononuclear complex Zn^{II}–Gal [22] and Zn^{II}–Kae [31]. As for Que, a dinuclear Zn^{II}–Que complex forms in different buffers [22,31]. In the complex, one Zn^{2+} is chelated by 4-CO and 3-OH on the C-ring with a high affinity, and the other Zn^{2+} is chelated by one hydroxyl (3′-OH) [31] or two hydroxyls (3′- and 4′-OH) [22] on the B-ring with a low affinity. Limited study on the formation of Zn^{II}–Myr complex was reported [27], and yet, it is expected that a preferred dinuclear Zn^{II}–Myr complex could likewise form due to the structural similarity of Myr to Que. In this way, Myr can provide at least one free B-ring hydroxyl that could be available to bind with BSA through hydrogen bonds. Hence, the exceptional behavior of Myr may be ascribed to the formation of dinuclear Zn^{II}–Myr complex accompanied by stronger hydrogen bonds between the free B-ring hydroxyl and BSA. In this way, different mode of BSA fluorescence quenching for Myr from other three flavonols may be involved.

3.2. Effect of Zn^{2+} ion on the fluorescence quenching type of BSA by Gal, Kae, Que or Myr

To gain more insight into the effect of Zn^{2+} ion on the interactions between the flavonols and BSA, the fluorescence quenching type for each flavonol were further investigated. The above results indicate that the flavonols, Zn^{2+} ion and the formed Zn^{II}–flavonol complexes all act as quenchers that are capable of quenching the fluorescence of BSA. Fluorescence quenching can be dynamic, resulting from collisional encounters between the fluorophores and quenchers, or static, resulting from the formation of a ground-state complex between them. The Stern–Volmer equation can be employed to describe the fluorescence quenching [45]. A linear Stern–Volmer plot is generally indicative of a single class of fluorophores, all equally accessible to the quenchers. Also, it means that only one mechanism (dynamic or static) of quenching occurs [39]. In many cases, the plot exhibits an upward curvature toward the *y*-axis at high concentration of the quenchers, which indicates that the fluorophores can be quenched both by collision (dynamic) and by complex formation (static) with the same quenchers [48]. The upward curvature may also indicate the involvement of a "sphere of action" model-defined static quench-

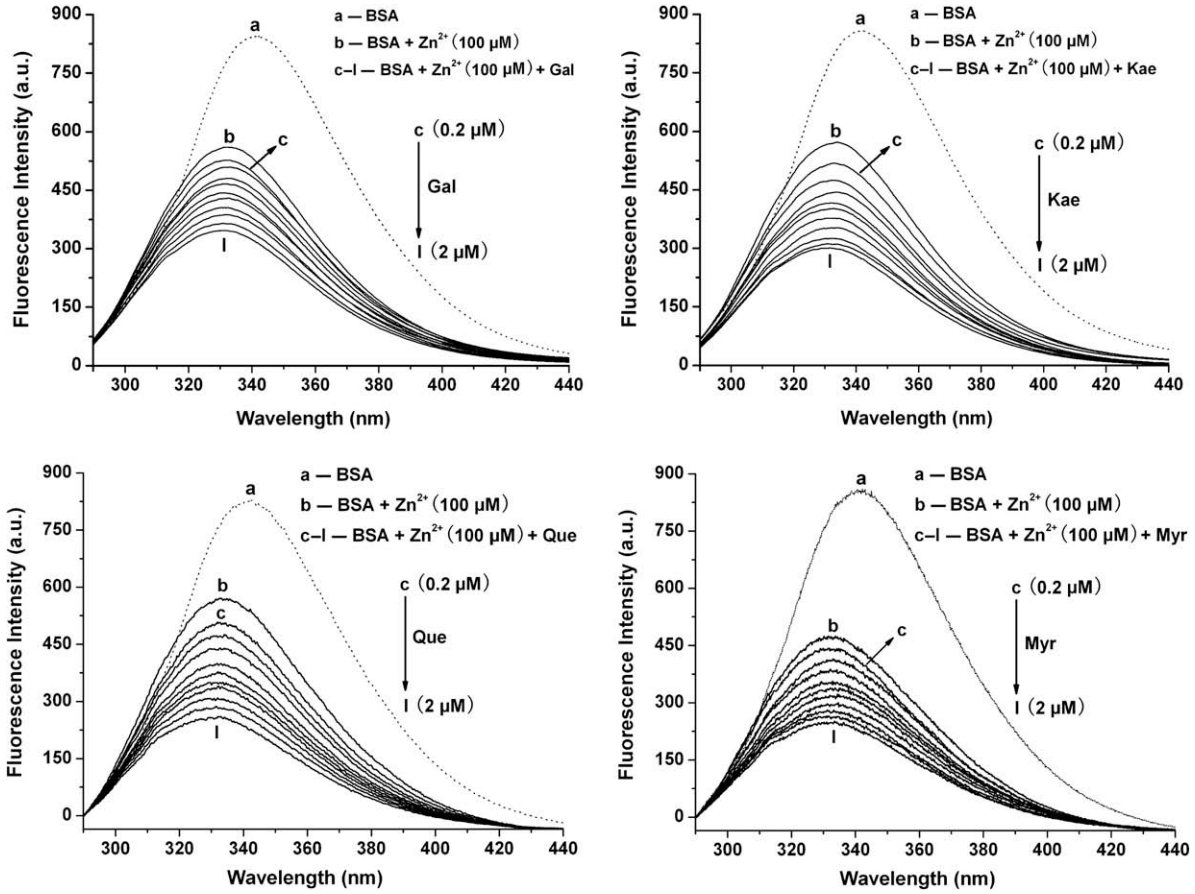

Fig. 5. The fluorescence quenching of BSA by Gal, Kae, Que or Myr in combination of quantitative Zn^{2+} ion. BSA, 1.00×10^{-6} M; Flavonol (from top to down), 0.20, 0.40, 0.60, 0.80, 1.00, 1.20, 1.40, 1.60, 1.80, 2.00 ($\times 10^{-6}$) M; Zn^{2+}, 10^{-4} M; λ_{ex} = 280 nm; T = 310 K; Tris–HCl buffer (pH 7.40).

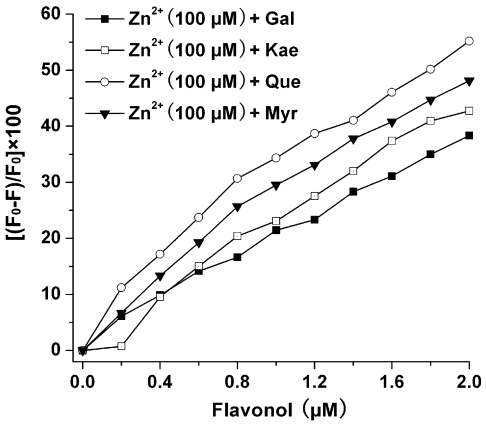

Fig. 6. The fluorescence quenching of BSA by each flavonol in combination of quantitative Zn^{2+} plotted as relative percent of $(F_0 - F)/F_0$ against the concentration variation of the flavonol. The fluorescence intensity was recorded at the maximum emission wavelength; the conditions are the same as those in Fig. 5.

ing, another case [43]. From the Stern–Volmer plot, the values of Stern–Volmer quenching constant (K_{SV}) and bimolecular quenching constant (k_q) for the quenchers can be determined. The parameter k_q can reflect the efficiency of quenching or the accessibility of the fluorophores to the quenchers. Moreover, it allows one to verify if the quenching is due to a complex formation with the protein that affects the microenvironment of Trp residues [43]. The maximum value ($k_{q(\max)}$) possible for a diffusion-controlled quenching (dynamic mechanism) in water typically gives rise to the quenching constant near 1×10^{10} M^{-1} s^{-1} [45]. In the case of complex formation between the protein and a quencher (static quenching), k_q is usually higher than $k_{q(\max)}$.

The Stern–Volmer plots of the BSA fluorescence quenching caused by Gal, Kae, Que or Myr in the absence and presence of Zn^{2+} ion are presented in Fig. 7. Given that the lifetime of BSA fluorophore is approximately 5 ns [45], the values of K_{SV} and K_q for each flavonol were calculated and summarized in Table 1. As shown in Fig. 7, the plots for Gal, Kae and Que in the absence of Zn^{2+} ion show concave toward the y-axis. As mentioned earlier, this characteristic implies that both static and dynamic quenching occurred for them. In the case of Myr, the curve displays a good linearity, which suggests that only one type of quenching (dynamic or static) occurred. In the presence of Zn^{2+} ion, the profile of the plots for Gal, Kae and Que hardly varied but only exhibited a decrease of the curvature. However, the plot for Myr changed, namely, the curve deviated from the original linearity and became slightly concave toward the y-axis at high concentration of Myr. These results indicate that, for Gal, Kae and Que, the presence of Zn^{2+} ion hardly changed the type of fluorescence quenching mechanism of BSA. The static quenching becomes preferential in terms of the curvature decrease. In contrast, for Myr, the fluorescence quenching of BSA occurred alternatively via a mixed mode (static and dynamic) rather than only one (static or dynamic). This result also justifies the deduction that Myr captured the BSA-bound Zn^{2+}, forming Zn^{II}–Myr complex. The association of the formed Zn^{II}–Myr complex with BSA through hydrogen bonds between one or two free hydroxyls on the B-ring of Myr and BSA is expected to contribute to the static quenching mechanism.

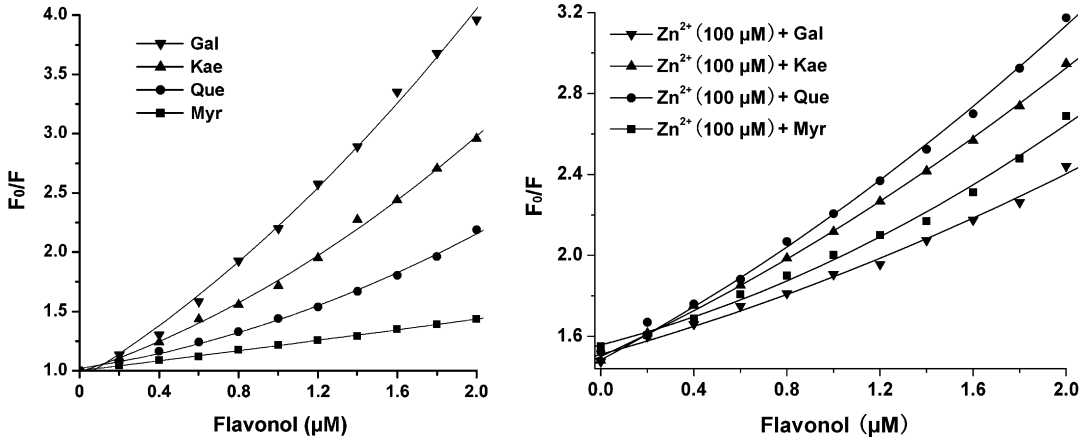

Fig. 7. Stern–Volmer plots of the fluorescence quenching of BSA caused by Gal, Kae, Que or Myr in the absence (left) and presence (right) of Zn^{2+} ion.

Table 1
The Stern–Volmer quenching constant K_{SV} ($\times 10^5$ M^{-1}) and the bimolecular quenching constant k_q ($\times 10^{13}$ M^{-1} s^{-1}) for the interactions of Gal, Kea, Que and Myr with BSA in the absence and presence of Zn^{2+} ion at 310 K and pH 7.40.

Zn^{2+} (M)		Gal	Kae	Que	Myr
0	K_{SV}	2.06	2.50	5.52	10.20
	k_q	4.10	5.00	11.00	20.40
1×10^{-4}	K_{SV}	4.49	6.78	8.17	5.00
	k_q	8.98	13.56	16.34	10.00

The values of K_{SV} and k_q for each flavonol in the absence of Zn^{2+} ion increased with the number of the B-ring hydroxyl. For Myr, they were almost equal to the sum of other three flavonols (Table 1). Study exhibited that compounds containing galloyl groups have more binding ability toward proteins [49]. The three hydroxyl groups on the B-ring together with a benzene ring of Myr render its highest affinity among the flavonols to BSA possibly through hydrogen bonds and hydrophobic interactions. This explains why Myr has the highest quenching efficiency. In the presence of Zn^{2+} ion, the values of K_{SV} and k_q for Gal, Kae and Que likewise increased with the number of B-ring hydroxyl. Each value was larger than that in the absence of Zn^{2+}. However, the presence of Zn^{2+} reversely decreased the values of K_{SV} (10.2×10^5 vs. 5.0×10^5 M^{-1}) and k_q (20.4×10^{13} vs 10.0×10^{13} M^{-1} s^{-1}) for Myr, which verifies the conversion of the fluorescence quenching type for Myr.

On the other hand, no matter Zn^{2+} ion is absent or present in the system, the values of k_q for the flavonols are far higher than $k_{q(max)}$, which suggests the formation of a complex between each flavonol/Zn^{II}–flavonol and BSA, corresponding to a static quenching mechanism. In this case, the interactions can further be interpreted by apparent static binding constant (K_a). The value of K_a can be calculated from the well-known equation: $\log[(F_0 - F)/F] = \log K_a + n\log[Q]$ [50]. The values of K_a for each flavonol associating with BSA in the absence or presence of Zn^{2+} ion are presented in Table 2. The results displayed a similar B-ring hydroxyl number-dependent tendency for each flavonol in the absence of Zn^{2+} ion. As discussed above, the trihydroxyl groups on the B-ring of Myr facilitate the formation of stronger hydrogen bonds between Myr and BSA than other flavonols. This case could account for the highest K_a of Myr among the flavonols, and furthermore, probably justify the dominance of the static quenching mechanism for Myr. In the presence of Zn^{2+} ion, the values of K_a for the flavonols decreased except for Gal, whose K_a increased slightly. The decreased magnitude of K_a value for Myr is the largest among the four flavonols. The results further verify the formation of Zn^{II}–flavonol complexes and the key role of B-ring hydroxyl in the interactions of the flavonols with BSA. The B-ring hydroxyl of the flavonols could establish hydrogen bonds with BSA in the absence of Zn^{2+} and act as donors for chelating in the presence of Zn^{2+}.

Table 2
Apparent static binding constant K_a for flavonol-BSA in the absence and presence of Zn^{2+} ion.

Zn^{2+} (M)	Gal	Kae	Que	Myr
0	1.89×10^5	5.08×10^6	3.70×10^7	2.15×10^8
1×10^{-4}	3.09×10^5	2.18×10^5	3.31×10^5	7.40×10^4

Taken above results together, it can be deduced that, in the absence of Zn^{2+} ion, flavonols Gal, Kae and Que quenched the fluorescence of BSA through the hydrogen bonds between each flavonol and BSA via the pathway of both collision (dynamic) and complex formation (static) with the latter as dominant. For Myr, only static quenching type involved. In the presence of Zn^{2+} ion, the fluorescence quenching of BSA for the four flavonols was principally resulted from interactions of the formed Zn^{II}–flavonol complexes with BSA via both static and dynamic quenching mechanism with the former as dominant.

4. Conclusions

The interactions of four flavonols bearing different number of hydroxyl substituent on the B-ring, Gal, Kae, Que and Myr, with BSA in the presence of Zn^{2+} ion in Tris buffer were studied by means of fluorescence quenching technique. The results demonstrated that Zn^{2+} ion exerted remarkable effect on the interactions of the flavonols with BSA, and the effect was distinct for the flavonol with different number of B-ring hydroxyl. The fluorescence quenching extent in the presence of Zn^{2+} ion was larger than that for corresponding flavonol alone, exhibiting an order of Que > Myr > Kae > Gal. However, the quenching extent in the absence of Zn^{2+} ion displayed an order of Myr > Que > Kae > Gal, which is in good agreement with the number variation of the B-ring hydroxyl on corresponding flavonol. The results suggest the key role of the B-ring hydroxyl and the distinct effect of B-ring hydroxyl number in the interactions. Moreover, the results indicate that each flavonol could catch the BSA-bound Zn^{2+} in the solution, forming Zn^{II}–flavonol complex that possibly quenched the fluorescence of BSA. The B-ring hydroxyl of the flavonols could establish hydrogen bonds with BSA in the absence of Zn^{2+} and act as donors for chelating in the presence of Zn^{2+} ion. From the fluorescence spectra obtained, the Stern–Volmer quenching constants K_{SV}, the

bimolecular quenching constant k_q and the apparent static binding constant K_a were determined. In the presence of Zn^{2+} ion, the values of K_a for Kae, Que and Myr decreased whereas K_{SV} and k_q for Gal, Kae and Que increased with the number of B-ring hydroxyl. The type of fluorescence quenching of BSA for Gal, Kae and Que hardly changed but the preference of static quenching increased in terms of the curvature decrease of Stern–Volmer curve and the higher values of k_q and K_a. In contrast, the fluorescence quenching of BSA for Myr occurred alternatively via a mixed mode (static and dynamic) instead of only one (dynamic or static). The values of K_{SV} and k_q for Myr remarkably decreased, which verifies the conversion of the fluorescence quenching type. These exceptional behaviors of Myr may be ascribed to the formation of dinuclear Zn^{II}–Myr complex and the additional hydrogen bonds between the free B-ring hydroxyl of Myr and BSA.

There are many essential "trace" metal ions that play important physiological roles in human body. The results obtained in this study are expected to increase the awareness of the peoples in the field of pharmaceutical research against the effects and remedies of the trace metals.

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