J Fluoresc (2011) 21:1879–1886
DOI 10.1007/s10895-011-0884-5

ORIGINAL PAPER

Binding of Quercetin to Lysozyme as Probed by Spectroscopic Analysis and Molecular Simulation

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Received: 20 November 2010 / Accepted: 23 March 2011 / Published online: 1 April 2011

Abstract The binding of quercetin to lysozyme (LYSO) in aqueous solution was investigated by fluorescence spectroscopy, UV-vis absorption spectroscopy and molecular simulation at pH 7.4. The fluorescence quenching of LYSO by addition of quercetin is due to static quenching, the binding constants, K_a, were 3.63×10^4, 3.31×10^4 and 2.85×10^4 $L\cdot mol^{-1}$ at 288, 298 and 308 K, respectively. The thermodynamic parameters, enthalpy change, ΔH, and entropy change, ΔS, were noted to be -7.56 $kJ\cdot mol^{-1}$ and 61.07 $J\cdot mol^{-1}\cdot K^{-1}$. The results indicated that hydrophobic interaction may play a major role in the binding process. The distance r between the donor (LYSO) and acceptor (quercetin) was determined as 3.34 nm by the fluorescence resonance energy transfer. The synchronous fluorescence spectroscopy showed the polarity around the tryptophan residues increased and the hydrophobicity decreased. Furthermore, the study of molecular simulation indicated that quercetin could bind to the active site (a pocket made up of 24 amino-acid residues) of LYSO mainly via hydrophobic interactions and that there were hydrogen interactions between the residues (Gln 57, Ile 98) of LYSO and quercetin. The accessible surface area (ASA) calculation verified the important roles of tryptophan (Trp) residues during the binding process.

Keywords Lysozyme · Quercetin · Fluorescence quenching · Molecular simulation

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Introduction

Quercetin (Fig. 1) is one of the most widely distributed flavonoids, ubiquitous in plants and vegetables. The flavonoids are a group of naturally occurring bioactive polyphenolic compounds. The basic nucleus of flavonoids is usually characterized by a phenyl benzo (γ) pyrone [1], that is, two aromatic rings (A and B), which are joined by a three-carbon linked γ-pyrone ring (C), hydroxyl, methoxyl or glycosyl are side groups. Since Rusznyàk Szent-Györgyi first recognized the therapeutical role of dietary flavonoids in 1936 [2], there are increasing evidences showing that flavonoids have a variety of biological effects in mammalian cell systems [3], including modifying eicosanoid biosynthesis (antiprostanoid and anti-inflammatory responses), protecting low-density lipoprotein from oxidation (prevent atherosclerotic plaque formation), preventing platelet aggregation (antithrombic effects), promoting relaxation of cardiovascular smooth muscle (antihypertensive, antiarrhythmic effects) and exerting antiviral and carcinostatic properties [4]. A large number of biochemical and molecular biological investigations have shown that therapeutically active flavonoids of both natural and synthetic origin frequently bind to proteins in mammals, forming complexes [5, 6]. So it is of fundamental significance to investigate the interaction between flavonoids and protein in order to understand the transportation and metabolic process for the flavonoids at a molecular level. Many previous studies have involved in the interactions of flavonoids with serum albumin and lysozyme [5–8]. However, the mechanisms of these processes were only partially understood, and the specific binding sites of flavonoids with proteins were scarcely explored.

Lysozyme (LYSO) is a 14.6 KDa single chain protein and composed of 129 amino-acid residues, containing 6

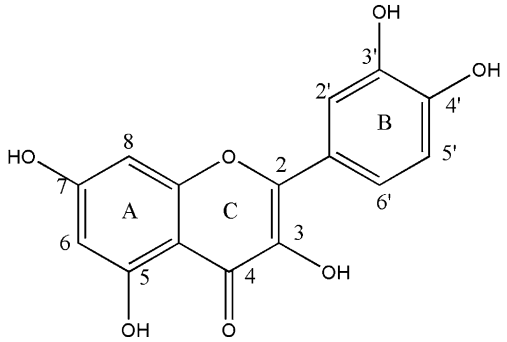

Fig. 1 Molecular structure of quercetin

tryptophanes (Trp), 4 disulfide bonds and 3 tyrosines (Tyr) [9, 10]. LYSO has many physiological and pharmaceutical effects, such as, antibacterium, antivirus and detumescence. So, it is extensively used in the pharmaceutical and food fields [11, 12]. Chicken egg white LYSO involves many aromatic amino acid residues which are suitable for this study. In addition, the three-dimensional structure is well-characterized and the interactions of flavonoids and LYSO have not been investigated, which make it a good target for the analysis at the molecular level of its interaction with drug. Thus, in this context, we conducted systematic analysis about the affinity of flavonoids towards LYSO in the hope of providing more information for elucidating the therapeutic effectiveness of drugs.

In this work, the interaction of LYSO with quercetin has been investigated in aqueous solutions at three temperatures under physiological conditions utilizing steady-state fluorescence technology in combination with synchronous fluorescence and UV-vis spectra. Furthermore, the molecular simulation was performed to demonstrate the binding mode of quercetin to LYSO.

Experimental

Materials

Lysozyme (LYSO), purchased from Amresco, was used without further purification. LYSO stock solution of 1.0×10^{-3} mol L^{-1} was prepared by directly dissolving in the doubly distilled water. Quercetin was of analytical grade, and purchased from the National Institute for Control of Pharmaceutical and Bioproducts, China. The stock solution (1.0×10^{-3} mol L^{-1}) of quercetin was prepared by dissolving appropriate amounts of quercetin in 10 ml anhydrous methanol. Tris base was of biochemical grade and was purchased from Beijing Solarbio Science and Technology Corporation (Beijing, China). Tris-HCl buffer solution (0.05 mol L^{-1} Tris) was used to keep the pH of the solution at 7.40. The stock solution of NaCl (analytical grade, 5 mol L^{-1}) was used to maintain the ion strength at 0.1. All other reagents were of analytical grade and doubly distilled water was used throughout all the experiments. All stock solutions were kept at 0–4 °C.

Apparatus and Methods

Fluorescence measurements were performed on a Cary Eclipse fluorescence spectrophotometer (Varian, America) equipped with a 150 W Xenon flash lamp. The widths of the excitation slit and emission slit were set at 2.5 nm and 10 nm, respectively. The UV-vis spectra was recorded at room temperature on a TU-1810 spectrophotometer (Puxi Analytic Instrument Co. Ltd., Beijing, China) equipped with 1.0 cm quartz cells. All pH measurements were made with a PHS-3C pH meter (Yilian Instruments Co. Ltd., Shanghai, China). The spectra measurements were thermostatically controlled by a SHP DC-0515 thermostatic bath (Hengping Instruments Co. Ltd., Shanghai, China).

Fluorescence Measurements

Certain volume of the stock solution of LYSO and various volume of quercetin solution were transferred to a 25 ml volumetric flask. 0.5 ml NaCl solution was added to maintain the ion strength at 0.1. The mixture was diluted to the experimental concentrations with 0.1 mol L^{-1} Tris-HCl buffer solution (pH 7.4) and equilibrated for 2 h at room temperature before measurements. To evaluate the effect of temperature on LYSO-quercetin interaction, fluorescence emission spectra were recorded at three different temperatures (288, 298 and 308 K) in the wavelength range of 300–450 nm (λ_{ex} = 280 nm). All the measurements were carried out at three times using freshly prepared samples and the results were reported as the average. The synchronous fluorescence spectra were obtained by scanning simultaneously the excitation and emission monochromator. The wavelength interval ($\Delta\lambda$) is fixed individually at 15 and 60 nm in the absence and presence of various amounts of quercetin.

UV-Vis Measurements

The UV-vis absorption spectra were obtained by scanning the solution on a TU-1810 spectrophotometer with the wavelength range of 200–550 nm. During the measurements, equal solution of quercetin was added to both complex solution and reference solution to eliminate the absorbance of quercetin itself.

Simulation Studies

The crystal structure of LYSO was taken from the Brookhaven Protein Data Bank (entry codes 6lyz).The

potential of the 3D structure of LYSO was assigned according to the Amber 4.0 force field with Kollman-all-atom charges. The initial structure of quercetin molecule was generated by molecular simulation software Sybyl 6.9 [13]. The geometries of this drug were subsequently optimized using the Tripos force field with Gasteiger-Hückel charges and a gradient of 0.005 kcal mol^{-1}. The FlexX software as part of the Sybyl suite was applied to calculate the possible conformation of the drug that binds to the protein. PyMol [14] was used to visualize the docked conformations and calculate the distances between possible hydrogen bonding partners.

The residues in the active site of LYSO (binding pocket obtained from the molecular docking model) were chose as target residues. Both of the accessible surface area (ASA) of the residues of LYSO (uncomplexed) and that of the LYSO-quercetin docked complex were computed using NACCESS [15]. The differences in ASA between all the active site residues of LYSO (uncomplexed) and that of the LYSO-quercetin docked complex are calculated as $\Delta ASA^i = ASA^i_{uncomplexed} - ASA^i_{complexed}$. All calculations were performed through SGI FUEL workstations.

Results and Discussion

Analysis of Fluorescence Spectra

Since quercetin was dissolved by methanol, it was necessary to evaluate the quenching effect of methanol and the effect of methanol on LYSO conformation. The ultimate percentages of methanol in all solutions were below 3% (v/v). The results showed that the effect of methanol on the interaction of quercetin with LYSO can be negligible in the amount used in our experiment [16]. Figure 2 shows that with the addition of quercetin, the fluorescence intensity of LYSO decreases regularly and there is a moderate red shift of the emission wavelength (about 5 nm). This indicates that quercetin can interact with LYSO and quench its intrinsic fluorescence, which will be further discussed below.

Investigation on the Quenching Mechanism

Fluorescence quenching refers to any process that decreases the fluorescence intensity of a sample. The quenching mechanisms of fluorescence may be static or dynamic. Both mechanisms can be distinguished from each other by the differences in temperature-dependent behavior. It is well known that dynamic quenching mainly depends upon diffusion. Since higher temperatures can result in larger diffusion coefficients, the bimolecular quenching rate constants are expected to increase with increasing temperature. In contrast, a static quenching process will lead to a decrease in the quenching rate constant with the increase in temperature [17]. In order to confirm the quenching mechanism, the fluorescence quenching was analyzed by Stern-Volmer equation [17]:

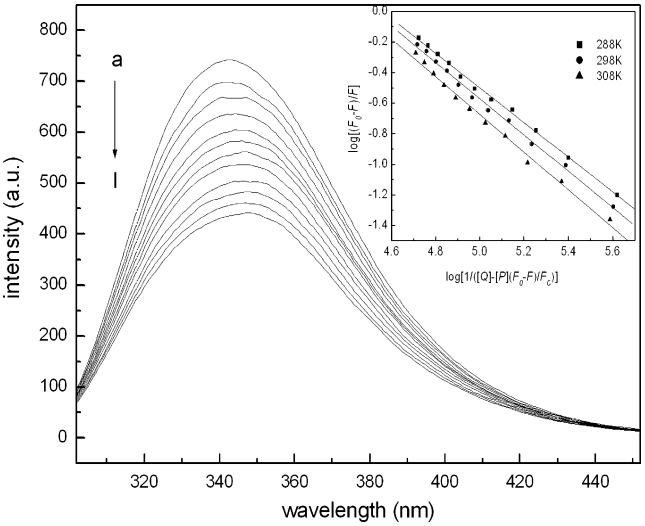

Fig. 2 The fluorescence spectra of the quercetin-LYSO system. [LYSO] = 1.0×10^{-5} mol L^{-1}, [quercetin] = 0, 3.0, 5.0, 7.0, 9.0, 11.0, 13.0, 15.0, 17.0, 19.0, 21.0, 23.0 ($\times10^{-6}$ mol L^{-1}); T = 288 K, pH = 7.4, λ_{ex} = 280 nm, λ_{em} = 342 nm. The inset corresponds to the plots of log[(F_0-F)/F] versus log[1/([Q]-[P](F_0-F)/F_0)]

$$\frac{F_0}{F} = 1 + k_q\tau_0[Q] = 1 + K_{sv}[Q] \quad (1)$$

where F_0 and F represent the steady-state fluorescence intensities in the absence and presence of quencher, respectively, K_{SV} is the Stern-Volmer dynamic quenching constant, $[Q]$ is the concentration of quencher. k_q stands for the bimolecular quenching rate constant, while τ_0 for the average life-time of fluorophore in the absence of quencher and its value is 10^{-8} s [18]. The values of K_{SV} and k_q with their correlation coefficients and standard deviations at different temperatures are presented in Table 1. The results in Table 1 show K_{SV} is inversely correlated with temperature, which suggests that the quenching may be not caused

Table 1 Stern-Volmer quenching constants of the quercetin-LYSO system at different temperatures

pH	T (K)	K_{sv} (10^4 L mol^{-1})	k_q (10^{12} L mol^{-1} s^{-1})	R^a	SD[b]
7.4	288	2.938	2.938	0.9949	0.0230
	298	2.674	2.674	0.9919	0.0264
	308	2.292	2.292	0.9874	0.0284

[a] is the correlation coefficient

[b] is the standard deviation of the fit

by dynamic collision but the formation of a complex. Moreover, values for k_q are much greater than the maximum diffusion collision rate constant of a variety of quenchers with biopolymer (2.0×10^{10} L mol^{-1} s^{-1}) [19]. This result indicates again that the fluorescence quenching process is mainly controlled by a static quenching mechanism rather than a dynamic quenching mechanism.

Determination of Binding Constant

When there are same and independent binding sites in a macromolecule, that is, at each binding site, the drug can bind to the macromolecule with the same capacity, the binding constant, K_a, and number of binding sites, n, of the quercetin-LYSO system can be calculated by the following equation [20]:

$$\log\left(\frac{F_0-F}{F}\right) = -n\log\left(\frac{1}{[Q]-[P]\frac{F_0-F}{F_0}}\right) + n\log K_a \quad (2)$$

where $[Q]$ and $[P]$ are total concentrations of quercetin and LYSO. The plots of log[(F_0-F)/F] versus log[1/([Q]-[P](F_0-F)/F_0)] for three temperatures are shown in the inset of Fig. 2, and the calculated results are listed in Table 2. The linear correlation coefficients are larger than 0.996 and the standard deviations are no more than 0.032, which indicates that the assumption underlying the derivation of Eq. (2) is satisfied. It can be observed that the values of K_a decrease with the increase of temperature, which is in accordance with the dependence of K_{SV} on temperature as mentioned above. This fact indicates that the interaction between quercetin and LYSO weakens when the temperature rises, resulting in the reduction of the stability of LYSO-quercetin complex. The values of n at different temperatures are approximately equal to 1, indicating there is one binding site of quercetin to LYSO.

Binding Mode

The thermodynamic parameters of binding reaction are the main evidences to determine the binding mode. Therefore, the thermodynamic parameters dependent on temperatures were analyzed in order to further characterize the acting forces between quercetin and LYSO. The thermodynamic parameters (ΔH^0 and ΔS^0) can be calculated based on Van't Hoff equation:

$$\ln K = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (3)$$

The free energy change ΔG^0 of the binding reaction at different temperatures is estimated from Eq. (4):

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (4)$$

In Eq. (3), K is analogous to the binding constant K_a at the corresponding temperature. The values of ΔG^0, ΔH^0 and ΔS^0 at different temperatures are also shown in Table 2. From Table 2, we can see that ΔH^0 is −7.558 kJ mol^{-1}, whereas ΔS^0 is 61.07 J mol^{-1} K^{-1}, which indicates that the binding process is exothermic reaction accompanied by a positive ΔS^0 value. The negative value for ΔG^0 indicates the spontaneity of the binding of quercetin to LYSO. Ross and Subramanian [21] have reported the characteristic sign and magnitude of the thermodynamic parameter associated with the various individual kinds of interaction that may take place in macromolecules association process. From the point of view of water structure, the positive ΔS^0 value of a drug-protein interaction indicates that the interaction is mainly hydrophobic [22]. The negative ΔH^0 value (−7.558 kJ mol^{-1}) observed cannot be mainly attributed to electrostatic interactions since the ΔH^0 of the electrostatic interactions is very small, almost zero [21, 23]. Moreover, the negative ΔH^0 value will be obtained whenever there is a hydrogen bonding in the binding reaction [21]. So it is not possible to account for the thermodynamic parameters according to a single intermolecular force model. Meanwhile, for quercetin-LYSO system, it is found that the main contribution to ΔG^0 value arises from ΔS^0 rather than ΔH^0. Therefore, the hydrophobic interactions most likely play a major role in the binding of quercetin to LYSO, but hydrogen bonding cannot be excluded.

Table 2 Binding constants K_a and the thermodynamic parameters of the quercetin-LYSO system at different temperatures

T (K)	K_a ($\times10^4$ L mol^{-1})	n	R^a	SD^b	ΔH^0 (kJ mol^{-1})	ΔG^0 (kJ mol^{-1})	ΔS^0 (J mol^{-1})	R^c
288	3.626	1.133	0.9985	0.0186	−7.558	−25.15	61.07	1.000
298	3.306	1.186	0.9977	0.0237		−25.76		
308	2.852	1.235	0.9962	0.0316		−26.37		

[a] is the correlation coefficient for the K_a values

[b] is the standard deviation of the fit according to Eq. (2)

[c] is the correlation coefficient for the Van't Hoff plot

Energy Transfer between LYSO and Quercetin

Förster non-radioactive energy transfer theory (FRET) has been used as a "spectroscopic ruler" for measuring molecular distances in biological and macromolecular systems [24]. FRET takes place when the fluorescence emission band of one molecule (donor) overlaps with an excitation band of a second molecule (acceptor) that is within 2–8 nm [25, 26]. Using FRET, the distance r between quercetin and LYSO could be calculated by the equation [17]:

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \tag{5}$$

where E denotes the efficiency of transfer between the donor and the acceptor, r is the average distances between acceptor and donor and R_0 is the critical distance when the transfer efficiency is 50%. The value of R_0 is calculated using the following equation:

$$R_0^6 = 8.8 \times 10^{-25} k^2 N^{-4} \Phi J \tag{6}$$

where k^2 is the spatial orientation factor of the dipole, N is the refractive index of medium, Φ is the fluorescence quantum yield of donor and J is the spectral overlap between the emission spectra of donor and the absorption spectra of acceptor, which is given by:

$$J = \frac{\int_0^\infty F(\lambda)\varepsilon(\lambda)\lambda^4 d\lambda}{\int_0^\infty F(\lambda) d\lambda} \tag{7}$$

where $F(\lambda)$ is the fluorescence intensity of the donor when the wavelength is λ and $\varepsilon(\lambda)$ is the molar absorption coefficient of the acceptor at same wavelength.

The overlap spectrum of the UV-vis absorption spectrum of quercetin and the fluorescence emission spectra of LYSO is shown in Fig. 3. Under the experimental conditions, it has been reported for LYSO that $k^2 = 2/3$, N=1.4, and Φ = 0.14 [26–28]. Consequently, we calculated that $J = 4.66 \times 10^{-15}$ cm^3 L mol^{-1}, R_0 = 2.15 nm and r = 3.34 nm. Obviously, the acceptor-donor average distance is in the range of 2–8 nm [25, 26], indicating that the energy transfer between LYSO and quercetin occurred with high possibility.

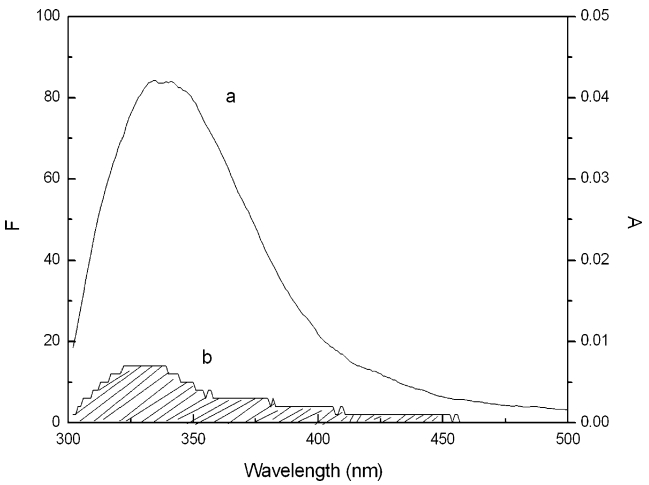

Fig. 3 Overlap of LYSO fluorescence emission (**a**) and quercetin absorption (**b**). [LYSO] = [quercetin] = 1.0×10^{-6} mol L^{-1}

Effect of Quercetin on the LYSO Conformation

Synchronous Fluorescence Studies

Brustein et al. [29] considered that maximum emission wavelength (λ_{em}) of the Trp residue is related to the polarity of microenvironment around it. So the changes of maximum emission wavelength of the chromophore will reflect the conformation changes of LYSO. As we all know, when the scanning interval $\Delta\lambda$ ($\Delta\lambda = \lambda_{em} - \lambda_{ex}$) was fixed at 15 and 60 nm respectively, the experiment on synchronous fluorescence of LYSO will provide the characteristic information for the Tyr residues and Trp residues [16].

Figure 4 shows the synchronous fluorescence spectra of LYSO with the addition of quercetin. It can be seen from Fig. 4a that the maximum emission wavelength keeps the position when $\Delta\lambda$ = 15 nm. However, an obvious red shift (about 6 nm) can be observed from Fig. 4b when $\Delta\lambda$ = 60 nm. At the same time, it is seen that the hypochromic effect of fluorescence emission of Trp residues is far more than that of Tyr residues. These facts indicate that the interaction of quercetin with LYSO do not affect the conformation of the region around the Tyr residues, but affect the conformation of the region around the Trp residues. So the binding of quercetin to LYSO is mainly located in close to the Trp residues. In addition, it also implies that the polarity around the tryptophan residues increases and the hydrophobicity decreases [30]. Moreover, we can see that the fluorescence intensity decreases with the addition of quercetin, which further demonstrates the occurrence of fluorescence quenching in the binding process.

UV/Vis Absorbance Spectroscopy Studies

To reconfirm the structural change of LYSO by the addition of quercetin, we measured the UV-vis absorption spectra of LYSO with various amounts of quercetin (Fig. 5). We can see that the absorption peaks at 280 nm increase obviously and have a moderate blue shift (about 5 nm) with the increase in the concentration of quercetin, which indicates that LYSO molecule associates with quercetin to form a LYSO-quercetin complex and the

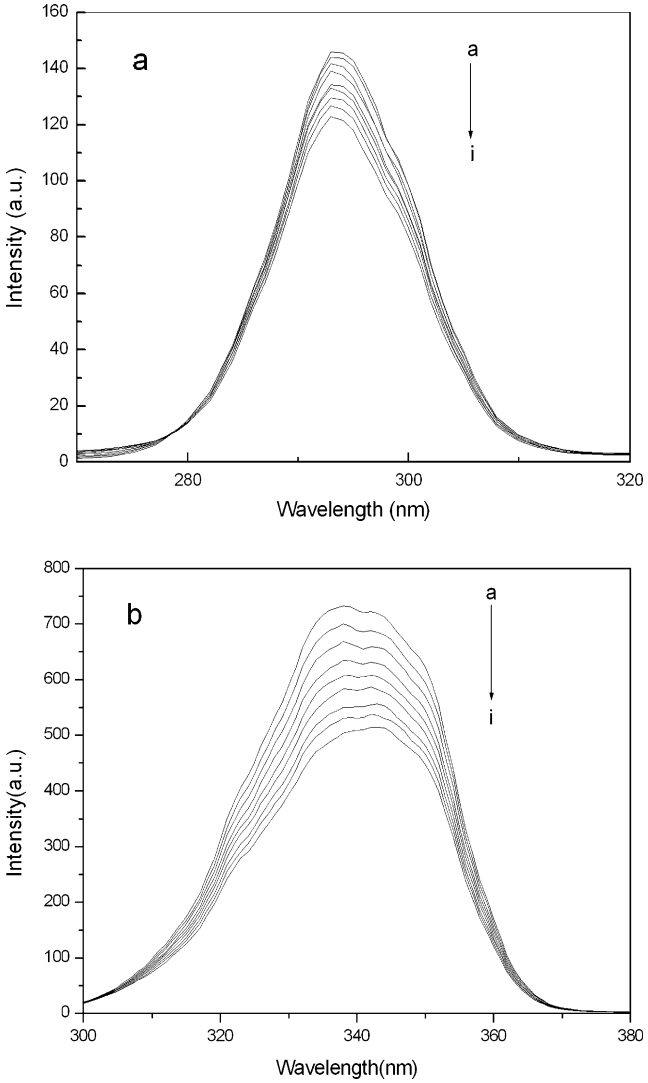

Fig. 4 Synchronous fluorescence spectra of LYSO in the presence of quercetin (pH 7.4). **a** $\Delta\lambda$ = 15 nm, **b** $\Delta\lambda$ = 60 nm; [LYSO] = 1.0×10^{-5} mol L^{-1}, [quercetin] = 0, 1.5, 3.0, 4.5, 6.0, 7.5, 9.0, 10.5, 12.0 ($\times10^{-6}$) mol L^{-1}

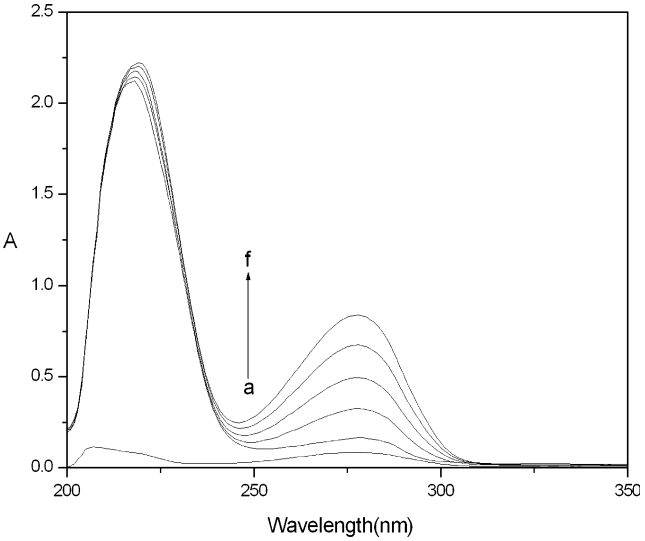

Fig. 5 The UV-vis absorption spectra of quercetin, LYSO and LYSO-quercetin system. **a** absorption spectra of quercetin only, **b** absorption spectra of LYSO only, c(LYSO) = c(quercetin) = 1.0×10^{-5} mol L^{-1}; **c**~**f** absorption spectra of LYSO-quercetin complex, c(LYSO) = 1.0×10^{-5} mol L^{-1}, c(quercetin) = 1.0, 3.0, 6.0, 9.0 ($\times10^{-5}$ mol L^{-1})

peptide strand extends even more. Meanwhile, it also suggests that the hydrophobicity around Trp residues decreases and the polarity increases [31], which is in accordance with the results of synchronous fluorescence spectra.

Molecular Simulation Studies

Molecular simulation studies have been applied to improve the understanding of the interactions between biomacromolecules and ligands. The important role of Trp residue for the enzymatic activity of LYSO has been sufficiently documented using various chemical methods [32, 33]. X-ray studies on LYSO and its complexes showed that there were 6 Trp residues in LYSO, and three of them (Trp 62, Trp 63, and Trp 108) located in the active sites [34]. As shown in Fig. 6a, quercetin binds to a pocket, which may be the active sites of LYSO. This pocket is made up of the residues: Phe 34, Glu 35, Ser 36, Ser 50, Thr 51, Asp 52, Tyr 53, Leu 56, Gln 57, Ile 58, Asn 59, Ser 60, Trp 62, Trp 63, Cys 64, Lys 97, Ile 98, Val 99, Asn 106, Ala 107, Trp 108 and Val 109, Ala 110. The best docked conformation of quercetin with LYSO is shown in Fig. 6b. From Fig. 6b, we find that Trp residues (Trp 62 and Trp 63) of LYSO are close to the C-ring of quercetin and Trp 108 is close to the B-ring, respectively, which suggests the existence of hydrophobic interaction between quercetin and LYSO. Moreover, this finding provides a good structural basis to explain the efficient fluorescence quenching of LYSO emission spectra in the presence of quercetin. The binding force model (hydrophobic interaction as dominated force) is in agreement with the results of the previous investigation [35]. Furthermore, Fig. 6b shows there are hydrogen bond interactions of 4'-OH hydrogen and 7-OH hydrogen of quercetin with Gln 57 and Ile 98 of LYSO, respectively. The values for the distances of hydrogen bonds are 1.95 and 2.04 Å, indicating that the hydrogen interactions between quercetin and LYSO are comparatively strong. Therefore, the results obtained from the docking studies indicated that interaction of LYSO with quercetin is dominated by hydrophobic force, but hydrogen bonding cannot be excluded, which is consistent with the spectra studies.

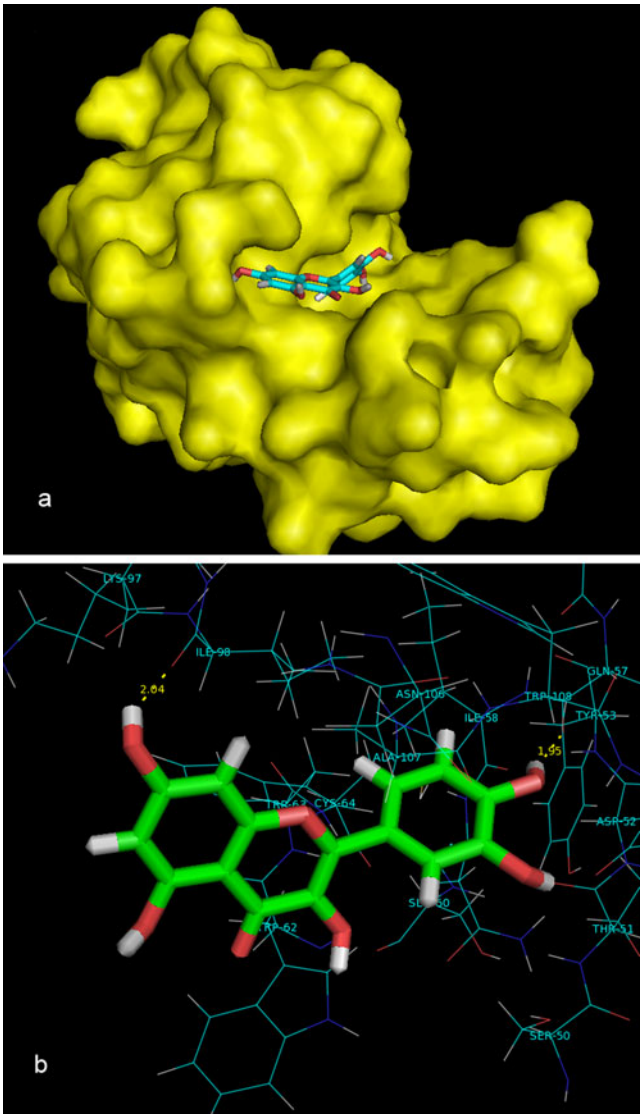

Fig. 6 Interaction model between quercetin and LYSO, only residues around 6Å of the ligand are displayed. The surface of the LYSO is displayed by QUICK method in (**a**) and the residues of the LYSO are presented using *line* in (**b**). The ligand structure is represented using stick model. The hydrogen bonds between the ligand and the protein are represented using *yellow broken line*

To further identify the residues taking part in the interaction, we calculated the differences in ASA between all the active site residues of LYSO (uncomplexed) and those of the LYSO-quercetin docked complex. The values for ASA of the residues in the binding pocket are given in Table 3. From Table 3, we can see that both of the values of ΔASA for Ile 58 and Ile 98 are 100%. This reason is probably that Ile is the most hydrophobic residues in the binding pocket and quercetin molecular will give priority to approach to it, thus Ile residue will be sheltered. Additionally, it is found that Tyr 53 has no change in its ASA, but all of the three Trp residues lose some of their ASA. This fact indicates the interaction between LYSO and quercetin does not affect the micro-region of Tyr but that of Trp residues, which corroborates the conclusions obtained from the synchronous fluorescence spectra. Furthermore, as illustrated in Table 3, the value of ΔASA for Trp 108 is 98.96%, which indicates that the quenching of fluorescence may probably be attributed to Trp 108.

Table 3 Accessible surface area (ASA) for the residues of uncomplexed and complexed forms of lysozyme with quercetin in $Å^2$

Residure	Uncomplexed	Complexed	% change
Glu 35	34.16	30.92	9.48
Asp 52	26.26	13.39	49.01
Tyr 53	19.26	19.26	0
Gln 57	13.17	3.54	73.12
Ile 58	2.59	0	100
Asn 59	32.13	8.82	72.55
Trp 62	120.57	78.80	34.64
Trp 63	48.01	11.79	75.44
Ile 98	12.29	0	100
Ala 107	53.51	6.87	87.16
Trp 108	10.59	0.11	98.96
Val 109	86.07	70.62	16.79

Conclusion

In this paper, the binding of quercetin to LYSO was investigated by fluorescence, synchronous fluorescence, UV-vis absorption and molecular simulation under simulative physiological conditions. This study shows that quercetin binds to LYSO with high affinity and quenches the intrinsic fluorescence of LYSO efficiently. The binding constants and thermodynamic parameters are evaluated to elucidate the binding mode of quercetin to LYSO. The distance between donor and acceptor is obtained according to Förster non-radioactive resonance energy transfer theory. The results of fluorescence, synchronous fluorescence and UV-vis absorption all suggest that the conformation of LYSO change obviously on the binding of quercetin. Spectroscopic experiments and molecular simulation indicate that the hydrophobic interaction may play an important role in the binding process. The docking studies together with the calculations of ASA further give us information on the residues involved in the interaction of quercetin with LYSO.

Acknowledgements The authors are grateful to the National Natural Science Foundation of China (Grant no. 20673034) and the Research Fund for the Doctoral Program of Higher Education of China (Grant no. 20060476001) for their financial supports. In addition, we thank Lanzhou University for supporting the molecular simulation software (Sybyl 6.9) and SGI FUEL workstations.

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