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Binding of (−)-epigallocatechin-3-gallate with thermally-induced bovine serum albumin/ι-carrageenan particles

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ABSTRACT

Novel thermally-induced BSA/ι-carrageenan particles are used as a protective carrier for (−)-epigallocatechin-3-gallate (EGCG). The addition of EGCG to BSA/ι-carrageenan particles can highly quench the intrinsic fluorescence of BSA, which is explained in terms of the binding of EGCG to the hydrophobic pockets of BSA mainly through the hydrophobic force. According to the double logarithm equation, the binding constant is determined as $1.1 \times 10^8\ M^{-1}$ for the binding of EGCG with BSA/ι-carrageenan particles. The high binding affinity is ascribed to both the molecular structure of EGCG and the partial unfolding state of BSA in BSA/ι-carrageenan particles. The circular dichroism spectra and calculated α-helix of BSA suggest that the bound EGCG leads to a more random secondary structure of BSA. Furthermore, BSA/ι-carrageenan particles are found to be superior to native BSA and pure BSA particles for improving the stability and radical scavenging activity of EGCG.

1. Introduction

Considerable attention has been paid to the particles constructed from natural biopolymers such as proteins and polysaccharides, owing to their broad applications in food, personal care, and pharmaceutical industries (Chen, Remondetto, & Subirade, 2006; Langer & Peppas, 2003; Liu, Jiao, Wang, Zhou, & Zhang, 2008). Recently, based on the studies on a series of globular protein/polysaccharide systems, McClements et al. showed that protein/polysaccharide particles can be created by directly heating of electrostatic complexes of proteins and polysaccharides above the protein's thermal denaturation temperature (Jones, Decker, & McClements, 2010; Jones, Lesmes, Dubin, & McClements, 2010; Jones & McClements, 2011). This approach of biopolymer particle formation relies on the electrostatic complex formation between proteins and polysaccharides, as well as the thermal denaturation and aggregation of globular protein molecules. Importantly, compared to the systems including native proteins, protein/polysaccharide complexes, and pure protein particles, thermally-induced protein/polysaccharide particles have improved resistance to aggregation/dissociation over wide ranges of pH, salt, and temperature (Jones & McClements, 2011). Thus, this novel kind of protein/polysaccharide particles is suggested to be a promising carrier for encapsulating, protecting, and delivering functional food ingredients, which greatly depends on the binding characteristics of bioactive components with protein/polysaccharide particles.

Green tea is one of the most popular beverages in the world primarily because of its extensively demonstrated biological and pharmacological effects such as antioxidant, antimutagenic, anticarcinogenic, antiviral, antiinflammatory, and anticancer activities (Valcic, Muders, Jacobsen, Liebler, & Timmermann, 1999). These health benefits of green tea consumption are most often attributed to tea catechins, a group of polyphenolic compounds including (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin, (−)-epicatechin-3-gallate, (−)-epicatechin, (+)-gallocatechin, and (+)-catechin. Amongst these tea catechins, epidemiological and experimental studies in animals and human suggest that EGCG (shown in Fig. 1) is the most effective one. Unfortunately, EGCG is vulnerable to degradation with the increase of either pH or temperature, which may restrict the application of EGCG in beverage and other food products (Li, Taylor, Ferruzzi, & Mauer, 2012).

There are extensive studies on the association of tea catechins with proteins to form soluble and insoluble complexes, derived by a combination of hydrophobic interactions and hydrogen bonds (Hasni et al., 2011; Kanakis et al., 2011; Wang, Ho, & Huang, 2007). The mixing of proteins with tea catechins can practically influence the physicochemical properties and bioactivities of both components. Recently, Shpigelman et al. have presented the possibility of using thermally-induced β-lactoglobulin particles as the protective vehicle for the delivery of EGCG (Shpigelman, Cohen, & Livney, 2012; Shpigelman, Israeli, & Livney, 2010). They found that β-lactoglobulin particles may encapsulate EGCG with higher

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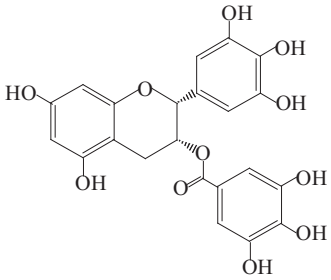

Fig. 1. Chemical structure of EGCG (Wang, Ho, & Huang, 2007).

binding affinity and give improved protection against EGCG degradation than does native β-lactoglobulin. Although holding the attractive advantages such as structural stability compared to other protein-based encapsulation carriers, preservation of tea catechins by protein/polysaccharide particles has not been reported. Therefore, it is valuable to investigate the benefits of protein/polysaccharide particles as the carrier for controlling the properties of tea catechins.

In the present work, we use thermally-induced bovine serum albumin (BSA)/ι-carrageenan particles as the protective carrier of EGCG. BSA is a model globular protein and its molecular and physicochemical properties have been well-investigated (Peters, 1996). In native state, one BSA molecule contains 583 amino acids in a single polypeptide chain with a molecular weight of about 66.4 kDa. ι-Carrageenan is extracted from red seaweed and commonly used as a functional ingredient in the food industry in the preparation of milk gels and to stabilize milk-based products. ι-Carrageenan is one sulfated linear polysaccharide with a structure composed of repeating D-galactose and 3,6-anhydro-D-galactose units (Dickinson & Pawlowsky, 1997). We have first studied the binding behaviours of EGCG with BSA/ι-carrageenan particles with steady-state fluorescence and circular dichroism. Then, compared to native BSA and pure BSA particles, the stability and the radical scavenging activity of EGCG with BSA/ι-carrageenan particles have been investigated.

2. Materials and methods

2.1. Materials

(−)-Epigallocatechin-3-gallate (EGCG) (purity ⩾ 98%) was purchased from J&K Scientific Co. Bovine serum albumin (BSA) was obtained from Aladdin Chemical Co. ι-Carrageenan and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma–Aldrich Chemical Co.

2.2. Methods

2.2.1. Surface tension measurement

The solutions of 0.15 wt% BSA, 0.03 wt% ι-carrageenan, and mixture of 0.15 wt% BSA and 0.03 wt% ι-carrageenan were prepared in pH 7 sodium phosphate buffer. The surface tensions of these samples were measured by a Dataphysics model DCAT11 tensiometer (Sartorius, Goetingen, Germany) using the Wilhelmy plate method at 25 °C. The plate was first rinsed with deionized water and then burned to red before each measurement. For comparability of equilibrium surface tension, the measurement was stopped when the standard deviation of the surface tension value was less than 0.01 mN/m.

2.2.2. Preparation of BSA/ι-carrageenan particles

The mixed solution of 0.15 wt% BSA and 0.03 wt% ι-carrageenan in pH 7 sodium phosphate buffer was heated at 80 °C in a water bath for 20 min, and cooled to room temperature to get the solution of BSA/ι-carrageenan particles finally. For comparison, the solution of pure BSA particles were obtained from 0.15 wt% BSA using the same procedure. Native BSA solution was also prepared with the concentration of 0.15 wt% BSA. EGCG freshly dissolved in pH 7 phosphate buffer was added to the solutions of native BSA, pure BSA particles, and BSA/ι-carrageenan particles at room temperature. The EGCG concentrations are fixed at 45 μM in all samples, except for the determination of binding constant.

2.2.3. Particle size and zeta potential measurements

The particle size and zeta potential of BSA/ι-carrageenan particles were determined using a Malvern Zetasizer Nano ZS (Malvern Instruments, London, England) at 25 °C. Whilst the particle size was controlled in situ by dynamic light scattering, the zeta potential was determined from the electrophoretic mobility according to the Smoluchowski equation. The mean particle diameter of BSA/ι-carrageenan particles is determined to be around 90 nm, which is much higher than the thickness of particle double layer (only a few nanometers). The Smoluchowski equation is applicable for the determination of zeta potential of biopolymer particles with thin electrical double layer (Cho, Decker, & McClements, 2009). During the measurements, the sample was diluted 10-times with pH 7 sodium phosphate buffer for analysis and each value was measured at least three times.

2.2.4. Fluorescence measurement

Steady-state fluorescence measurement for the binding of EGCG with BSA/ι-carrageenan particles was carried out on a Hitachi F-4500 FL Spectrophotometer at 25 °C. The emission spectra of BSA were taken from 300 to 450 nm with an excitation wavelength of 295 nm. The slit widths of excitation and emission were kept at 2.5 and 5 nm, respectively. By varying EGCG concentration from 0 to 180 μM, the binding constant of EGCG with BSA/ι-carrageenan particles can be calculated from the maximum fluorescence of BSA according to the double logarithm equation (Bourassa, Kanakis, Tarantilis, Pollissiou, & Tajmir-Riahi, 2010; Wei, Xiao, Wang, & Bai, 2010).

2.2.5. Circular dichroism measurement

Circular dichroism (CD) spectra of BSA in the samples of native BSA, pure BSA particles, BSA/ι-carrageenan particles, and BSA/ι-carrageenan particles with EGCG were measured on a Chirascan automatic recording spectrophotometer at 25 °C. The CD spectra were recorded at 1 mm pathlength in the range of 180–240 nm. The scan speed was 120 nm/min with a response time of 2 s. Three scans were accumulated for each spectrum. The results were expressed as ellipticity in millidegrees (mdeg). The α-helical content of BSA was calculated using CDNN, a deconvolution program for protein secondary structure analysis from CD data (Chirascan).

2.2.6. Measurement of EGCG loading efficiency

Immediately after the end of preparing BSA/ι-carrageenan particles encapsulated with EGCG, the product was transferred into an Amicon Ultra-3000 centrifugal filter device (Millipore Corp., Billerica, MA, USA), and then centrifuged at 14,000 rpm for 30 min in order to separate the unencapsulated EGCG (in the tube) from the encapsulated form (in the filter). The mass of free EGCG content in the tube was analysed according to EGCG standard curve through the measurement of maximum absorbance of EGCG at 278 nm by UV–vis spectroscopy (Shimadzu, UV-1800). The EGCG loading efficiency in BSA/ι-carrageenan particles was calculated to be about 78%, which was higher than the value of 71% for EGCG encapsulated in pure β-lactoglobulin particles (Shpigelman et al., 2012).

2.2.7. Evaluation of EGCG stability

Since the appearance of yellow colour was found in EGCG solution in neutral and alkaline conditions due to the degradation of EGCG (Hong et al., 2002; Mizooku, Yoshikawa, Tsuneyoshi, & Arakawa, 2003), the absorbances of EGCG samples of free EGCG, EGCG with native BSA, EGCG with pure BSA particles, and EGCG with BSA/ι-carrageenan particles were measured at 425 nm for 60 h at 25 °C on a UV–vis spectrophotometer (Shimadzu UV-1800) in order to investigate the stability of EGCG at pH 7.

2.2.8. DPPH scavenging activity measurement

The antioxidant activity of EGCG was measured using a DPPH method (Niu et al., 2012). Free EGCG or the sample of EGCG with native BSA, pure BSA particles, or BSA/ι-carrageenan particles was mixed with the ethanolic solution of DPPH. The absorbance of the mixed solution was measured at 517 nm at 25 °C on a UV–vis spectrophotometer (Shimadzu, UV-1800). Whilst DPPH has the characteristic maximum absorbance at 517 nm, other compounds (EGCG, BSA and ι-carrageenan) have almost no absorbance above 475 nm. So, there is no UV–vis crossover or crosstalk at 517 nm in DPPH scavenging activity measurement.

3. Results and discussion

3.1. Properties of BSA/ι-carrageenan particles

The surface tension measurement was first carried out for confirming the formation of soluble BSA/ι-carrageenan complexes in mixed solution of BSA and ι-carrageenan at pH 7. Pure 0.15 wt% BSA solution has a surface tension value of 58.1 mN/m, which is smaller than the value of 72.0 mN/m for pure water due to the adsorption of BSA molecules to the interface to lower the interfacial free energy. However, compared to pure water, the nearly unchanged surface tension value for pure 0.03 wt% ι-carrageenan solution (71.6 mN/m) suggests that ι-carrageenan is not surface active. The surface tension value of mixed solution of 0.15 wt% BSA and 0.03 wt% ι-carrageenan is 60.9 mN/m, which is 2.8 mN/m higher than BSA alone. The reduction of surface activity of BSA in the presence of ι-carrageenan is consistent with the surface tension study on the same system from Dickinson and Pawlowsky (1997). This result might be caused by BSA/ι-carrageenan complexes forming due to an electrostatic attraction between cationic patches on BSA and anionic sulphate groups on ι-carrageenan even though both BSA and ι-carrageenan have net negative charges at pH 7.

Meanwhile, through the studies on the thermal treatment of electrostatic complexes of globular proteins and anionic polysaccharides, McClements et al. found that sulfated carrageenan combined with proteins may form biopolymer particles, which usually have smaller size than carboxylated polysaccharides (Jones et al., 2010). Recently, our group found BSA/ι-carrageenan complexes could be an effective carrier for enhancing the stability and health-promotion activities of curcumin, a natural polyphenolic ingredient from the turmeric powder (Yang, Wu, Li, Zhou, & Wang, 2013). In the current work, we further select the system of BSA and ι-carrageenan to prepare biopolymer particles by heating a mixed solution of 0.15 wt% BSA and 0.03 wt% ι-carrageenan at pH 7. The mean particle diameter of BSA/ι-carrageenan particles is determined to be around 90 nm, and the zeta potential of BSA/ι-carrageenan particles is about −40 mV. The obtained BSA/ι-carrageenan particles often exhibit good stability in one month storage, which may be attributed to their extremely small particle size and high negative charge.

3.2. Binding characteristics of EGCG with BSA/ι-carrageenan particles

Fig. 2 depicts the spectra of steady-state fluorescence of BSA in BSA/ι-carrageenan particles as a function of EGCG concentration at pH 7. Without addition of EGCG, BSA in BSA/ι-carrageenan particles exhibits a characteristic fluorescence maximum at 345 nm, which is mainly resulted from the intrinsic fluorescence of tryptophan residues (Sulkowska, 2002). The added EGCG to the solution of BSA/ι-carrageenan particles is observed to remarkably decrease the fluorescence intensity of BSA with an obvious red shift. Because ι-carrageenan is a non-fluorescent molecule and EGCG cannot give fluorescence at the excitation wavelength of 295 nm used for BSA fluorescence measurement, the fluorescence quenching of BSA indicates that the binding of EGCG with BSA/ι-carrageenan particles has occurred.

Although tea polyphenols may bind with neutral polysaccharides like amylose through hydrophobic interaction or hydrogen bonding (Chai, Wang, & Zhang, 2013), there is almost no interaction between ι-carrageenan and EGCG because of the dominant anionic backbone of ι-carrageenan, which can be demonstrated by the almost identical absorption curves of EGCG with and without ι-carrageenan. In contrast, BSA is a globular protein composed of three structurally similar domains (I, II, and III), each containing a number of hydrophobic pockets (Bourassa et al., 2010). It is usually accepted that tryptophan 214 (Trp 214), which is located in a hydrophobic pocket of BSA, is responsible for the intrinsic fluorescence of BSA at 345 nm (Sulkowska, 2002). When EGCG is added, the phenolic rings of EGCG could be involved in the hydrophobic binding of EGCG, that is, the hydrophobic interaction of EGCG with the hydrophobic groups near Trp 214 results in fluorescence quenching of BSA. This is in agreement with previous findings suggesting that the aryl groups of polyphenols hydrophobically bind to the hydrophobic pockets of proteins (Kawamoto, Mizutani, & Nakatsubo, 1997). The dominant hydrophobic interaction between EGCG and BSA has been also verified by stronger EGCG adsorption on BSA surface at higher temperature (Wang et al., 2007). Meanwhile, the phenolic hydroxyl groups in EGCG may cause additional hydrogen bonding with BSA. On the other hand, it was reported that the fluorescence of BSA may be blue shifted if Trp 214 is buried within a native protein, and its fluorescence may shift to longer wavelengths when BSA is unfolded, resulting in greater exposure

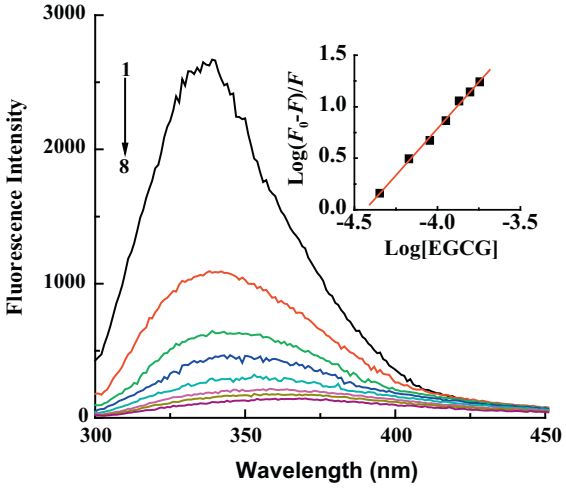

Fig. 2. Fluorescence spectra of BSA in BSA/ι-carrageenan particles at various EGCG concentrations in pH 7 sodium phosphate buffer at 25 °C. Spectra 1–8 correspond to 0, 45, 67.5, 90, 112.5, 135, 157.5 and 180 μM EGCG, respectively. The inserted linear plot of $\log(F_0 - F)/F$ *vs* log[EGCG] was drawn using the changes in maximum fluorescence of BSA.

of Trp 214 to solvent (Lakowicz, 1999; Soares, Mateus, & De Freitas, 2007). Therefore, the observed red shift of fluorescence maximum of BSA induced by EGCG addition suggests that the binding of EGCG causes BSA to remain in a more unfolded state in BSA/ι-carrageenan particles, which is in agreement with the binding of other polyphenols with native BSA (Bourassa et al., 2010). This conformational change of protein may be just involved in the tertiary structure, or both in the secondary structure and tertiary structure.

Fluorescence quenching of tryptophan residues of proteins with polyphenolic compounds is generally initiated by the static quenching from the formation of ground-state complexes between the protein and the quencher (Shpigelman et al., 2010). It is assumed that there are '*n*' same and independent binding sites in BSA where EGCG binds according to the following model:

$$P + nL \xrightarrow{K_b} PL_n \quad (1)$$

where '*P*' denotes BSA, '*L*' is EGCG and 'PL_n' is BSA–EGCG complexes. For this static quenching, the double logarithm equation can be written as (Bourassa et al., 2010; Wei et al., 2010):

$$\log\frac{(F_0 - F)}{F} = \log K_b + n\log[\mathrm{EGCG}] \quad (2)$$

In the present case, F_0 and F are the fluorescence intensities of BSA before and after the addition of EGCG, respectively; [EGCG] is EGCG concentration; K_b is the binding constant; and n is the number of binding sites.

As given in the inset of Fig. 2, the linear fitting plot of the double logarithm curve of $\log(F_0 - F)/F$ *vs* $\log[EGCG]$ was drawn using the changes in maximum fluorescence of BSA. From the slope and intercept of the plot, the value of K_b for the binding of EGCG with BSA/ι-carrageenan particles was obtained as $1.1 \times 10^8\ M^{-1}$. The value of K_b was found to be in the range of 10^3 to $10^7\ M^{-1}$ for the interactions of polyphenols including tea catechins with bovine serum albumin or human serum albumin (Roy et al., 2012; Shpigelman et al., 2010; Sáez-Ayala et al., 2013; Yang et al., 2013), depending on the properties of the system and the environmental conditions as well as the fitting models. Here, the observed high K_b value of $1.1 \times 10^8\ M^{-1}$ for EGCG with BSA/ι-carrageenan particles could be attributed to the structure of EGCG and the characteristics of BSA/ι-carrageenan particles. On one hand, EGCG contains three phenolic rings that are involved in the hydrophobic interaction with BSA. The interaction between polyphenol and protein was observed to be enhanced with the increasing number of phenolic groups (Wang et al., 2007), which could increase the conformational mobility of polyphenol and thus maximise the polyphenol/protein interactions. On the other hand, Yazdi reported that heated milk had an increased binding affinity for polyphenolic compounds compared to unheated milk, which can be attributed to the heat induced unfolding of whey proteins (Yazdi & Corredig, 2012). In our system, heating of BSA/ι-carrageenan complexes may cause partial unfolding of BSA and in turn increase the exposure of the hydrophobic pockets of BSA, to which more EGCG molecules can bind. The more intimate contact facilitated by the unfolding and the hydrophobic interactions may also assist the formation of hydrogen bonds of phenolic hydroxyl groups in EGCG with BSA. As a result, EGCG finally shows great binding activity toward BSA/ι-carrageenan particles.

3.3. Conformation changes of BSA upon EGCG binding

Circular dichroism (CD) is one of the strong and sensitive spectroscopic techniques to explore the secondary structure of proteins and also their binding with small molecules (Kelly, Jess, & Price, 2005). Thus, the changes of conformation of BSA in BSA/ι-carrageenan particles by the binding of EGCG were further investigated

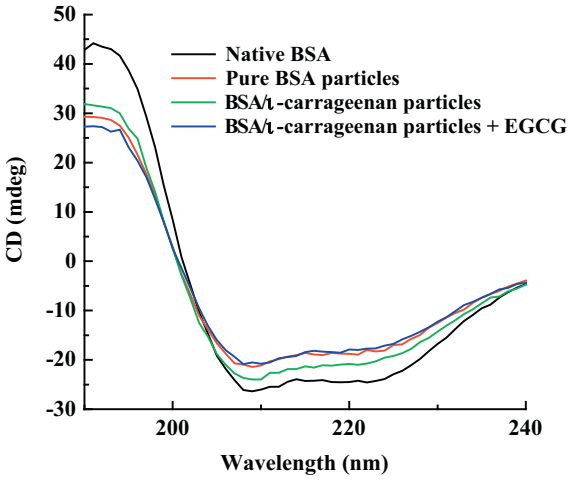

Fig. 3. CD spectra of BSA in the samples of native BSA, pure BSA particles, BSA/ι-carrageenan particles, and BSA/ι-carrageenan particles with EGCG in pH 7 sodium phosphate buffer at 25 °C.

using CD measurement. Fig. 3 gives CD spectra of BSA in BSA/ι-carrageenan particles with and without EGCG, together with CD spectra of native BSA and pure BSA particles for comparison. Native BSA exhibits two negative bands in the far UV region at 208 and 222 nm characteristic of α-helices (Kelly et al., 2005; Norde & Giacomelli, 2000). Compared to native BSA, the decreases in the ellipticity magnitude of BSA in 200–230 nm are observed in pure BSA particles and BSA/ι-carrageenan particles. However, the ellipticity magnitude of BSA in pure BSA particles is smaller than that in BSA/ι-carrageenan particles, which is further decreased when EGCG is added. After quantitative analysis of the CD spectra, the contents of α-helix of BSA were calculated to be 64.4%, 48.1%, 50.0%, and 47.8% in native BSA, pure BSA particles, BSA/ι-carrageenan particles, and BSA/ι-carrageenan particles with EGCG, respectively. The calculated α-helix content of native BSA is within the range observed in other people's work (Norde & Giacomelli, 2000). The decreases in ellipticity and α-helix of BSA in pure BSA particles and BSA/ι-carrageenan particles indicate a partial protein unfolding. Murayama and Tomida (2004) observed the denature of α-helix and the formation of an irreversible intermolecular β-sheet of proteins at high temperatures. Meanwhile, higher amounts of ellipticity and α-helix of protein in BSA/ι-carrageenan particles than in pure BSA particles could give the evidence for the stabilization effect of ι-carrageenan on the folded structure of BSA. However, the further decrease in ellipticity and α-helix of BSA in BSA/ι-carrageenan particles in the presence of EGCG suggests that the binding of EGCG with BSA/ι-carrageenan particles leads to a loss in the helical content of BSA. The formation of a more random secondary structure of BSA upon adding EGCG is supported by the red shift in the fluorescence of BSA shown in Fig. 2. The conformation of BSA was previously reported to undergo partial unfolding upon binding of polyphenols, indicated by a decrease of α-helix and an increase of β-sheet structure (Bourassa et al., 2010).

3.4. Stability of EGCG with BSA/ι-carrageenan particles

EGCG degradation occurs in neutral and alkaline conditions mainly through oxidation and dimer formation (Hong et al., 2002; Mizooku et al., 2003), and the resulted change of the solution from colourless to yellow can be used to test the stability of EGCG. The absorbance changes of EGCG samples of free EGCG, EGCG with native BSA, EGCG with pure BSA particles, and EGCG with BSA/ι-carrageenan particles were recorded at 425 nm for 60 h at pH 7, as shown in Fig. 4. The observed absorbance changes of EGCG

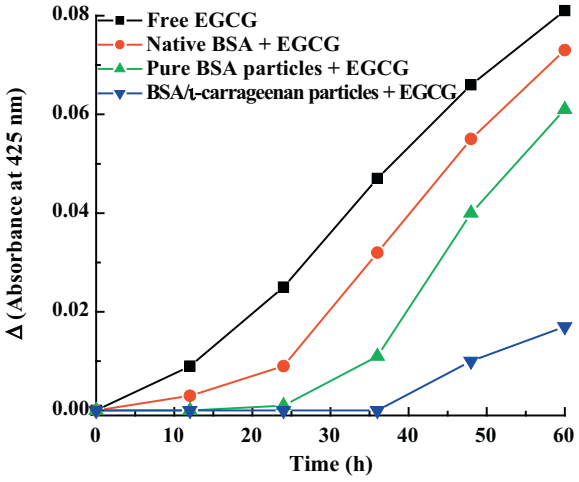

Fig. 4. Change of absorbance of EGCG at 425 nm in the samples of free EGCG, EGCG with native BSA, EGCG with pure BSA particles, and EGCG with BSA/ι-carrageenan particles as a function of time in pH 7 sodium phosphate buffer at 25 °C.

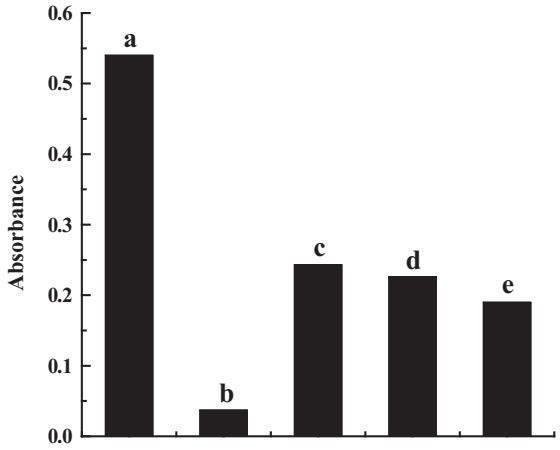

Fig. 5. DPPH absorbance at 517 nm in various samples in pH 7 sodium phosphate buffer at 25 °C. (a) DPPH alone; (b) DPPH with free EGCG; (c) DPPH with EGCG and native BSA; (d) DPPH with EGCG and pure BSA particles; (e) DPPH with EGCG and BSA/ι-carrageenan particles.

samples are within the range of 0.08 in the experimental conditions, which are related to the low concentration of 45 μM EGCG contained in our samples. However, four samples tested may be ranked in order of increasing protective effect on EGCG as follows: free EGCG < EGCG with native BSA < EGCG with pure BSA particles < EGCG with BSA/ι-carrageenan particles. Free EGCG that is not protected shows the fastest degradation at pH 7, which is in agreement with the high instability of EGCG in neutral and alkaline solutions in the work from other people (Li et al., 2012; Zhu, Zhang, Tsang, Huang, & Chen, 1997). Mizooku et al. (2003) analysed the pH-dependent oxidation of EGCG by liquid chromatography/mass spectrometry and found the oxidation species of EGCG corresponds to the destruction of galloyl group, where two hydrogen atoms are removed and one oxygen atom was added. The somewhat increased suppressions of the degradation of EGCG with native BSA and pure BSA particles may be ascribed to the mild binding of EGCG. When thermally-induced BSA/ι-carrageenan particles are added, the highest stability of EGCG corresponds well to the high binding affinity of EGCG with BSA/ι-carrageenan particles. Strong binding of EGCG to the hydrophobic pockets of BSA can effectively retard the contact of galloyl group of EGCG with water, resulting in higher stability of EGCG with BSA/ι-carrageenan particles than with native BSA and pure BSA particles.

3.5. DPPH scavenging activity of EGCG with BSA/ι-carrageenan particles

DPPH radical scavenging method has been usually used to test the radical scavenging ability of various antioxidant substances due to the simple, rapid, sensitive, and reproducible procedure. Upon the reduction by an antioxidant, DPPH is scavenged and its characteristic maximum absorbance at 517 nm decreases proportionally to the increase of the amount of non-radical form of DPPH (Niu et al., 2012). Fig. 5 highlights the values of maximum absorbance of DPPH alone and in the presence of free EGCG, EGCG with native BSA, EGCG with pure BSA particles, and EGCG with BSA/ι-carrageenan particles. Pure EGCG can reduce DPPH absorbance by about 93%. This strong scavenging ability of EGCG may be attributed to the donation of H from the hydroxyl groups on its galloyl moiety to DPPH (Nanjo et al., 1996). It is observed that the mixing of EGCG with native BSA leads to a weaker scavenging action compared to free EGCG, as indicated by bigger absorbance value of DPPH combined with EGCG and native BSA than the value only with free EGCG. The reducing DPPH radical scavenging ability of EGCG with native BSA may be attributed to the masking effect of proteins on the antioxidant capacity of polyphenols (Arts et al., 2002), which could be caused by the formation of hydrogen bond that prevents the phenolic hydroxyl groups from donating H atoms to the radicals. However, when combined with two kinds of protein particles, EGCG exhibits better DPPH radical scavenging activities than with native BSA. Moreover, compared to native BSA and pure BSA particles, the structure changes of BSA in BSA/ι-carrageenan particles discussed above may provide a more proper microenvironment for EGCG to donate H to reduce DPPH into non-radical form more easily.

4. Conclusions

Overall, the results indicate that thermally-induced BSA/ι-carrageenan particles can allow more hydrophobic groups of BSA to be exposed to EGCG molecules, exhibiting high binding affinity of EGCG with BSA/ι-carrageenan particles. Measurements of fluorescence and CD reveal that EGCG is bound in the hydrophobic pockets of BSA, which significantly affects the molecular conformation of BSA in BSA/ι-carrageenan particles. Moreover, hydrophobically BSA-bound EGCG gives more enhanced stability and stronger DPPH radical scavenging ability in BSA/ι-carrageenan particles compared to native BSA and pure BSA particles. Whilst protein/polysaccharide particles have been extensively investigated in terms of their preparation conditions and physicochemical properties (Jones & McClements, 2011), this work may indicate that novel protein/polysaccharide particles are worthy of further investigation in an attempt to enhance the bioavailability of the natural product in functional foods.

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