

How black tea pigment theaflavin dyes chicken eggs: Binding affinity study of theaflavin with ovalbumin

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ABSTRACT

Theaflavin (TF), which is the key pigment in black tea, is a health-promoting food component with beneficial effects on humans. However, the interactions by which these effects are transferred and exerted into protein-rich foods are unclear. Here, egg ovalbumin (OVA) was selected as a representative dietary protein to ascertain their binding mechanism. Steady-state, time-resolved fluorescence and isothermal titration calorimetric results showed that TF can interact well with OVA with an affinity magnitude of 10^4 . The noncovalent binding was mainly driven by hydrophobic interaction and hydrogen bonds. Structural analysis displayed that the TF binding pocket significantly overlapped with one of the surrounding specific IgE-binding epitopes, thereby causing a subtle structural adjustment on the secondary conformation of OVA. The biological complexation model that was delineated here will help understand how black tea dyes egg white in tea egg products and for the development of protein-rich carriers in functional foods.

1. Introduction

Egg is an excellent protein source with abundant nutritional values in human diet. In different splendid food cultures, eggs are cooked into various forms and high value-added products to present unique tastes or functional improvements. Tea egg is a traditional and highly popular food commodity in several Asia countries. Generally, fresh chicken eggs are boiled in water and marinated with black tea after cracking or peeling the shells to impart characteristic color and tea flavor on eggs (Chen, Lu, Chien, & Chen, 2010). During marinating process, egg white is easily dyed by the orange-red theaflavins and reddish-brown thearubigins (see Figure Abstract in Supporting Information), which are the two major pigments in black tea (Stodt et al., 2014), and thereby interactions between egg white proteins and these bioactive pigments are vigorously carried out.

Among the black tea pigments, theaflavin (TF) is an abundant compound in the manufacture of black tea and gathered by catechin enzymatic oxidation and decarboxylation during fermentation (Chowdhury et al., 2018; Teng & Chen, 2018). Over the past decades, TF is well-known as a unique antioxidant and has been proved to own anticancer, vasorelaxant, hypolipidemic, and protective effects against

microtubule dysfunction in laboratory models (Chakrabarty et al., 2019; Chen et al., 2019; Chen, Teng, & Cao, 2019; Leung et al., 2016; Sur & Panda, 2017; Tanaka & Kouno, 2003). In a number of subsequent applications, TF can be functionally used as health-supplement and ingested in combination with protein-rich foods to exert its biological activities. The complexation can enhance the solubility or photostability for bioactive food coloring agents (Chen, Feng, Molnár, & Chen, 2019; Liu et al., 2018). To our knowledge, approximately 40 different proteins are found in egg white (Nafchi, Tabatabaei, Pashania, Rajabi, & Karim, 2013). Therefore, tea-flavored eggs dyed using black tea are good nutritional sources of bioactive TF in diet.

The main components of egg white protein are ovalbumin (OVA), ovomucoid, ovotransferrin, and lysozyme. Among these components, OVA, which consists of 385 amino acids, is the most prevalent component, accounting for 50% to 60% in total and representing an allergen-specific phosphoglycoprotein with IgE-mediated response (Geng et al., 2019; Mine & Rupa, 2003; Sara, Iván, Rosina, & Elena, 2013). Scientific studies have shown that OVA could noncovalently interact with other food components or plant extracts; its functional properties are closely related with the complexation between bioactive substances. For example, the emulsification of OVA can be improved by tannic acid

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binding (Chen et al., 2018) and gelation can enhance the complex coacervation to carboxymethylcellulose (Xiong et al., 2017). Specifically, Ognjenović and coworkers achieved the interaction between OVA and epigallo-catechin 3-gallate (EGCG), which was extracted from green tea with similar physiological activities to TF, showed that EGCG partly overlaps with IgE recognition epitope (Ognjenović et al., 2014). This occurrence showed that the binding of the health-promoting components from tea to ovalbumin may reduce egg white sensitization and may be beneficial to its gastrointestinal stability (Chen, Gnanaraj, Arulselvan, El-Seedi, & Teng, 2019).

Given the benefits of complexation, it is essential to take a closer look at the interaction between ovalbumin and the bioactive theaflavin pigment. The interaction not only transfer characteristic black tea color to egg white, but also may affect the texture and nutrition of protein carriers, and thereby to protect and controlled-release the bioactivities of TF in tea eggs. Here, we present a detailed analysis of the binding mechanism between TF to OVA via fluorescence, isothermal titration calorimetric (ITC), circular dichroism (CD), and molecular docking/dynamic simulations. This work attempted to unveil the TF feature recognition of OVA, as a potential carrier applied in value-added functional foods and understand the mechanism by which black tea pigments dye chicken eggs.

2. Experiment section

2.1. Reagents and chemicals

Purified ovalbumin from egg white (A605084-0005, > 98%) was purchased from Sangon Biotech (Shanghai, China). OVA was dissolved in 0.01 M phosphate buffer saline (PBS) at pH 7.4 with an ionic strength of 0.1 M NaCl to obtain a 20 μ M stock solution. Theaflavin was obtained from Sigma–Aldrich (St Louis, USA) and prepared at a concentration of 50 μ M in ultrapure water. All other reagents were used of analytical grade or the highest grade available. All the stock solutions stored at 4 °C in the dark.

2.2. Steady-state fluorescence

Steady-state fluorescence were carried out with a 1.0 cm quartz cell using a Cary Eclipse fluorescence spectrophotometer (Varian, USA). The excitation and emission slits were set at 5 and 10 nm, respectively. An excitation wavelength (λ_{ex}) was set to 280 nm in intrinsic fluorescence and the emission spectra were recorded in the range of 300–450 nm at 298, 304 and 310 K, respectively. Hence, to avoid inner filter effect, the fluorescence intensity was corrected using the same method in our pervious research (Wu, Yan, Wang, Wang, & Li, 2015). Fluorescence titrations were conducted by fixing the OVA concentration at 2.0 μ M while varying the TF concentration from 0 to 28 μ M at increments of 4 μ M. Prior measurements, the vortex-mixed solutions as well as a blank sample containing the buffer and appropriate TF concentration were thermally equilibrated for 1 h. All assays were conducted in triplicate. Stern-Volmer equation (Lakowicz, 2006a,b) was performed to preliminarily distinguish the quenching mechanism:

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

where F_0 and F are the fluorescence intensities of OVA with and without TF, respectively; K_{sv} is the Stern-Volmer quenching constant can be determined using the linear regression of the plot of F_0/F against the concentration of TF [Q]. The modified Stern–Volmer equation below can be further calculated the values of n , refers to the Hill coefficient in OVA/TF cooperativity, and equilibrium constant K_a in static quenching (Ghosh, Mondal, & Mukherjee, 2015; Lakowicz, 2006a,b):

$$\log(F_0 - F)/F = \log K_a + n \log [Q] \quad (2)$$

Synchronous fluorescence spectra of OVA varying TF concentrations were recorded under a fixed excitation wavelength ($\Delta\lambda = \lambda_{em} - \lambda_{ex}$)

(15 or 60 nm) with parameters set same as those recorded in steady-state fluorescence measurements, and thus, the spectra show the spectroscopic behaviour of tyrosine (Tyr) and tryptophan (Trp) residues, respectively (Dantas et al., 2017).

To obtain the three-dimensional (3D) fluorescence spectra of OVA (2 μ M) and OVA/TF (1:5), the excitation wavelength was set to 200–400 nm with 5 nm increment, and the emission spectra were from 200 nm to 500 nm. All other parameters were the same as the intrinsic fluorescence experiments.

2.3. Time-resolved fluorescence

Fluorescence lifetimes of OVA and OVA–TF system were obtained by time-correlated single-photon counting technique with a Horiba Jobin Yvon FluoroLog-3 spectrofluorometer (HORIBA, France) at $\lambda_{ex} = 280$ nm and $\lambda_{em} = 336$ nm at 298 K. The concentration of OVA was 2 μ M, and varying concentrations of TF from 0 to 10 μ M ([OVA]: [TF] = 1:0, 1:1, 1:5). Bi-exponential iterative fitting was used to analyze the time-resolved fluorescence data, and the quality of each fit was assessed by the chi-square (χ^2) values. The average fluorescence lifetime (τ) can be calculated via bi-exponential on the basis of decay times (τ_i) and normalized pre-exponential factors (α) by using the following equation (Lakowicz, 2006a,b):

$$\tau = \sum \alpha_i \tau_i \quad (3)$$

2.4. Isothermal titration calorimetric

Calorimetric studies of TF binding to OVA were carried out on an isothermal titration calorimeter (ITC) CN-ITC200 (MicroCal, USA) to obtain their thermodynamic parameters at 298 and 310 K, respectively. For each assay, 50 μ M OVA solution in 1 mM PBS buffer (pH 7.4) was loaded in the sample cell and continuously stirred at 450 rpm by twenty successive injections containing the aqueous solution of TF (600 μ M) into syringe. The respective control was conducted by injecting the titrant into PBS buffer without OVA to correct the heat change because of mixing and dilution. The raw data were automatically collected in each titration and calculated by iterative curve fitting of binding isotherms using Origin 9.0 software (Microcal, LLC ITC200). The binding changes of free energy (ΔG) and entropy (ΔS) can be calculated from the values of binding constant (K_a) and enthalpy (ΔH) based on the Van't Hoff equation (Kitamura, Nakatani, Takaha, & Okada, 1999; Wu, Liu, Zhang, Zhang, & Li, 2018):

$$\Delta G = -RT \ln K_a \quad (4)$$

$$\Delta G = \Delta H - T \Delta S \quad (5)$$

2.5. Circular dichroism

Far-UV CD spectra were measured on CD spectrometer (CD Model 400, AVIV, USA) at 298 K in nitrogen atmosphere. Each spectrum was taken from the average of five successive readings. The spectra of OVA (2 μ M) and TF ([OVA]: [TF] = 1:0, 1:1, 1:5) system were recorded from 250 nm to 190 nm wavelengths in a 1 mm path length cell with 1 nm step size, 1 nm band width, and a scan speed of 50 nm/min. As described previously (Wu et al., 2019), the observed CD data can be described as residue-average molar ellipticity to calculate the α -helical content of protein.

2.6. Molecular simulations

The available crystal structure of OVA (ID: 1OVA) was gathered from RCSB Protein Data Bank. Docking simulations of TF (3D structure generated from PubChem CID: 114777) to OVA were implemented using the FlexX platform interfaced in LeadIT. All the water molecules

and ligand atoms were removed from the structure. The ligand (TF) and receptor (OVA) were optimized by AMBER14 force field and AM1-BCC model (Jakalian, Jack, & Bayly, 2010) to ensure the proper protonation states and energy minimization before docking. An incremental construction algorithm implemented in FlexX (Knegtel et al., 1999) was used to place TF into OVA. Docking simulations were performed with 100 runs covering the whole OVA ($98 \text{ \AA} \times 98 \text{ \AA} \times 98 \text{ \AA}$ along the x, y, and z-axes), thereby estimating the reasonable modes of the TF-OVA complex. The system with the lowest docking energy was selected for visualization in Discovery Studio 3.1 software and examined by YASARA program (Version 18.3.23) for Molecular dynamics (MD) simulations.

MD simulations with an explicit water model were performed at 298 K (pH 7.4). The size of the water box (containing 0.9% NaCl) covering the docking conformation at $98 \text{ \AA} \times 98 \text{ \AA} \times 98 \text{ \AA}$ along the x, y, and z-axes. Long range Coulomb interactions were included with a cut-off of 9 Å using the smooth particle mesh Ewald summation. During 40,000 ps simulation running time, raw data were collected per 10 ps with multiple time steps of 1.25 fs for intramolecular and 2.5 fs for intermolecular forces. MD trajectories were analysed via the root mean square deviation (RMSD), radius of gyration (Rg), and the second conformation of OVA/TF-OVA complex.

2.7. Statistics analysis

All assays were conducted in triplicate in the interaction studies. Statistical difference together with the mean values and standard deviations were evaluated by analysis of variance. All statistic data in present work were carried out using the OriginPro Software (Northampton, USA).

3. Results

3.1. OVA-TF complex formation

Basically, the binding of TF to OVA may alter the ground and excited state behaviors in their fluorescence with the variation of different spectroscopic characteristics, such as shape, intensity, and lifetime. Fig. 1(A) displays the raw fluorescence data of OVA with various TF amounts at 280 nm excitation wavelength. As a monophosphorylated glycol-protein, OVA exhibits a strong fluorescence emission peak at 335 nm and its intrinsic fluorescence is mainly derived from three tryptophan residues (Ognjenović et al., 2014). Furthermore, free TF did not exhibit fluorescence emission in the range of 300–440 nm, which had no effect on OVA. With the addition of TF, the fluorescence intensities of OVA gradually shrank with insignificant band shifting. After the improvements of inner filter effect corrections, the fluorescence intensity of OVA still diminished with the addition of TF; hence, the quenching mechanism may be complex formation (static) instead of collision (dynamic). In interpreting the quenching of OVA from steady-state fluorescence, the type of quenching mechanism occurring between TF and OVA must be determined. With the analysis of quenching data according to Eq. (1), the corresponding Stern–Volmer plots at different temperatures are shown in Fig. 1(B) inset. K_{SV} was obtained by the linear regression of the plot of F_0/F against TF concentrations and the calculated values were 2.95×10^4 (298 K), 2.23×10^4 (304 K), and 2.01×10^4 L/mol (310 K), respectively, which is opposite correlated with temperature increasing. The possible OVA fluorescence quenching mechanism by TF was static type as higher temperature would generally result in dissociation.

Essentially, the quenching mechanism can be remarkably determined by time-resolved fluorescence spectroscopy. Static quenching is ordinarily associated with stable fluorescence lifetime values, whereas dynamic quenching alters the fluorescence lifetime values evidently (Lakowicz, 2006a,b). Fig. 1(B) presents the typical spectra for the fluorescence decay of OVA with the addition of TF. Obviously, the

different amounts of TF had minimal effects on the lifetime of OVA, and the data are presented in Table 1. The decay curve of free OVA is a single exponential with 4.68 ns lifetime. Meanwhile, upon the addition of TF even at the maximum ratio, the average excited state lifetime of OVA displayed insignificant changes, thereby indicating that the fluorescence quenching of OVA due to TF was essentially a static mechanism; this result was in good agreement with the conclusion derived from K_{SV} analysis. Therefore, the binding of theaflavin to egg ovalbumin depends on the formation of a ground-state complex.

3.2. Binding parameters and thermodynamic analysis

As noted above, the static quenching resulted from the attacks of TF into OVA and subsequent TF-OVA complex formation. For the static quenching process, the complex forming phenomena can be delineated using double logarithmic equation (Eq. (2)). For different systems at 298, 304, and 310 K, the values of the binding constant K_a (intercept) and Hill coefficient n (slope) were calculated by plotting $\log(F_0-F)/F$ against $\log[Q]$. In the present case, the n value was close to unity, as described by the fact that 1:1 binding stoichiometry was observed for the TF-OVA interaction process. The calculated K_a values (4.25×10^4 , 3.39×10^4 , and 2.76×10^4 L/mol) decreased with the increase in temperature. Generally, in the case of static quenching, the binding ability related to TF-OVA interaction diminishes with increase in temperature. To our knowledge, the 10^4 magnitude affinity computed for the TF-OVA implies a moderate interaction in high-affinity (Digilio et al., 2014; Li et al., 2017; Peng, Ding, Peng, Jiang, & Zhang, 2013). Additionally, the binding affinity was simultaneously demonstrated by the outcomes of the calorimetric studies via the ITC approach.

In contrast to conventional steady-state fluorescence, the thermodynamic parameters of evolving heat in the interaction process were directly obtained to estimate the binding properties for individual sites through ITC (Kilmister, Faulkner, Downey, Darby, & Falconer, 2016; Norberg et al., 2010; Xiong et al., 2017). Fig. 1(C) shows the representative thermogram of the complexation between TF and OVA at 298 K. The single injection of TF into OVA was reflected by each heat flow versus time profiles as shown in the panel above. Meanwhile, the integrated plot per injection at different molar ratios of TF to OVA is presented below to show the quantity of liberated heat. The calorimetric titration curve indicated that the interaction was an exothermic process when the binding event occurred. The K_a and enthalpy change (ΔH) at 298 K were observed to be 4.30×10^4 L/mol and -1.41 kJ/mol, respectively. Hence, the values of the changes in Gibbs free energy (ΔG) and entropy (ΔS) were computed to be -26.44 kJ/mol and 83.99 J/mol/K, respectively. The binding affinity obtained using ITC experimental data is approximately consistent with that obtained through the steady-state fluorescence analysis at 298 K. From the ITC thermogram of the system at 310 K (Fig. S1), the calculated K_a , ΔH , ΔG , and ΔS values were 3.04×10^4 L/mol, -0.13 kJ/mol, -26.60 kJ/mol, and 85.41 J/mol/K, respectively. The positive/negative sign of the same kind of thermodynamic parameters remained consistent at two temperatures. The negative ΔG signified that TF-OVA interaction was spontaneous in nature. When TF binds to OVA, associated solvent molecules will then be released due to the advancement of binding. Therefore, the release of solvent molecules is responsible for the positive entropy of the whole system during TF attachment on OVA. As comprehensively illustrated and empirically summarized by Ross (Ross & Subramanian, 1981; Wiesinger & Hinz, 1986), negative ΔH and positive ΔS values briefly correspond to the involvement of hydrophobic interaction and electrostatic attraction in the binding process. Subsequently, these forces mutually supported and competed to accelerate the aggregation of the TF adjacent to OVA binding pocket. Spectrofluorimetry and ITC results revealed that TF binds to OVA with the formation of a relatively stable complex in solution.

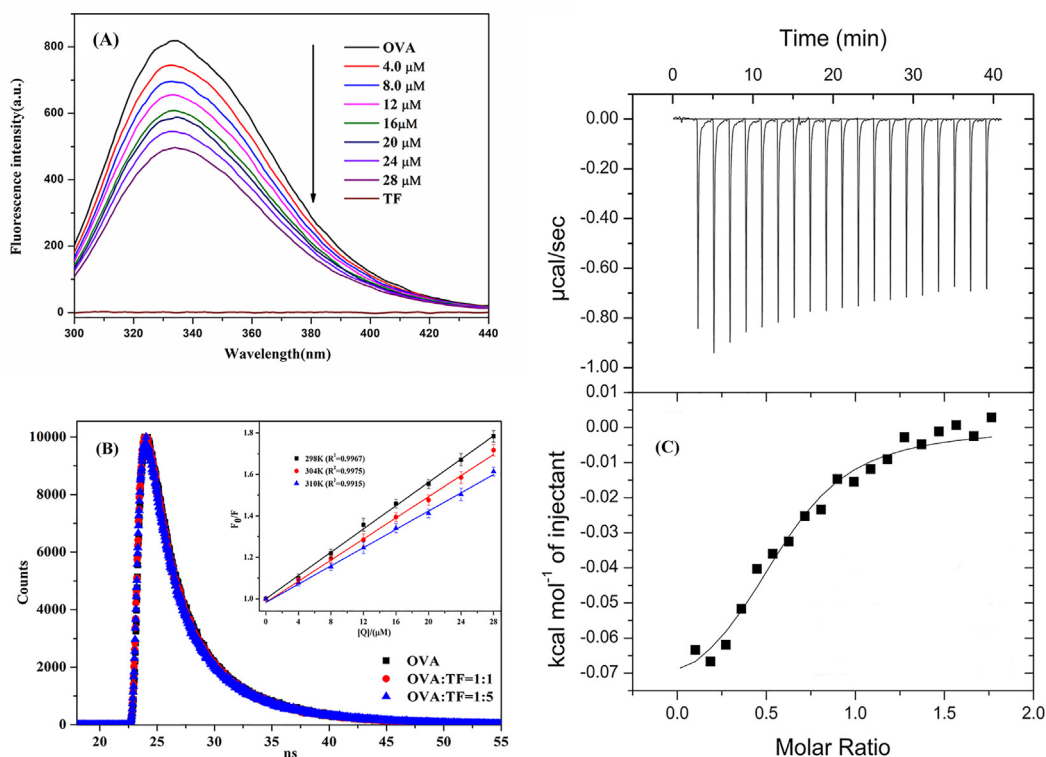


Fig. 1. The effect of TF on the OVA fluorescence and ITC measurements indicates the complexation. (A) The fluorescence quenching spectra of OVA (pH 7.4, $T = 25\text{ }^{\circ}\text{C}$) at different concentrations of TF. (B) Time-resolved fluorescence lifetime of OVA various amounts of TF and the insert is the Stern-Volmer plots for the TF-OVA complex at 298, 304, and 310 K, respectively. (C) The ITC thermogram at 298 K reveals that the binding of TF to OVA is associated with the exothermic enthalpy changes. The original data (top) and the best fitting curve (below) on sequential titration of TF solutions into OVA.

Table 1

Fluorescence lifetime decay of OVA ($2\text{ }\mu\text{M}$) as a function of the ratios of TF.

Sample	τ_1 (ns)	τ_2 (ns)	α_1	α_2	τ (ns)	χ^2
Free OVA	0.94 ± 0.04	5.45 ± 0.12	0.17 ± 0.02	0.83 ± 0.05	4.68	1.09
OVA: TF = 1:1	0.90 ± 0.10	5.43 ± 0.07	0.18 ± 0.02	0.82 ± 0.03	4.61	1.06
OVA: TF = 1:5	0.91 ± 0.07	5.39 ± 0.09	0.17 ± 0.04	0.83 ± 0.05	4.63	1.10

3.3. Synchronous and 3D fluorescence studies

During TF-OVA interaction, the intermolecular forces that maintain the secondary and tertiary conformation of OVA may be transformed. Synchronous and 3D fluorescence were applied to reflect the TF binding vicinity environment in OVA. The specific spectral intervals ($\Delta\lambda$) were selected at 15 and 60 nm to obtain the characteristic information on molecular microenvironment in the vicinity of Tyr and Trp residues in synchronous fluorescence, respectively (Lloyd, 1971). The OVA spectra under different TF concentrations are presented in Fig. 2A–B. Synchronous fluorescence intensities gradually decreased with the increase in TF concentration, and quenching was stronger for Trp residues than that for Tyr residues. TF also caused a 4 nm bathochromic shift when $\Delta\lambda = 60\text{ nm}$. This finding indicated that the Trp residues of OVA considerably contributed to static quenching. The hydrophobicity decreased, while polarity increased with TF complexation.

The contours and corresponding data of OVA tridimensional fluorescence spectra in the absence and presence of TF are shown in Fig. 2C–D. The typical fluorescence signals of free OVA and TF-OVA complex presented three main peaks. Peak 1 represents the Rayleigh scattering ($\lambda_{\text{ex}} = \lambda_{\text{em}}$) peak that was scattered at the same wavelength in all directions. Peaks 2 and 3 correspond to the emission of Trp/Tyr residues and polypeptide backbone of OVA, respectively (Dantas et al., 2017). The intensity of peak 1 decreased upon TF addition, thereby

suggesting that TF-OVA complex induced changes in the OVA molecular diameter of the analytes. Meanwhile, the fluorescence intensities of peaks 2 and 3 were reduced to approximately 63% and 26%, respectively, along with a trivial red shift. As noted above, the reduction of peak 2 was attributed to the changes in the microenvironments around Trp/Tyr residues, and the behavior of peak 3 was possibly due to the modification in the polypeptide chain, thereby changing the original folding degree in OVA (Xie, Wehling, Ciftci, & Zhang, 2017). Thus, the binding of TF with OVA may have influenced the conformation of OVA; this corollary has been documented well in studies on CD and molecular simulation.

3.4. Docking model analysis and molecular dynamic simulation

Molecular docking is a convenient and efficient approach with a good prospect of application. In ligand-protein noncovalent interaction, docking analysis can provide comprehensive information. The present study focuses on two aspects, as follows: first is the prediction of favorable binding modes/geometries and for TF in the binding site of OVA; second, the estimation of the binding free energy and interaction forces of the TF-OVA system. The optimum binding results from FlexX docking are presented below and described in Fig. 3.

Global docking analysis (Fig. 3A) showed that TF binding to OVA can be mainly clustered into two classes, and the 63% top binding

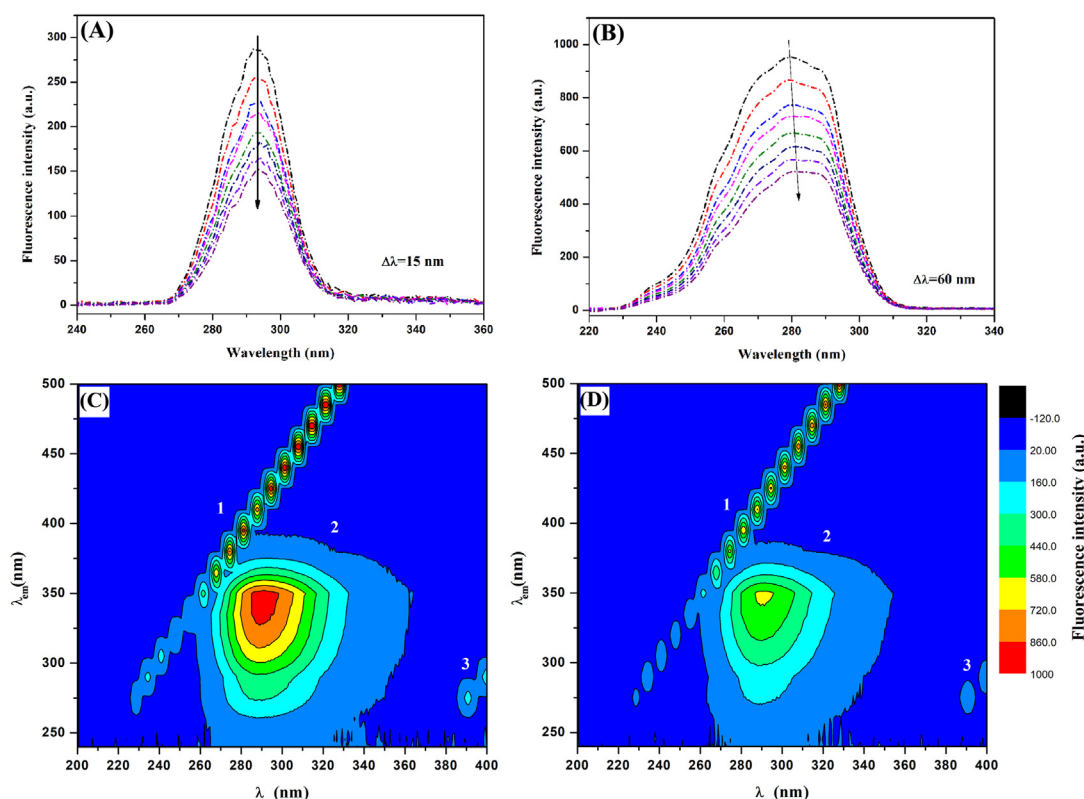


Fig. 2. Synchronous fluorescence spectra of OVA varying concentrations of TF under the fixed excitation wavelength $\Delta\lambda = 15$ nm (A) and $\Delta\lambda = 60$ nm (B). Contour diagrams of 3D fluorescence spectra of 2 μ M OVA (C) and OVA/TF complex at ratio of 1:5 (D).

modes in the same cluster that ranked first were close to the immunodominant allergic IgE epitope, which was designated as DVYSF-SLA (position started from Asp108 and ended at Ala115; the numbering from the PDB ID 1OVA is used throughout) (Mine & Rupa, 2003; Sara et al., 2013). According to the docking process, the top TF structures with slightly different orientations were observed in this pocket, and the optimum pose with the lowest energy scale at -29.12 kJ/mol was selected as the representative to analyze the high-affinity binding details. As displayed in Fig. 3B–C, the binding site was composed of residues from several secondary structural elements and formed a

hydrophobic cavity in the OVA surface to hold TF. The Pro106 residue of OVA was a major contributor that was involved to form mixed π/π alkyl hydrophobic force with TF. In addition, the hydroxyl hydrogens from TF molecular interact with side-chain oxygen atoms from Asn107, Lys 105, Thr104, Phe112, Leu114, and Gln164 residues through conventional H bridge. The remaining active amino acids of TF-OVA binding were Asp108, Tyr110, Val109, Ser111, Ile103, Ser113, Ser116, Gly140, Arg139, and Asn166 within $5 \text{ \AA}$ in the proximity of TF, and these residues were favorable to van der Waals forces. These interactions stabilized the docking mode and were significant to understand

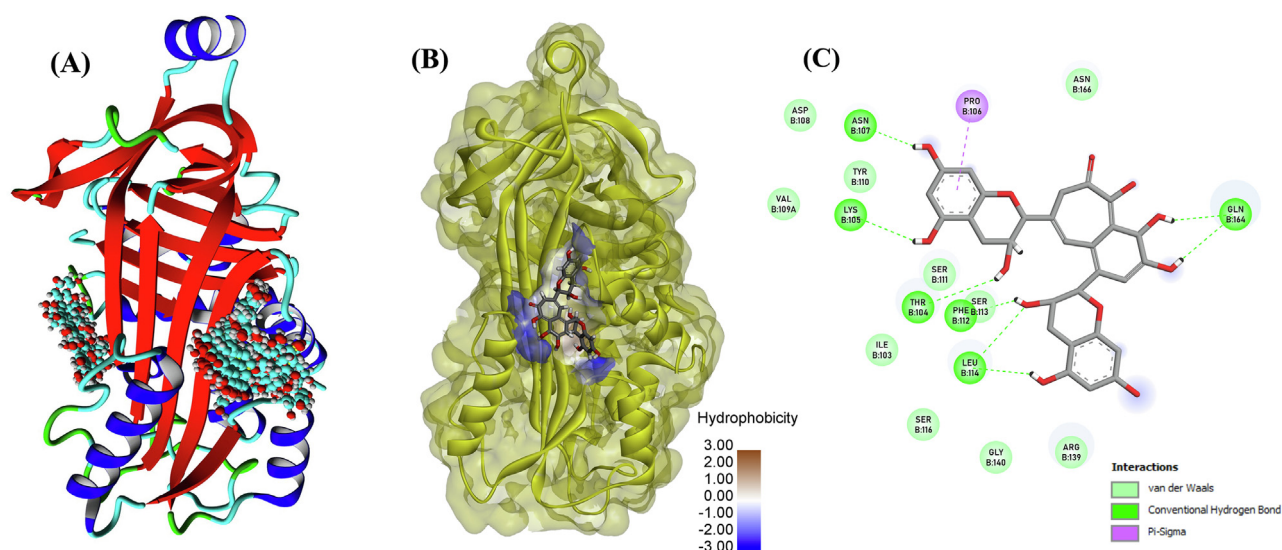


Fig. 3. (A) Cluster analysis for the global docking confirmations of TF binding to OVA. (B) The optimum pose of the TF-OVA complex with hydrophobic cavity shown in OVA surface form. (C) Two-dimensional representation of the interaction maps between TF with participation of several active amino acid residues of OVA.

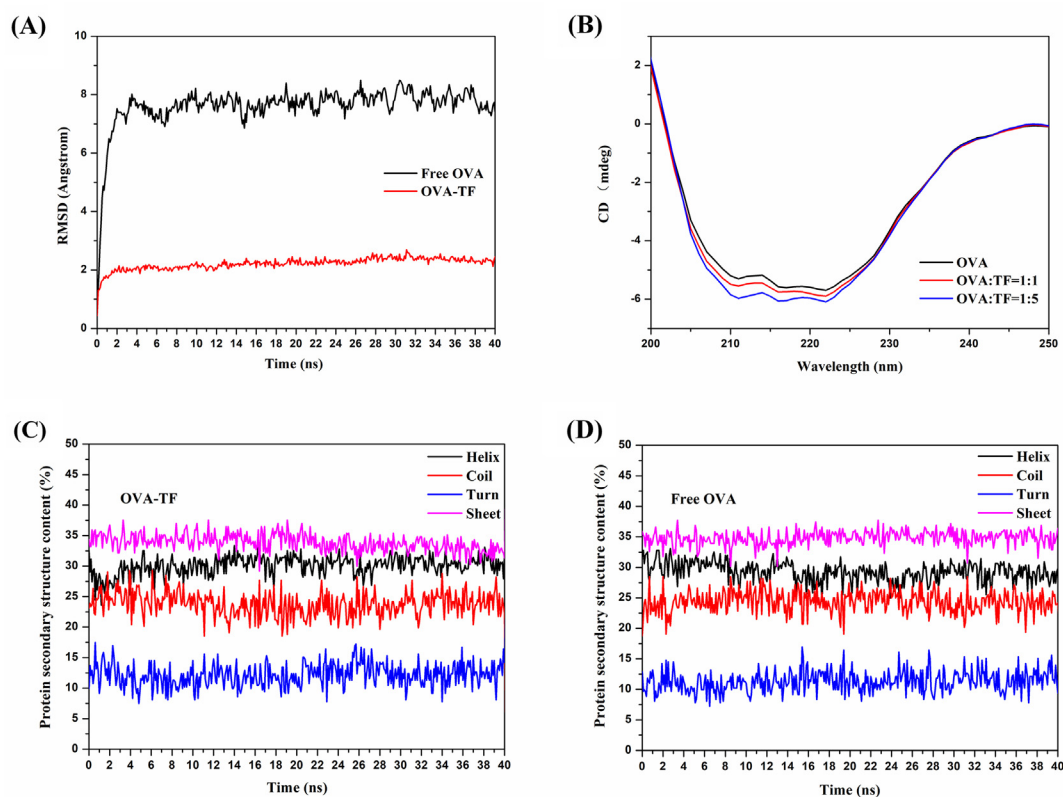


Fig. 4. (A) RMSDs of the MD simulations of the free OVA and OVA-TF complex systems. (B) CD spectra of OVA with the absence and presence of gradient proportions of TF. Contents of the secondary structure for OVA-TF complex (C) and free OVA (D).

the changes in fluorescence of OVA in the presence of TF as mentioned above.

The dynamical behavior of the binding system was explored at the pH of 7.4 on the basis of the simulation platform of YASARA software at a duration of 40 ns. As mentioned above, the rank one conformation of TF-OVA was further investigated in atomic perspective. The root mean square deviation (RMSD), which is the offset distance of OVA backbone atoms inside simulation times relative to their initial position (Sharifi, Ghayeb, & Mohammadi, 2017). Here, the RMSD plots of the free OVA and TF-OVA complex are displayed in Fig. 4A to assess their stability in MD simulation. The RMSD value results showed, the TF-OVA system possessed smaller skeletal deviation (2.09 Å in average) than that of sole OVA, and the complex fluctuated in balanced position within the whole trajectory period. Overall, OVA was inclined to adjust to a stable structure after interaction with a relatively high-affinity TF binding site.

3.5. Secondary conformational changes of OVA

As set forth, the TF binding affected the OVA structure. For further verification of the conformational changes in the dietary protein, circular dichroism spectroscopy and MD simulations were performed to analyze the secondary structural composition changes in OVA quantitatively. As demonstrated in Fig. 4B, the spectrum of pure OVA displayed a broad negative band in the far-UV region from 208 nm to 222 nm, which was the characteristic shape of polypeptide chains representing α -helix structure inside OVA (Geng et al., 2014). With TF addition, the overall shape of OVA remained stable, whereas the α -helix content increased. This effect was observed at 1:1 M ratio and persisted at high TF ratio. The increase was presumably a consequence of the reduction of β -sheet. As displayed in Fig. 4C–D, the OVA secondary structure contents in the presence and absence of TF were determined via MD simulations at a dynamic time of 40 ns. The results also suggested that the insertion of TF increased the disturbance of random coil,

whereas the average contents of random coil and β -sheet remained nearly unchanged. On the basis of the information from CD spectra and MD simulations, we concluded that binding resulted in a tight embedding of TF with OVA and complex affected the secondary structures of pure OVA due to conformational change.

4. Discussion

In addition to its uses as beverage, black tea is also widely used as an ingredient in food processing, of which coloring is one of its most important application. Anciently, black tea has been used for hundreds of years in egg product processing. However, the molecular mechanism of black tea coloration, that is the interaction between its main pigment TF and the main constituent protein in eggs, has not yet been studied. In the present study, the details of the interaction between TF and OVA and the complex formation were described for the first time, and the coloring mechanism of black tea on food protein was explained at the molecular level. Generally, the color of the commercial tea eggs can remain stable for 6 months to 12 months under the condition of transparent packaging, storage and sales at room temperature. The formation of the stable complex between TF and OVA explained the high color stability on tea eggs.

As mentioned above, the computational prediction of the high-affinity TF-OVA binding pocket partly overlapped the designated IgE recognition epitope. Thus, the interaction with TF may affect not only the secondary conformation but also, and perhaps more interestingly, the structure of IgE epitopes. The changes in the CD results of OVA conformation indicated subtle structural adjustment during TF binding in the allergen. As has been argued, the high-affinity TF binding point overlapped an immunodominant allergic IgE epitope. Hence, the TF modification to OVA conformation may influence the IgE-binding capacity. Future experiments should investigate and whether TF can directly inhibit IgE binding.

As set forth, the effect of the interaction is two-way; thus, the complexation of OVA binding also has potential biological significance for TF. Although TF was relatively stable in nature, its structure and biological activities may be threatened in specific food processing applications. At the same time, gastrointestinal digestion also challenged the maintenance of TF structure, thereby decreasing its biological accessibility and utilization rate. Using OVA as carrier would be a novel idea to construct an embedding and delivery system in improving TF bioavailability. This work provided insight to the interaction between theaflavin and ovalbumin, which is a globular dietary protein from chicken eggs, by fluorescence, ITC, CD spectrometry, and molecular docking/dynamic simulations. A high-affinity binding mechanism was important in complex food systems. In the case TF-OVA complex, specifically, the noncovalent binding of TF to OVA mainly included two processes, as follows: first, TF met OVA and spontaneously entered a hydrophobic pocket to form a complex under electrostatic attraction and hydrophobic interaction, which resulted in static fluorescence quenching; second, the TF action related to the stability of complex was enhanced by interaction forces (e.g. hydrogen bonds) with the surrounding OVA amino acids, thereby causing secondary structural changes in OVA. Overall, it is foreseeable that the interaction between the bioactive orange-red pigment TF and OVA are occurred during dyeing of egg white by using the black tea. Meanwhile, the biological complexation model delineated here will be useful for the development of protein-rich carriers to exert health-promoting components in functional foods.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2019.125407>.

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Supporting Information

How black tea pigment theaflavin dyes chicken eggs: Binding affinity study of theaflavin with ovalbumin

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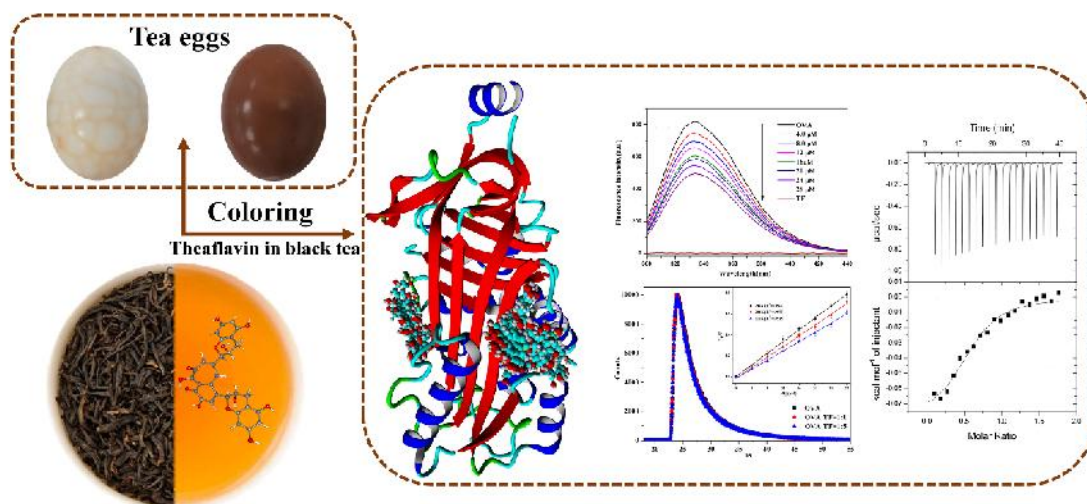


Figure Abstract

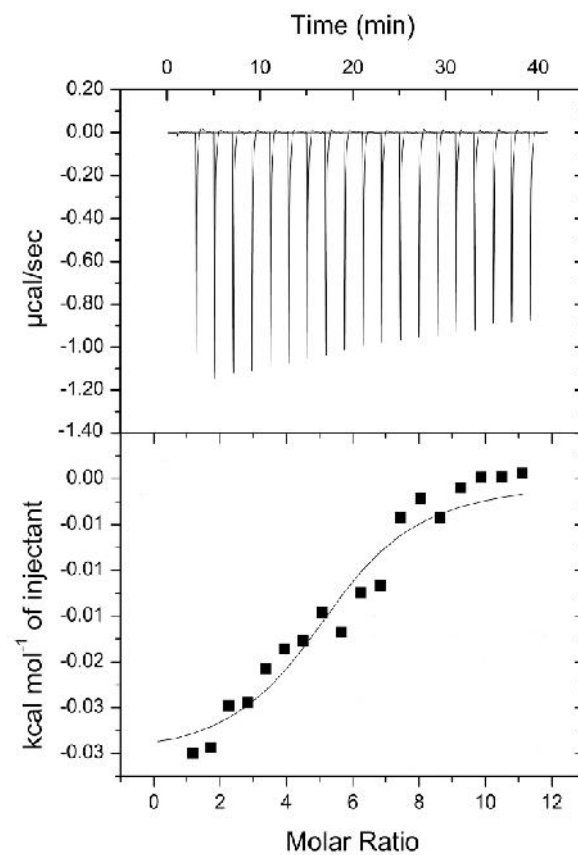


Figure. S1 The ITC thermogram at 310 K, the original data (top) and the best fitting curve (below) on sequential titration of TF solutions into OVA.