JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Binding of Curcumin with Bovine Serum Albumin in the Presence of ι-Carrageenan and Implications on the Stability and Antioxidant Activity of Curcumin

Mingling Yang, Yue Wu, Jinbing Li, Haibo Zhou, and Xiaoyong Wang*

School of Chemistry and Molecular Engineering, East China University of Science and Technology, Shanghai 200237, China

ABSTRACT: This work studied the influences of formation of BSA/ι-carrageenan complexes on the binding, stability, and antioxidant activity of curcumin. In the presence of BSA and ι-carrageenan, curcumin gives higher intensities of absorption and fluorescence than free curcumin and curcumin only combined with BSA. The added ι-carrageenan is observed to promote curcumin for quenching the instrinsic fluorescence of BSA. These results are explained in terms of the formation of BSA/ι-carrageenan complexes, which help to stabilize the folded structure of BSA for providing curcumin with a more hydrophobic microenvironment. The small difference in anisotropy values of curcumin with BSA alone and of BSA/ι-carrageenan complexes suggests that ι-carrageenan acts as outer stretch conformation in BSA/ι-carrageenan complexes but does not directly disturb the hydrophobic pockets inside BSA, where curcumin is hydrophobically located. The determined values of the binding constant are higher for curcumin with BSA/ι-carrageenan complexes than with BSA alone. Moreover, BSA/ι-carrageenan complexes are found to be superior to single BSA for enhancing the stability and DPPH radical-scavenging ability of curcumin.

KEYWORDS: *curcumin, BSA, ι-carrageenan, fluorescence, stability*

INTRODUCTION

Curcumin, a natural polyphenolic compound isolated from turmeric powder, has recently drawn significant interest owing to its numerous biological and pharmacological activities, including antioxidant, antitumor, anti-inflammatory, anticancer, and other desirable medicinal properties.[1−4] Curcumin is a diferuloylmethane molecule containing two ferulic acid residues joined by a methylene bridge. Hydroxyl groups of the benzene rings, double bonds in the alkene part, and the central β-diketone moiety are suggested to play crucial roles in the beneficial activities of curcumin.[5] However, the major problem with the limitation of application of curcumin as an effective therapeutic promoting agent is its low bioavailability.[6] Only a trace amount of curcumin appears in blood plasma, whereas most orally administered curcumin is excreted in the feces and the urine after rapid metabolization in the intestine to form several reduced products. One reason is the poor solubility of curcumin in water at acidic and neutral pH values, which makes curcumin hard to absorb from the gastrointestinal tract after oral administration. Another cause of low bioavailability of curcumin is due to its limited stability against gastrointestinal fluids and neutral/basic pH conditions.[7,8]

Proteins, holding the attractive advantages of being natural, nontoxic, and widely available, are avid binders of curcumin and have been widely used to improve the water solubility, stability, and bioavailability of curcumin. Esmaili et al. recently reported that β-casein can markedly increase the solubility of curcumin above 2500 times at pH 7, compared to the high insolubility in water at acidic and neutral conditions.[9] The work from Wang et al. in 1997 showed that about 90% of curcumin is decomposed within 30 min in phosphate buffer of pH 7.2, whereas in the presence of 10% serum proteins, <20% of curcumin is decomposed within 1 h and about 50% of curcumin remained after incubation for 8 h.[8] Later, many investigators also observed the stabilizing effects on curcumin of various proteins including milk casein,[10,11] β-lactoglobulin,[12] soy protein,[13,14] and bovine serum albumin (BSA).[15,16] Furthermore, Kunwar et al.[17] and Tapal et al.[13] found that the complexations of curcumin with human serum albumin and soy protein exhibit enhanced contributions compared to free curcumin for cellular uptake and antioxidant activities.

However, it is well-known that protein molecules tend to undergo obvious aggregations at pH values close to the isoelectric point, when the ionic strength is above a certain level, and when the temperature exceeds the thermal denaturation temperature. Thus, one of the important drawbacks associated with the use of proteins as encapsulation carriers is their structural instability at certain environmental conditions. Meanwhile, as two important food components, the interactions between proteins and polysaccharides have been extensively investigated and reviewed.[18−20] Protein molecules carrying heterogeneously distributed charges can electrostatically bind on polysaccharide chains to form soluble and insoluble protein/polysaccharide complexes in solution, depending on a series of electrostatic parameters such as pH, ionic strength, protein surface charge density, and polysaccharide linear charge density. McClements et al. previously used protein/polysaccharide complexes to produce food emulsions with improved environmental stability compared with those stabilized by a protein single layer because of the increase in interfacial thickness and rheology.[21] Here, we assume that the presence of polysaccharide could alter the binding behavior of curcumin with protein and then influence

Received: May 2, 2013
Revised: July 2, 2013
Accepted: July 2, 2013
Published: July 2, 2013

the stability and antioxidant activity of curcumin. It would be advantageous to employ the benefits of protein/polysaccharide complexes to get the carrier for curcumin superior to single protein to control the physicochemical properties and bioactivities of curcumin.

BSA is a globular protein. In its native state, BSA has a molar mass of 66.4 kDa and possesses about 583 amino acids with 17 disulfide bonds and 1 free cysteine group. ι-Carrageenan is one sulfated linear polysaccharide of D-galactose and 3,6-anhydro-D-galactose and extensively used in the food industry as a gelling-stabilizing and viscosity-building agent. Dickinson et al. have already demonstrated the complex formation between BSA and ι-carrageenan using a surface tension technique.[22] It is interesting to further investigate how the formation of BSA/ι-carrageenan complexes affects the binding, stability, and antioxidant activity of curcumin. In the present work, the characteristics of binding of curcumin to the system combined by BSA plus ι-carrageenan at pH 7 have been studied using absorption and fluorescence measurements first. After that, we have studied the stability and the free radical scavenging ability of curcumin with BSA/ι-carrageenan complexes in comparison with free curcumin and curcumin combined with BSA alone.

MATERIALS AND METHODS

Materials. Curcumin, BSA, ι-carrageenan, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich Chemical Co. and used without further purification. All other chemical reagents used were of analytical grade, and water was double distilled.

Preparation of Curcumin Samples. As curcumin is poorly soluble in water, a solution of curcumin dissolved in ethanol at 1 g/L was used as stock.[7,8,10] At the same time, sodium phosphate buffer of pH 7 was used to prepare BSA solutions with and without ι-carrageenan. A small quantity of curcumin stock solution was added to sodium phosphate buffer or biopolymer solutions to achieve a constant concentration of 10 mg/L curcumin. The finally obtained curcumin samples include curcumin alone, curcumin with 0.5 g/L BSA, and curcumin with 0.5 g/L BSA and 0.5, 1.0, or 1.5 g/L ι-carrageenan. All curcumin samples are clear solutions without curcumin precipitates even after centrifugation at 12000 rpm. Compared to the solvent of sodium phosphate buffer, the concentration of ethanol in final curcumin solutions is negligible (~1%).

UV−Vis Absorption Measurement. The absorption spectra of fresh curcumin samples were acquired using a Shimadzu UV-2450 spectrophotometer at 25 °C by circulating water through the thermostated cuvette holder. In the test of the stability of curcumin, curcumin samples were protected from light. The maximum absorption of curcumin at 428 nm was recorded at different time intervals within 1000 min.

Steady-State Fluorescence Measurement. Steady-state fluorescence measurements were performed with an Edinburgh FLS900 spectrofluorophotometer at 25 °C by circulating water through the thermostated cuvette holder. The fluorescence spectra of 10 mg/L curcumin in different samples were taken from 450 to 700 nm with the excitation wavelength at 424 nm. Intrinsic fluorescence of 0.5 g/L BSA was measured for the samples that contain BSA alone, BSA with 10 mg/L curcumin, and BSA with 10 mg/L curcumin and 0.5, 1.0, or 1.5 g/L ι-carrageenan. The emission spectra of BSA were recorded from 300 to 390 nm at an excitation wavelength of 280 nm.

In the determination of binding constants of curcumin with BSA and BSA/ι-carrageenan complexes, titrations were conducted by increasing curcumin concentration. The data recorded at maximum fluorecence of BSA at 337 nm were used to estimate the binding constant from the equation[15]

$$\log \frac{(F_0 - F)}{F} = \log K_b + n \log[\mathrm{Cur}] \quad (1)$$

where F_0 and F are the fluorescence intensities from BSA at 337 nm in the absence and in the presence of different concentrations of curcumin, respectively; K_b is the binding constant; n is the number of binding sites; and [Cur] is curcumin concentration.

Fluorescence Polarization Technique. An Edinburgh FLS900 spectrofluorophotometer with parallel and perpendicular polarizers was used to determine the fluorescence anisotropy (r) of curcumin at pH 7. Curcumin was excited at 424 nm, and the emission spectra were scanned from 500 to 600 nm. The fluorescence intensities were obtained at 0°−0°, 0°−90°, 90°−0°, and 90°−90° angle settings of the excitation and emission polarization accessories at 25 °C. The value of r was calculated according to[23]

$$r = (I_{\parallel} - G \times I_{\perp})/(I_{\parallel} + 2G \times I_{\perp}) \quad (2)$$

where $I_{\parallel}$ and $I_{\perp}$ are the polarized fluorescence intensities in the directions parallel and perpendicular, respectively, and G is the grating correction factor, which is the ratio of sensitivities of the instrument for vertically and horizontally polarized light.

DPPH-Scavenging Activity Measurement. The radical-scavenging activity of curcumin was examined according to the DPPH method.[24] The procedure for dissolving DPPH was similar to that of curcumin. For DPPH samples without curcumin, DPPH was first dissolved in ethanol as stock, and then a small quantity of DPPH stock was mixed with sodium phosphate buffer with and without BSA and ι-carrageenan. For the samples of DPPH with curcumin, DPPH and curcumin were dissolved in ethanol together as stock. A small quantity of this stock was mixed with various curcumin samples. The values of maximum absorption of DPPH at 534 nm were recorded using a Shimadzu UV-2450 spectrometer at 25 °C. To avoid the influence of variation of ethanol concentration on the absorption of DPPH, the ethanol amount (~1%) is small and the same in all DPPH samples.

RESULTS AND DISCUSSION

The spectra of absorption and fluorescence of curcumin are often used as a tool to monitor the interaction of curcumin with micelles[25] and liposomes,[26] as well as proteins,[12,14] because of the sensitive responses of the intensity and positition of absorption maximum and fluorescence maximum of curcumin to the medium environment. Figure 1 depicts the spectra of UV−vis absorption and steady-state fluorescence of freshly

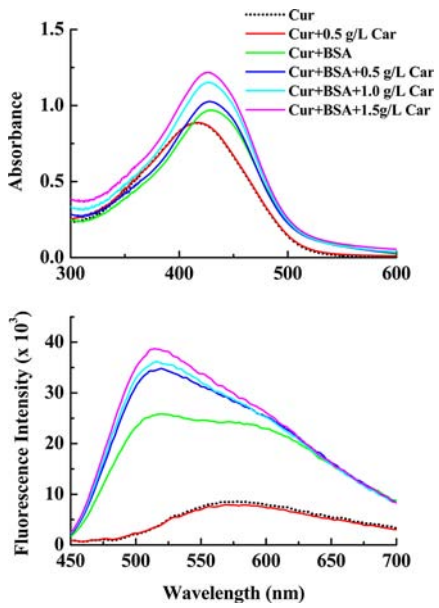

Figure 1. Spectra of absorption and fluorescence of curcumin samples at pH 7. Curcumin concentration, 10 mg/L; BSA concentration, 0.5 g/L.

dx.doi.org/10.1021/jf401827x | J. Agric. Food Chem. 2013, 61, 7150−7155

prepared curcumin samples at pH 7. Whereas curcumin in water exhibits intense absorption in the wavelengths from 300 to 500 nm with maximum absorption at 418 nm, free curcumin also shows a low broad fluorescence peak at around 575 nm. These observations are similar to the absorption and fluorescence features of curucmin in aqueous buffers.[27] When 0.5 g/L ι-carrageenan is added, the spectra of both absorption and fluorescence of curcumin give curves almost identical to those of free curcumin, suggesting no interaction between curcumin and ι-carrageenan. However, in the presence of BSA, the curves of absorption and fluorescence of curcumin show striking changes. The addition of BSA greatly improves the absorption and fluorescence intensities of curcumin compared to those in water, together with an obvious red shift of absorption maximum from 418 to 428 nm and blue shift from 575 to 517 nm in the fluorescence peak. Similar results were reported for curcumin mixed with proteins.[12] The addition of BSA can promote the movement of curcumin from a hydrophilic to a hydrophobic microenvironment, because the aryl groups of curcumin feasibly hydrophobically bind to the hydrophobic pockets of BSA.

After the addition of ι-carrageenan to the mixed solution of curcumin and BSA, the intensities of absorption and fluorescence of curcumin further increase to higher values with a small blue shift than those of curcumin with BSA alone. This result may be attributed to the formation of BSA/ι-carrageenan complexes owing to the electrostatic interaction. It is well established that electrostatic attraction may take place between cationic patches (i.e., $-NH_3^+$) on BSA and anionic sulfate groups (i.e., $-SO_4^-$) on ι-carrageenan to form soluble BSA/ι-carrageenan complexes at pH 7.[22] The binding of curcumin was reported to alter BSA conformation with a major reduction of α-helix and an increase in β-sheet and turn structures, indicating a partial protein unfolding.[16] However, the complexation of BSA with ι-carrageenan could stabilize the folded structure of BSA, which is supported by slightly increased molar ellipticity of protein in the presence of sulfated polysaccharides.[28] Although curcumin may bind to the hydrophobic pockets of BSA, the retained folding structure of BSA induced by the complexation of ι-carrageenan may promote BSA to offer curcumin with a more hydrophobic microenvironment, which in turn increases the absorption and fluorescence intensities of curcumin. Moreover, compared to the appearance of a marked shoulder at 570 nm in the fluorescence curve of curcumin with BSA, pronounced fluorecence maximum at 517 nm but without a shoulder peak at 570 nm is seen for curcumin combined with BSA and ι-carrageenan. This result gives evidence that curcumin locates in a more hydrophobic microenvironment with BSA/ι-carrageenan complexes than with BSA alone.

The changes of intrinsic fluorescence of BSA can be employed to understand the binding of curcumin with BSA and BSA/ι-carrageenan complexes. As shown in Figure 2, BSA alone shows a characteristic fluorescence maximum at 337 nm. The addition of curcumin to BSA solution causes a great decrease in the fluorescence intensity of BSA, as well as a clear blue shift. The intrinsic fluorescence of BSA is mainly due to tryptophan 213 (Trp 213) that is located in the hydrophobic pockets inside the tertiary structure of BSA.[29] Considering the high hydrophobicity of curcumin, it is expected that the access of curcumin to the hydrophobic sites close to Trp 213 results in the quenching effect on the fluorescence of BSA. With increasing ι-carrageenan concentration from 0.5 to 1.5 g/L,

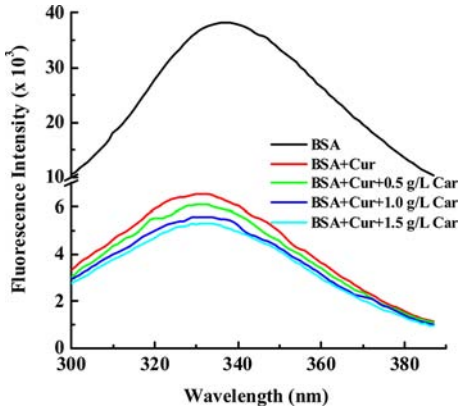

Figure 2. Intrinsic fluorescence spectra of BSA with and without curcumin as a function of ι-carrageenan concentration. BSA concentration, 0.5 g/L; curcumin concentration, 10 mg/L.

the added ι-carrageenan leads to gradual decreasing of BSA fluorescence. This result suggests that the complexation of BSA with ι-carrageenan is beneficial for curcumin to quench the fluorescence of BSA. The tightening effect of BSA structure induced by the added ι-carrageenan seems to bury Trp 213 in a more hydrophobic microenvironment. As a result, curcumin can penetrate inside to establish more hydrophobic contact with Trp 213 in BSA/ι-carrageenan complexes than that of single BSA. Additionally, after the addition of ι-carrageenan, the decrease in BSA fluorescence in Figure 2 corresponds to the increase in fluorescence due to curcumin in Figure 1. This observation indicates a possibility of energy transfer between curcumin and Trp 213 of BSA, which is enhanced in the presence of ι-carrageenan.

Fluorescence polarization measurement can directly provide information on the anisotropy (r) of curcumin, which indicates the extent of the rotation restriction of curcumin. Usually, a higher r value reveals greater restriction of the rotation of curcumin. Figure 3 presents r values of curcumin in various

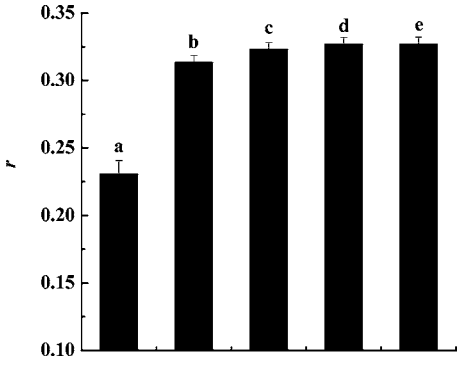

Figure 3. Values of the anisotropy of curcumin in different samples: (a) curcumin alone; (b) curcumin with 0.5 g/L BSA; (c) curcumin with 0.5 g/L BSA and 0.5 g/L ι-carrageenan; (d) curcumin with 0.5 g/L BSA and 1.0 g/L ι-carrageenan; (e) curcumin with 0.5 g/L BSA and 1.5 g/L ι-carrageenan.

samples, including free curcumin, curcumin with BSA, and curcumin with BSA/ι-carrageenan complexes. The r values of curcumin in water and BSA are 0.23 and 0.32, respectively. The higher r value of curcumin in the presence of BSA suggests that curcumin is bound in the hydrophobic pockets inside tertiary structure of BSA, where curcumin experiences a restricted

motion and, therefore, an increase in the anisotropy occurs. A similar increase in r values was also observed by Barik et al. in the case of curcumin bound to proteins.[15] After the addition of ι-carrageenan to the mixture of curcumin and BSA, curcumin continues to give a slight increase of r value. This can support our above discussion that the addition of ι-carrageenan may promote BSA to offer curcumin with a more hydrophobic microenvironment. However, it should be noted that the changes of r values of curcumin after the addition of 0.5−1.5 g/L ι-carrageenan are not significant, compared to those in water and in BSA. Although cationic $-NH_3^+$ groups of BSA may electrostatically interact with anionic $-SO4^-$ groups on ι-carrageenan, cationic $-NH_3^+$ groups are located on the surface of BSA. Therefore, ι-carrageenan appears as outer stretch conformation in BSA/ι-carrageenan complexes. The small difference in r values of curcumin with BSA and BSA/ι-carrageenan complexes reveals that formation of BSA/ι-carrageenan complexes does not directly disturb the hydrophobic pockets inside BSA where curcumin is located hydrophobically.

The binding constant (K_b) values of curcumin with only BSA and BSA/ι-carrageenan complexes have been determined by recording BSA fluorescence at various curcumin concentrations. Figure 4 shows the representative changes of fluorescence

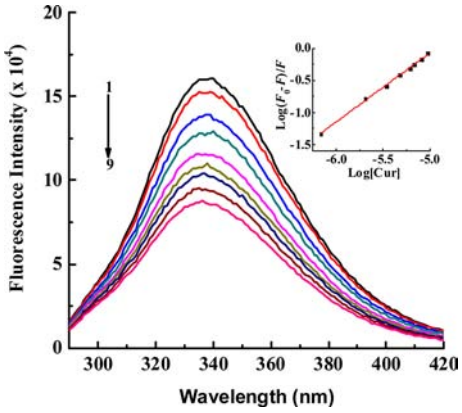

Figure 4. Variations of fluorescence spectra of BSA as a function of curcumin concentration. Spectra 1−9 correspond to the spectra of 5 g/L BSA with 0, 0.7, 2.1, 3.4, 4.8, 6.1, 6.8, 8.2, and 9.5 μM curcumin, respectively. (Inset) Linear plot of $\log((F_0 - F)/(F))$ vs log[Cur] for the determination of the binding constant of curcumin with BSA alone.

spectra of BSA in the presence of different curcumin concentrations. It can be seen that maximum fluorescence intensity of BSA at 337 nm decreases gradually with increasing curcumin concentration. The changes in maximum fluorescence were used to calculate K_b value of curcumin with BSA from the linear plot of $\log((F_0 - F)/(F))$ versus log[Cur], as shown in the inset of Figure 4. The K_b values of curcumin with BSA/ι-carrageenan complexes were obtained in the same way. The acquired K_b values are 2×10^5, 2.7×10^6, 1.2×10^7, and 1.5×10^7 M^{-1} for curcumin with 0.5 g/L BSA alone, 0.5 g/L BSA and 0.5 g/L ι-carrageenan, 0.5 g/L BSA and 1.0 g/L ι-carrageenan, and 0.5 g/L BSA and 1.5 g/L ι-carrageenan, respectively. The K_b value of curcumin with BSA without ι-carrageenan is close to the reported K_b value of curcumin with proteins.[17] In the presence of ι-carrageenan, the values of K_b are higher for curcumin with BSA/ι-carrageenan complexes than for curcumin with BSA alone, indicating increased affinity for curcumin with BSA/ι-carrageenan complexes. This result may be ascribed to the more hydrophobic microenvironment in BSA in the presence of ι-carrageenan than that of single BSA.

The changes of the relative intensity of the characteristic maximum absorption of curcumin as a function of time are used to evaluate the stability of curcumin in the samples of free curcumin, curcumin with BSA, and curcumin with BSA/ι-carrageenan complexes. As shown in Figure 5, about 53% of

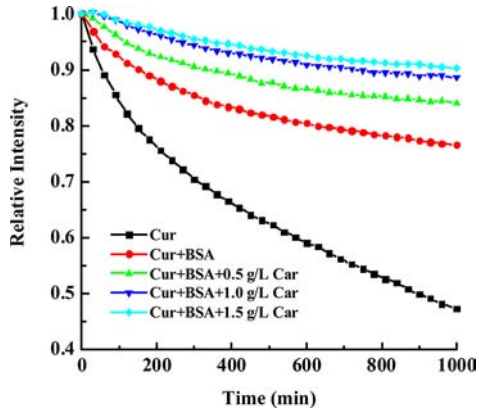

Figure 5. Relative intensity of the maximum absorption of curcumin in different samples as a function of time. Curcumin concentration, 10 mg/L; BSA concentration, 0.5 g/L.

free curcumin is decomposed rapidly after 1000 min of incubation, whereas curcumin combined with BSA is degraded about 24%, and the additions of BSA with 0.5, 1.0, and 1.5 g/L ι-carrageenan let curcumin degrade about 16, 12, and 10%, respectively. The rapid degradation of free curcumin at pH 7 is consistent with the reported work from Tønnesen et al.[7] Free curcumin is decomposed induced by the destruction of conjugated diene structure due to the loss of a proton from the activated carbon atom in the keto−enol group of curcumin.[30] When curcumin binds to the hydrophobic pockets of BSA, the greatly enhanced stability of curcumin indicates that the keto−enol group of curcumin is effectively protected from aqueous medium, like high stability of curcumin with casein[10] and β-lactoglobulin.[12] Higher suppression yields of BSA/ι-carrageenan complexes than BSA alone correspond well to higher K_b values of curcumin with BSA/ι-carrageenan complexes. Stronger binding of curcumin to the hydrophobic pockets of BSA in the presence of ι-carrageenan can inhibit the interaction of curcumin with water more sufficiently, leading to higher yields of suppression of curcumin degradation.

Finally, the DPPH scavenging method is employed to test the radical-scavenging ability of BSA/ι-carrageenan complexes. When DPPH encounters a proton-donating substance, such as an antioxidant, the radical is scavenged and the absorbance of DPPH is reduced.[24] Figure 6 outlines the values of maximum absorption of DPPH alone and in the presence of pure curcumin, pure BSA, pure ι-carrageenan, curcumin with BSA, and curcumin with BSA/ι-carrageenan complexes. Whereas pure ι-carrageenan almost cannot reduce DPPH absorption, addition of BSA alone can reduce the absorption of DPPH about 5%. Proteins and protein hydrolysates from plant and animal sources were reported to possess antioxidative acitivity, which is highly related to the level, sequence, and composition of amino acids and peptides.[13,31,32] It is observed that pure curcumin can reduce the absorption of DPPH about 10%,

dx.doi.org/10.1021/jf401827x | J. Agric. Food Chem. 2013, 61, 7150−7155

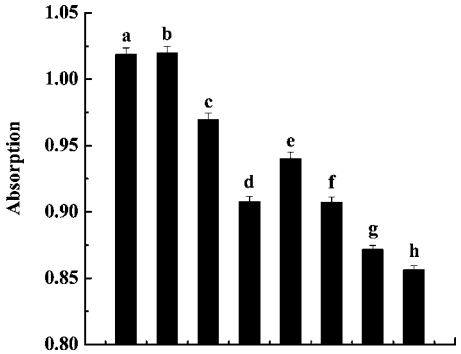

Figure 6. DPPH absorption at 534 nm in different samples: (a) DPPH alone; (b) DPPH with 0.5 g/L *ι*-carrageenan; (c) DPPH with 0.5 g/L BSA; (d) DPPH with 10 mg/L curcumin; (e) DPPH with 10 mg/L curcumin and 0.5 g/L BSA; (f) DPPH with 10 mg/L curcumin, 0.5 g/L BSA, and 0.5 g/L *ι*-carrageenan; (g) DPPH with 10 mg/L curcumin, 0.5 g/L BSA, and 1.0 g/L *ι*-carrageenan; (h) DPPH with 10 mg/L curcumin, 0.5 g/L BSA, and 1.5 g/L *ι*-carrageenan.

which reveals the higher scavenging ability of curcumin when compared to pure BSA. The antioxidative effectiveness of curcumin is manily contributed to the ability to donate its hydrogen atom to DPPH.[30] Although the addition of curcumin with BSA alone leads to smaller absorption of DPPH compared to free DPPH, the absorbance value of DPPH in the presence of curcumin with BSA alone is quite higher than the value with free curcumin. The reducing DPPH radical-scavenging ability of curcumin with single BSA may be attributed to the restraining effect of BSA on curcumin to offer the H atom. However, when BSA and *ι*-carrageenan are combined to form complexes, the DPPH radical-scavenging ability of curcumin performs even better than free curcumin. In this case, the structure stability of BSA induced by the complexation of *ι*-carrageenan brings about a more proper microenvironment for the reaction of curcumin with DPPH. Hence, curcumin can donate an H atom to lead DPPH to be reduced into nonradical form more easily.

In conclusion, BSA/*ι*-carrageenan complexes are formed through the electrostatic attraction between cationic $-NH_3^+$ patches on BSA and anionic $-SO_4^-$ groups on *ι*-carrageenan at pH 7. The formation of BSA/*ι*-carrageenan complexes may stabilize the folded structure of BSA to provide a more hydrophobic microenvironment to curcumin. Compared to curcumin combined with BSA alone, the added *ι*-carrageenan makes hydrophobically BSA-bound curcumin to give higher absorption and fluorescence intensities, bigger binding constant values, more enhanced stability, and stronger DPPH radical-scavenging ability. The present work reveals that protein/polysaccharide complexes could be used as an effective carrier to encapsulate bioactive compounds in functional foods.

AUTHOR INFORMATION

Corresponding Author

*(X.W.) Phone: 86-21-64252012. E-mail: xiaoyong@ecust.edu.cn.

Funding

This work is supported by the National Natural Science Foundation of China (Grant 21173081), the Natural Science Foundation of Shanghai (Grant 11ZR1408600), and the Fundamental Research Funds for the Central Universities (Grant WK1213003).

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Sharma, O. P. Antioxidant activity of curcumin and related compounds. *Biochem. Pharmacol.* **1976**, *25*, 1811−1812.

(2) Chan, M. M.; Huang, H. I.; Fenton, M. R.; Fong, D. In vivo inhibition of nitric oxide synthase gene expression by curcumin, a cancer preventive natural product with anti-inflammatory properties. *Biochem. Pharmacol.* **1998**, *55*, 1955−1962.

(3) Khar, A.; Ali, A. M.; Pardhasaradhi, B. V.; Begum, Z.; Anjum, R. Antitumor activity of curcumin is mediated through the induction of apoptosis in AK-5 tumor cells. *FEBS Lett.* **1999**, *445*, 165−168.

(4) Aggarwal, B. B.; Kumar, A.; Bharti, A. C. Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res.* **2003**, *23*, 363−398.

(5) Ruby, A. J.; Kuttan, G.; Babu, K. D.; Rajasekharan, K. N.; Kuttan, R. Anti-tumour and antioxidant activity of natural curcuminoids. *Cancer Lett.* **1995**, *94*, 79−83.

(6) Anand, P.; Kunnumakkara, A. B.; Newman, R. A.; Aggarwal, B. B. Bioavailability of curcumin: problems and promises. *Mol. Pharmaceutics* **2007**, *4*, 807−818.

(7) Tønnesen, H. H.; Karlsen, J. Studies on curcumin and curcuminoids. VI. Kinetics of curcumin degradation in aqueous solution. *Z. Lebensm. Unters. Forsch.* **1985**, *180*, 402−404.

(8) Wang, Y. J.; Pan, M. H.; Cheng, A. L.; Lin, L. I.; Ho, Y. S.; Hsieh, C. Y.; Lin, J. K. Stability of curcumin in buffer solutions and characterization of its degradation products. *J. Pharm. Biomed. Anal.* **1997**, *15*, 1867−1876.

(9) Esmaili, M.; Ghaffari, S. M.; Moosavi-Movahedi, Z.; Atri, M. S.; Sharifizadeh, A.; Farhadi, M.; Yousefi, R.; Chobert, J.-M.; Haertlé, T.; Moosavi-Movahedi, A. A. *β* casein-micelle as a nano vehicle for solubility enhancement of curcumin; food industry application. *LWT−Food Sci. Technol.* **2011**, *44*, 2166−2172.

(10) Sneharani, A. H.; Singh, S. A.; Rao, A. G. A. Interaction of α_{S1}-casein with curcumin and its biological implications. *J. Agric. Food Chem.* **2009**, *57*, 10386−10391.

(11) Yazdi, S. R.; Corredig, M. Heating of milk alters the binding of curcumin to casein micelles. *Food Chem.* **2012**, *132*, 1143−1149.

(12) Sneharani, A. H.; Karakkat, J. V.; Singh, S. A.; Rao, A. G. A. Interaction of curcumin with *β*-lactoglobulin—stability, spectroscopic analysis, and molecular modeling of the complex. *J. Agric. Food Chem.* **2010**, *58*, 11130−11139.

(13) Tapal, A.; Tiku, P. K. Complexation of curcumin with soy protein isolate and its implications on solubility and stability of curcumin. *Food Chem.* **2012**, *130*, 960−965.

(14) Teng, Z.; Luo, Y.; Wang, Q. Nanoparticles synthesized from soy protein: preparation, characterization, and application for nutraceutical encapsulation. *J. Agric. Food Chem.* **2012**, *60*, 2712−2720.

(15) Barik, A.; Priyadarsini, K. I.; Mohan, H. Photophysical studies on binding of curcumin to bovine serum albumin. *Photochem. Photobiol.* **2003**, *77*, 597−603.

(16) Bourassa, P.; Kanakis, C. D.; Tarantilis, P.; Pollissiou, M. G.; Tajmir-Riahi, H. A. Resveratrol, genistein, and curcumin bind bovine serum albumin. *J. Phys. Chem. B* **2010**, *114*, 3348−3354.

(17) Kunwar, A.; Barik, A.; Pandey, R.; Priyadarsini, K. I. Transport of liposomal and albumin loaded curcumin to living cells: an absorption and fluorescence spectroscopic study. *Biochim. Biophys. Acta* **2006**, *1760*, 1513−1520.

(18) Schmitt, C.; Sanchez, C.; Desobry-banon, S.; Hardy, J. Structure and technofunctional properties of protein−polysaccharide complexes: a review. *Crit. Rev. Food Sci. Nutr.* **1998**, *38*, 689−753.

(19) de Kruif, C. G.; Weinbreck, F.; de Vries, R. Complex coacervation of proteins and anionic polysaccharides. *Curr. Opin. Colloid Interface Sci.* **2004**, *9*, 340−349.

(20) Wang, X.; Ruengruglikit, C.; Wang, Y.; Huang, Q. Interfacial interactions of pectin with bovine serum albumin studied by quartz crystal microbalance with dissipation monitoring: effect of ionic strength. *J. Agric. Food Chem.* **2007**, *55*, 10425−10431.

(21) McClements, D. J. *Food Emulsions: Principles, Practices, and Techniques*, 2nd ed.; CRC Press: Boca Raton, FL, 2004.
(22) Dickinson, E.; Pawlowsky, K. Effect of ι-carrageenan on flocculation, creaming, and rheology of a protein-stabilized emulsion. *J. Agric. Food Chem.* **1997**, *45*, 3799−3806.
(23) Shinitzky, M.; Barenholz, Y. Fluidity parameters of lipid regions determined by fluorescence polarization. *Biochim. Biophys. Acta* **1978**, *515*, 367−394.
(24) Goupy, P.; Dufour, C.; Loonis, M.; Dangles, O. Quantitative kinetic analysis of hydrogen transfer reactions from dietary polyphenols to the DPPH radical. *J. Agric. Food Chem.* **2003**, *51*, 615−622.
(25) Adhikary, R.; Carlson, P. J.; Kee, T. W.; Petrich, J. W. Excited-state intramolecular hydrogen atom transfer of curcumin in surfactant micelles. *J. Phys. Chem. B* **2010**, *114*, 2997−3004.
(26) Takahashi, M.; Uechi, S.; Takara, K.; Asikin, Y.; Wada, K. Evaluation of an oral carrier system in rats: bioavailability and antioxidant properties of liposome-encapsulated curcumin. *J. Agric. Food Chem.* **2009**, *57*, 9141−9146.
(27) Began, G.; Sudharshan, E.; Sankar, K. U.; Rao, A. G. A. Interaction of curcumin with phosphatidylcholine: a spectrofluorometric study. *J. Agric. Food Chem.* **1999**, *47*, 4992−4997.
(28) Zhang, G.; Foegeding, E. A.; Hardin, C. C. Effect of sulfated polysaccharides on heat-induced structural changes in β-lactoglobulin. *J. Agric. Food Chem.* **2004**, *52*, 3975−3981.
(29) Sahoo, B. K.; Ghosh, K. S.; Dasgupta, S. Investigating the binding of curcumin derivatives to bovine serum albumin. *Biophys. Chem.* **2008**, *132*, 81−88.
(30) Jovanovic, S. V.; Steenken, S.; Boone, C. W.; Simic, M. G. H-atom transfer is a preferred antioxidant mechanism of curcumin. *J. Am. Chem. Soc.* **1999**, *121*, 9677−9681.
(31) Thiansilakul, Y.; Benjakul, S.; Shahidi, F. Antioxidative activity of protein hydrolysate from round scad muscle using alcalase and flavourzyme. *J. Food Biochem.* **2007**, *31*, 266−287.
(32) Raghavan, S.; Kristinsson, H. G. Antioxidative efficacy of alkali-treated tilapia protein hydrolysates: a comparative study of five enzymes. *J. Agric. Food Chem.* **2008**, *56*, 1434−1441.