JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 3431–3435 3431
DOI:10.1021/jf803671s

# Spectroscopy Study on the Interaction of Quercetin with Collagen

Xiaozhan Yang,† Dacheng Wu,‡ Zongliang Du,‡ Ruixia Li,‡ Xulong Chen,‡ and Xiaohui Li*,†

†Institute of Materia Medica, College of Pharmay, Third Military Medical University, Chongqing 400038, People's Republic of China, and ‡Textile Institute, Sichuan University, Chengdu 610065, People's Republic of China

In order to understand the interaction between quercetin and collagen clearly, the UV−vis, FTIR-HATR, and fluorescence spectroscopy were used, and the data obtained by these experiments suggested that quercetin could bind to collagen. Results of FTIR-HATR and UV−vis absorption spectra suggested that the interaction of quercetin and collagen did not alter the conformation of collagen. The fluorescence spectra revealed that collagen could cause the quenching of quercetin fluorescence through a dynamic quenching procedure. The calculated quenching constant $K_{SV}$ and bimolecular quenching rate constant $k_q$ suggested that diffusion played a major role in quenching. In addition, the interaction of quercetin and collagen was evaluated by calculating (determining) the number of binding sites (*n*) and apparent binding constant $K_A$.

**KEYWORDS: Quercetin; collagen; fluorescence; quenching; spectroscopy; interaction**

## INTRODUCTION

Quercetin (3,5,7,3′,4′-pentahydroxyflavone) is a member of flavonoids, which are ubiquitous phenolic secondary metabolites (*1*−*3*). As shown in **Scheme 1**, quercetin consists of two aromatic rings (A and B) linked by an oxygen containing heterocycle (ring C), and it possesses 5-OH and 3-OH substituents. Because of its structural characteristics, quercetin has been widely used as a beneficial food supplement that is recommended for the prevention and suppression of many diseases associated with oxidative stress (*4*, *5*). As quercetin is widely used as a food supplement, it is important to improve our understanding about the behavior and actions of this food component (*6*).

Collagen is the main component of connective tissue, occurring in skin, tendons, bone, and membranes. It is a strongly hydrophilic protein that explains the ability of collagen materials to bind a large amount of water in its internal structure (*7*). The triple helix is maintained by hydrogen bonding between the −NH group of glycine and the carbonyl group C═O of residues from another polypeptide chain or by hydrogen bonding with water molecules. Moreover, the stabilization of the collagen helix is also due to short-range interactions such as van der Waals forces, hydrophobic, or electrostatic interactions.

Studies on drug−protein binding are important in pharmacology because drug−protein binding affects pharmacological activities and drug distribution. A number of biochemical and molecular biological studies have revealed that proteins (including enzymes) are the frequent target for therapeutically active flavonoids of both natural and synthetic origin (*8*, *9*). The nature and dynamics of the binding of small molecules to biomacromolecules represent an active area of investigation. By means of spectroscopy, the molecular interactions of quercetin, rutin, and other flavonoids with proteins such as human serum albumin (HSA) and bovine serum albumin (BSA) have been investigated successfully (*6*, *10*, *11*). However, compared with HSA and BSA, collagen has a different molecular structure, and most importantly, it has different biological functions. Because collagen possesses interesting biological properties such as nontoxicity and large availability, it is extensively used as a source biomaterial in medicine, pharmaceutics, and cosmetic industries (*12*−*15*). Therefore, studying the interaction between quercetin and collagen is important and can obtain some information regarding drug action.

In this article, the interaction between quercetin and collagen was investigated using UV−vis, FTIR, and fluorescence spectroscopy. The results showed that quercetin could bind to collagen. The values of the binding constants and the number of the binding sites were obtained through a fluorescence quenching study.

## MATERIALS AND METHODS

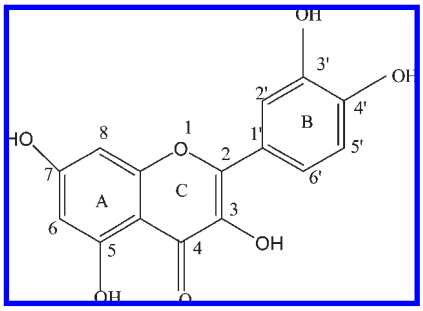
**Reagents.** Quercetin was extracted from buckthorn in our laboratory, and the purity is ≥98.5% (HPLC). Collagen was extracted from the adult cattle hide, its average molecular weight is 300 kD, and the purity is ≥99%. All other reagents were commercially available and used as received.

**Preparation of Sample Solutions.** As demonstrated, 25% ethanol does not affect protein structure, while higher ethanol

*Corresponding author. Tel: 86-23-68752318. Fax: 86-23-68753397-82. Mobile: 86-13883008816. E-mail: Lpsh008@yahoo.com.cn; xhl@mail.tmmu.com.cn.


Published on Web 3/27/2009

**Scheme 1.** Molecular Structure of Quercetin




content (50%) can alter protein structure (*16*). Meanwhile, ethanol and 1% acetic acid (HAc) solution did not affect the spectral measurement result. Collagen was dissolved in 1% acetic acid (HAc) solution (pH 2). Quercetin was dissolved in anhydrous alcohol, which was then diluted with 1% HAc. The volume ratio of anhydrous alcohol to 1% HAc was 1:3, and the quercetin concentration was $1.0 \times 10^{-4}$ mol/L.

For the UV−vis measurements, collagen concentration was 0.6 mg/mL. The quercetin−collagen solutions were prepared by mixing the quercetin and collagen solution to obtain sample solutions with quercetin/collagen ratios ranging from 4:1 to 1:4.

For the FTIR measurements, the collagen sample was prepared by titrating the collagen solution (0.6 mg/mL, 8 mL) with 1% HAc (2 mL), and the quercetin−collagen (1:4) sample was prepared by titrating the collagen solution (0.6 mg/mL, 8 mL) with quercetin solution ($1.0 \times 10^{-4}$ mol/L, 2 mL). The quercetin sample was prepared by titrating the quercetin solution ($1.0 \times 10^{-4}$ mol/L, 8 mL) with 1% HAc (2 mL), and the quercetin−collagen (4:1) sample was prepared by titrating the quercetin solution ($1.0 \times 10^{-4}$ mol/L, 8 mL) with collagen solution (0.6 mg/mL, 2 mL). All samples were left to stand for 2 h before measurements. In all of the FTIR spectra of samples, the IR asorbance of the solvent had been subtracted using the solvent as background.

In the case of fluorescence measurements, the quercetin solution ($1.0 \times 10^{-4}$ mol/L, 10 mL) was titrated with collagen solution (1.0 mg/mL) with volumes from 0.05 to 0.45 mL, and then 1% HAc was added to these samples until the ultimate volume was 11 mL.

**Spectroscopic Measurements.** Fluorescence spectra were recorded on a 970CRT fluorescence spectrophotometer (Shanghai Analytical Apparatus Company, China). The slit width for both excitation and emission was set to 10 nm, and the excitation wavelength was 295 nm. FTIR-HATR (Fourier transform infrared spectroscopy− horizontal attenuated total reflection) spectra were acquired on a FTS3000 FTIR spectrometer (Digilab Company, USA) equipped with a HATR accessory. The scanning range was 4000−650 $cm^{-1}$, and the resolution was 4 $cm^{-1}$, and all spectra were recorded after 32 scans. Spectra processing procedures: spectra of solvents were collected under the same conditions. Then, the absorbance of the solvent was subtracted from the spectra of sample solutions to get the FT-IR spectra of quercetin, collagen, and quercetin−collagen complex. In addition, the spectrometer was continuously purged with dry air to remove excess water vapor. The UV−vis spectra were recorded on a UV-1700 spectrophotometer (Shimadzu, Japan) equipped with a xenon lamp source and $10 \times 10 \times 40$ mm quartz cell in the range of 190−500 nm.

**Principles of Fluorescence Quenching.** Fluorescence quenching is the decrease of the quantum yield of fluorescence from a fluorophore induced by a variety of molecular interactions with the quencher molecule. Fluorescence quenching can be dynamic, resulting from collision encounters between the fluorophore and quencher, or static, resulting from the formation of a ground-state complex between the

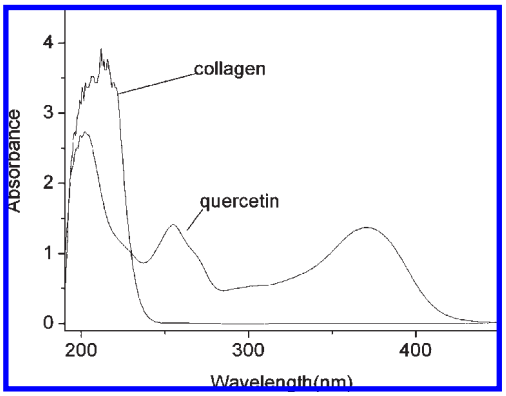



**Figure 1.** Absorption spectra of quercetin ($1.0 \times 10^{-4}$ mol/L) and collagen (0.6 mg/mL).

fluorophore and quencher. Application of the fluorescence quenching technique can also reveal the accessibility of the fluorophores to quenchers (*17*).

Fluorescence quenching is described by the Stern−Volmer equation (*17*, *18*):

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

Here, $F_0$ and $F$ are the fluorescence intensities before and after the addition of the quencher, respectively, $K_q$ is the bimolecular quenching constant, $\tau_0$ is the lifetime of the fluorophore in the absence of quencher ($\tau_0 = 10^{-8}$ s), $[Q]$ is the concentration of the quencher, and $K_{SV}$ is the Stern−Volmer quenching constant. Hence, eq **1** was applied to determine $K_{SV}$ by linear regression of a plot of $F_0/F$ against $[Q]$. For higher ligand concentration, in excess of available specific protein binding sites, this approximation is valid. According to eq **1**,

$$K_q = K_{SV}/\tau_0 \quad (2)$$

In many cases, the fluorophore can be quenched both by collision and complex formation with the same quencher. In this case, the Stern−Volmer plot exhibits an upward curvature, concave toward the $y$-axis at high $[Q]$, and $F/F_0$ is related to $[Q]$ by the following modified form of the Stern−Volmer equation:

$$\frac{F_0}{F} = (1 + K_D[Q])(1 + K_S[Q]) \quad (3)$$

Here, $K_D$ and $K_S$ are the dynamic and static quenching constants, respectively. The first factor on the right-hand side in eq **3** describes the dynamic quenching, and the second factor describes the static quenching. There are further cases when quenching can occur because of specific binding interactions and in such cases, the quenching appears to arise from static quenching. In these cases, the bimolecular quenching constant is calculated and compared to the maximum value possible for diffusion-limited quenching in water ($\sim 10^{10}$ $M^{-1}$ $s^{-1}$).

**Calculation of Binding Parameters.** The apparent binding constant $K_A$ and binding sites $n$ can be calculated from the following equation (*19*, *20*):

$$\log\frac{F_0 - F}{F} = n\log K_A - n\log\left(\frac{1}{[D_t] - (F_0 - F)[P_t]/F_0}\right) \quad (4)$$

Here, $[D_t]$ and $[P_t]$ are the total quencher concentration and the total protein concentration, respectively. By the plot of $\log(F_0 - F)/F$ versus $\log(1/([D_t] - (F_0 - F)[P_t]/F_0))$, the number of binding sites $n$ and binding constant $K_A$ can be

obtained (*13*). In this study, however, we exchanged the [$D_t$] and [$P_t$] because of the different method we adopted.

## RESULTS AND DISCUSSION

**UV−Vis Measurement.** The interaction of quercetin with collagen was assessed by UV−vis spectroscopy. The UV−vis spectra of quercetin and collagen are illustrated in **Figure 1**. In the range of 200−450 nm, quercetin possesses two absorption peaks at 372 and 256 nm, while collagen exhibits no evident peak at 210 nm. As for the spectrum of quercetin, band I at 372 nm is supposed to be associated with the absorption of the cinnamoyl system (B + C ring), and band II at 256 nm corresponds to the absorption of the benzoyl moiety formed by the A + B ring (**Scheme 1**) (*21*).

**Figure 2** shows the absorbance changes of quercetin/collagen mixtures with different weight ratios. As seen, the absorbance varied with the ratio change, and after further analysis based on standard curve, the variation of the absorbance was mainly due to the concentration variation. No significant peak shift was found in **Figure 2**; this result indicated that there was no obvious interaction between quercetin and collagen and that the electronic level structure of quercetin did not alter.

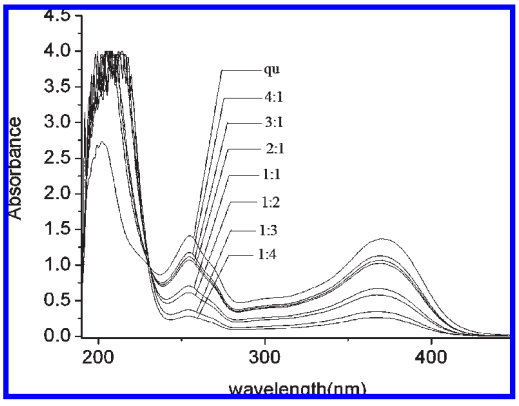



**Figure 2.** Spectra of quercetin ($1.0 \times 10^{-4}$ mol/L) and the quercetin−collagen (0.6 mg/mL) mixture.

**FTIR-HATR Measurement.** Infrared spectroscopy has long been used as a powerful method for investigating the secondary structures of proteins and their dynamics (*17*, *21*). In the IR region, the frequencies of bands due to amide I, II, and III vibrations are sensitive to the secondary structure of proteins. Particularly, the amide I band is useful for the secondary structure studies. The protein amide I band at 1600−1700 $cm^{-1}$ (mainly C=O stretch) and amide II band at 1548 $cm^{-1}$ (C−N stretch coupled with N−H bending mode) both have a relationship with the secondary structure of protein. The top part of **Figure 3** shows the FTIR spectra of collagen and the quercetin−collagen sample with a ratio of 1:4. The bottom part of **Figure 3** shows the FTIR spectra of quercetin and the quercetin−collagen sample with a ratio of 4:1. If there was a change of collagen conformation, a shift in the peak of the amide I band or a disappearance of the peak corresponding to the N−H residual amide II band should be observed. However, neither of them occurred, which revealed that no change in the secondary structure of collagen occurred. The interaction between quercetin and collagen caused only a slight decrease in the band intensity at all wavelengths, which indicated that quercetin could induce a slight decrease in the helix structure content of collagen. However, in the bottom part of **Figure 3**, the peak of quercetin at 1132 $cm^{-1}$ disappeared after the reaction with collagen; this peak was related to stretching vibration of the

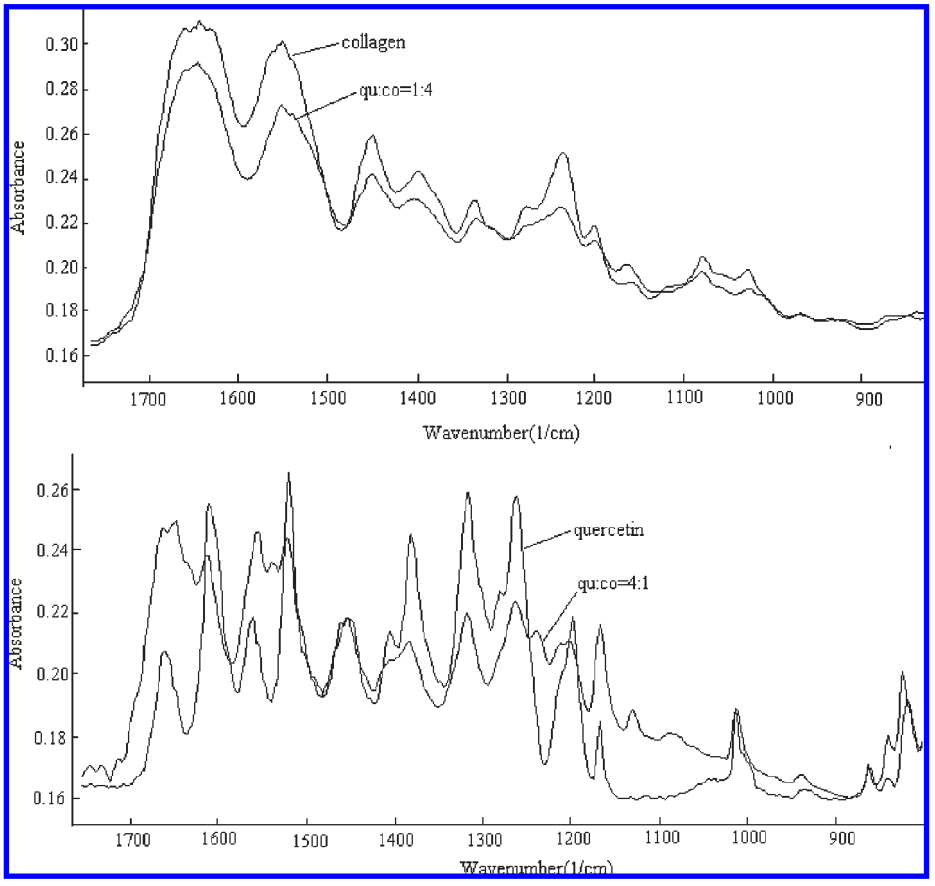



**Figure 3.** FTIR spectra of collagen (0.6 mg/mL), quercetin ($1.0 \times 10^{-4}$ mol/L), and the quercetin−collagen mixture.

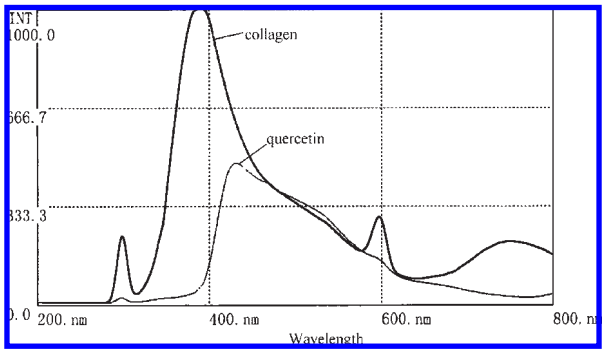



**Figure 4.** Fluorescence spectra of quercetin ($1.0 \times 10^{-4}$ mol/L) and collagen (0.6 mg/mL).

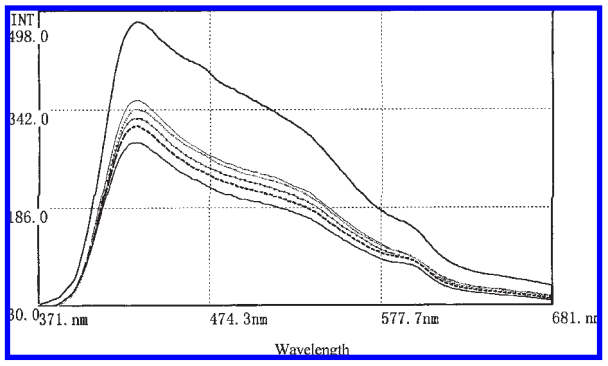



**Figure 5.** Effect of collagen on the fluorescence intensity of quercetin (from top to bottom: qu, qu:co = 0.05, 0.15, 0.25, 0.35, and 0.45 mL).

ether linkage (C−O−C) in the C ring, and the disappearance of this peak indicated that ether linkage might be the active group binding to collagen.

**Fluorescence Measurement.** An intrinsic fluorescence study was performed to evaluate the changes in the tertiary structure of collagen caused by the interaction of quercetin with collagen. It was found that the fluorescence band of 1% HAc had little overlap with the band of collagen and did not affect the band of quercetin. In addition, after collagen was added to the quercetin solution, the characteristic band of quercetin did not vary. Accordingly, we adopted the method of titrating the quercetin solution with the collagen solution.

**Figure 4** showed the fluorescence spectra of quercetin and collagen. One could observe that the emission peaks of collagen and quercetin were at 393 and 430 nm, respectively. The effect of collagen on the fluorescence intensity of quercetin is shown in **Figure 5**. The fluorescence intensity of quercetin was gradually decreased when the collagen solution was added, which indicated that collagen quenched the fluorescece intensity of quercetin. This result suggested that quercetin could bind to the collagen (*18*). In order to discuss the results within the concentration range where the concentration has a linear correlation with intensity, the experiment was carried out within the linear part of the Stern−Volmer dependence ($F_0/F$ against [$Q$]). **Figure 6** displays the Stern−Volmer plots of quercetin fluorescence quenching by collagen at room temperature (25 °C). The plot shows that within the investigated concentrations, the results agree with the Stern−Volmer equation (eq **1**). The Stern−Volmer plot did not show significant deviation toward the $y$-axis at the experimental concentration range, and the configuration of the absorption spectra did not change,

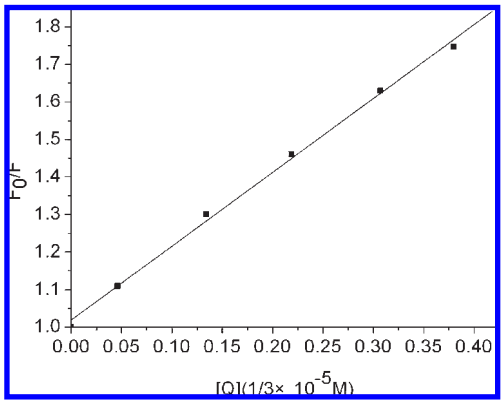



**Figure 6.** Stern−Volmer plot for the quenching of quercetin by collagen at room temperature (25 °C).

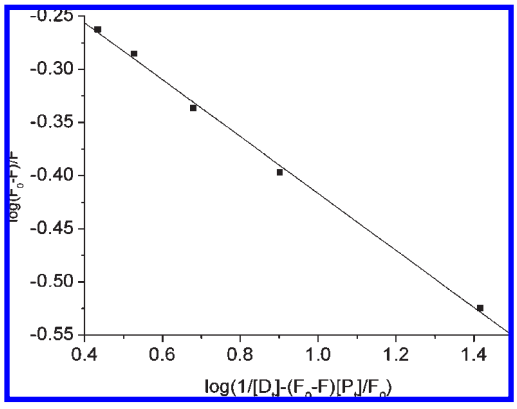



**Figure 7.** Plot of $\log(F_0 - F)/F$ vs $\log(1/([D_t] - (F_0 - F)[P_t]/F_0))$ for collagen.

which is an indication that dynamic quenching was predominant (*17*). The average quenching constant for quercetin was 610 $M^{-1}$; thus, $k_q = 6.1 \times 10^{10}$ $M^{-1}$ $s^{-1}$. This value was almost the same order of magnitude with the diffusion-limited quenching in water ($\sim 10^{10}$ $M^{-1}$ $s^{-1}$), which suggested that the quenching was dominated by the diffusion.

As for the effect of collagen on the fluorescence spectra of quercetin, there was no apparent $\lambda_{em}$ shift in **Figure 5**. This result indicated that the energy level of quercetin was not influenced; therefore, there was no active group to influence the amino acid residues in collagen. This result suggested that there was no other change in the microenvironment of the amino acid residues except that the quercetin molecule was located near the amino acid residues to cause the quenching effect. Therefore, the protein molecular conformation was not evidently affected.

**Figure 7** shows the plot of $\log(F_0 - F)/F$ versus $\log(1/([D_t] - (F_0 - F)[P_t]/F_0))$ for the quercetin−collagen system obtained from the fluorimetric titration at room temperature (25 °C). From the data in **Figure 7**, the binding constant ($K_A$) and the number of binding sites ($n$) could be calculated according to eq **4**. The calculation result showed that the binding constant ($K_A$) was $0.83 \times 10^5$ $mol^{-1}$ and that the number of binding sites ($n$) was 2−3, which indicated that quercetin can bind to one class of sites in collagen. This result well agrees with the number of binding between quercetin and HSA (*13*, *21*). Because the collagen molecular weight is nearly 100 times the quercetin molecular weight, it is proposed that quercetin most likely binds to the hydrophobic pocket located in the subdomain of collagen (*22*). Consequently, storage and conveying of quercetin can be undertaken by collagen in the body.

## DISCUSSION

In this study, the mechanism of quercetin interaction with collagen was investigated by a spectroscopic method. Experimental results suggested that collagen could bind with quercetin and could quench the fluorescence of quercetin. The binding constant and the number of binding sites ($n$) of the quercetin−collagen system at room temperature (25 °C) were calculated. On the basis of the Stern−Volmer equation, the Stern−Volmer quenching constant ($K_{SV}$) and bimolecular quenching constant ($k_q$) were obtained, and their values suggested that fluorescence quenching was a dynamic process.

According to the present experimental results, the binding mode of quercetin and collagen was also be analyzed. Because the binding of quercetin and collagen did not evidently alter the configuration of quercetin and collagen, the binding was weak, and the occurrence probability of the covalent interaction between quercetin and collagen was low. There are essentially four types of noncovalent interactions that may play a role in the binding of quercetin with collagen. These are hydrogen bonds, van der Waals forces, and electrostatic and hydrophobic interactions (*19*). On the basis of quenching measurements and UV−vis and FTIR results, the interactions between collagen and quercetin were considered to be through two modes: (1) the quercetin molecules bind within the hydrophobic pockets of collagen, and (2) these small molecules surround the protein molecule mainly through hydrogen bonds. The former mode of association agrees with that of polyols binding with quercetin (*23*), and the latter mode of association agrees with earlier results where isothermal titration calorimetry was employed for the investigation of protein−tannin interactions (*24*).

## LITERATURE CITED


(1) Robert, J. W.; Jeremy, P. E. S.; Catherine, R. E. Flavonoids: antioxidants or signalling molecules?. *Free Radical Biol. Med*. **2004**, *36*, 838–849.

(2) Thomas, W. Absorption and metabolism of flavonoids. *Free Radical Biol. Med*. **2004**, *36*, 829–837.

(3) Catherine, R. E. Flavonoids and isoflavones: absorption, metabolism, and bioactivity. *Free Radical Biol. Med*. **2004**, *36*, 827–828.

(4) Falkovskaia, E.; Sengupta, P. K.; Kasha, M. Photophysical induction of dual fluorescence of quercetin and related hydroxyflavones upon intermolecular H-bonding to solvent matrix. *Chem. Phys. Lett*. **1998**, *297*, 109–114.

(5) Cherviakovsky, E. M.; Bolibrukh, D. A.; Baranovsky, A. V.; Vlasova, T. M.; Kurchenko, V. P.; Gilep, A. A.; Usanov, S. A. Oxidative modification of quercetin by hemeproteins. *Biochem. Biophys. Res. Commun*. **2006**, *342*, 459–464.

(6) Mark, I. K.; Walle, U. K.; Hester, V. D.W.; Joellyn, M. M.; Thomas, W. Covalent binding of the flavonoid quercetin to human serum albumin. *J. Agric. Food Chem*. **2005**, *53*, 4194–4197.

(7) Sionkowska, A.; KaminÂska, A. Changes induced by ultraviolet light in fluorescence of collagen in the presence of $\beta$-carotene. *J. Photochem. Photobiol., A* **1999**, *120*, 207–210.

(8) Herwig, O. G.; Yvonne, H.; Barbara, K.; Alexander, F. Specific interactions of quercetin and other flavonoids with target proteins are revealed by elicited fluorescence. *Biochem. Biophys. Res. Commun*. **2004**, *318*, 490–495.

(9) Tian, J. N.; Liu, J. Q.; He, W. Y.; Hu, Z. D.; Yao, X. J.; Chen, X. G. Probing the binding of scutellarin to human serum albumin by circular dichroism, fluorescence spectroscopy, FTIR, and molecular modeling method. *Biomacromolecules* **2004**, *5*, 1956–1961.

(10) Sengupta, B.; Sengupta, P. K. The interaction of quercetin with human serum albumin: a fluorescence spectroscopic study. *Biochem. Biophys. Res. Commun*. **2002**, *299*, 400–403.

(11) Harshadrai, M. R.; Dorte, C.; Sascha, R.; Jurgen, K. Interaction of different phenolic acids and flavonoids with soy proteins. *Int. J. Biol. Macromol*. **2002**, *30*, 137–150.

(12) Wisniewsk, M.; Sionkowska, A.; Kaczmarek, H.; Lazare, S.; Tokarev, V.; Belin, C. Spectroscopic study of a KrF excimer laser treated surface of the thin collagen films. *J. Photochem. Photobiol., A* **2007**, *188*, 192–199.

(13) Brendan, A. H.; Janet, H. L.; Emilio, C. C. M. S.; Lorna, J. G. Mechanical characterization of collagen−glycosaminoglycan scaffolds. *Acta Biomater*. **2007**, *3* (4), 463–474.

(14) Geng, Y. Q.; David, M. Q.; Peter, J. R. SLRP interaction can protect collagen fibrils from cleavage by collagenases. *Matrix Biol*. **2006**, *25*, 484–491.

(15) Barbara, R.; Giovanni, T.; Eliana, L.; Bruna, P.; Susanna, V.; Flavio, F.; Maria, A. V. Intact collagen and atelocollagen sponges: Characterization and ESEM observation. *Mater. Sci. Eng., C* **2007**, *27*, 802–810.

(16) Kanakis, C. D.; Tarantilis, P. A.; Polissious, M. G.; Diamantoglou, S.; Taimir-Riahi, H. A. Antioxidant flavonoids bind human albumin. *J. Mol. Struct*. **2006**, *798*, 69–74.

(17) Athina, P.; Rebecca, J. G.; Richard, A. F. interaction of flavonoids with bovine serum albumin: a fluorescence quenching study. *J. Agric. Food Chem*. **2005**, *53*, 158–163.

(18) Wang, Y. Q.; Zhang, H. M.; Tao, G. H.; Tang, S. H. Interaction of the flavonoid hesperidin with bovine serum albumin: a fluorescence quenching study. *J. Lumin*. **2007**, *126*, 211–218.

(19) Bi, S. Y.; Song, D. Q.; Tian, Y.; Zhou, X.; Liu, Z. Y.; Zhang, H. Q. Molecular spectroscopic study on the interaction of tetracyclines with serum albumins. *Spectroc. Acta, Part A* **2005**, *61*, 629–636.

(20) Bi, S. Y.; Ding, L.; Tian, Y.; Song, D. Q.; Zhou, X.; Liu, X.; Zhang, H. Q. Investigation of the interaction between flavonoids and human serum albumin. *J. Mol. Struct*. **2004**, *703*, 37–45.

(21) Ferenc, Z.; Zsolt, B.; Miklos, S. Probing the binding of flavonoid, quercetin to human serum albumin by circular dichroism, electronic absorption spectroscopy and molecular modelling methods. *Biochem. Pharmacol*. **2003**, *65*, 447–456.

(22) Sulkowska, A. Interaction of drugs with bovine and human serum albumin. *J. Mol. Struct*. **2002**, *614*, 227–232.

(23) Usha, R.; Raman, S. S.; Subramanian, V.; Ramasami, T. Role of polyols (erythritol, xylitol and sorbitol) on the structural stabilization of collagen. *Chem. Phys. Lett*. **2006**, *430*, 391–396.

(24) Frazier, R. A.; Papadopoulou, A.; Mueller-Harvey, I.; Kissoon, D.; Green, R. J. Probing protein-tannin interactions by isothermal titration microcalorimetry. *J. Agric. Food Chem*. **2003**, *51*, 5189–5195.


**Received for Review November 25, 2008. Accepted March 10, 2009. Revised manuscript received February 17, 2009. This work was supported by the National Natural Science Foundation of China (No. 50473050).**