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Characteristics of the interaction mechanism between tannic acid and sodium caseinate using multispectroscopic and thermodynamics methods

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ABSTRACT

The spectroscopy and thermodynamics method was applied to study the binding of tannic acid (TA) to sodium caseinate (SC) in a phosphate buffer solution at pH 7.0. The turbidity of TA/SC complexes in phosphate buffer solution increased with increasing of TA/SC mass ratio. It tended to equilibrium until the TA/SC mass ratio was over the value of 1:1. The presence of TA changed the particle size of TA/SC complexes, which decreased with increasing of TA/SC mass ratio (TA/SC mass ratio less than 1:1). The intrinsic fluorescence and synchronous fluorescence spectra measurements demonstrated the complexes of SC with TA, which leads to the formation of TA/SC complexes. The far-UV CD results indicated that the TA induced a progressive increased in proportion of β-sheet structure, however, the proportion of α-helical, β-turn and random coil all decreased. Fourier transform infrared spectroscopy (FTIR) further confirmed that TA induced the transformation of SC structure. Isothermal titration calorimetry (ITC) measurements, which were used to characterize the binding mechanism of TA/SC complexes in phosphate buffer solution (pH 7.0) at 25 °C, clearly showed that the binding between TA and SC was mainly driven by the noncovalent interaction (e.g. H-bonding).

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1. Introduction

In recent years, the interaction mechanism between protein and polyphenols have been studied due to their wide range of applications in the food industry. Milk proteins are natural vehicles for bioactive substances because of several unique properties, including foaming, gelling, self-assembly behavior and emulsification, these structural and physicochemical properties facilitate their functionalities in the food industry (Livney, 2010). Milk casein is the main component of milk proteins, accounting for approximately 80%, which is a heterogeneous mixture from the view of the composition or structure. Caseinate is a proline-rich protein with a random coil structure that is more flexible than typical globulin (Bandyopadhyay, Ghosh, & Ghosh, 2012; Maiti, Ghosh, & Dasgupta, 2006; Papadopoulou & Frazier, 2004). Proline residues are reportedly important for interactions of proteins and polypeptides with polyphenols (Siebert, Troukhanova, & Lynn, 1996). Sodium caseinate (SC) which is typically produced from acid casein, principally consists of four fractions called α_{s1}, α_{s2}, β and κ-casein (De Kruif & Holt, 2003). A significant body of research has been generated to understand the interaction between sodium caseinate and polysaccharides. Sodium caseinate/gum arabic complexes formed via electrostatic interaction (Ye, Flanagan, & Singh, 2006). The interaction between sodium caseinate and xanthan gum is pH-dependent (Kobori, Matsumoto, & Sugiyama, 2009). Chitosan and sodium caseinate formed nanoparticles in aqueous solutions (Anal, Tobiassen, Flanagan, & Singh, 2008). However, not only proteins were able to form complexes with polysaccharides, but also polyphenols, such as epigallocatechin gallate, gallic acid (Arts et al., 2002; Shpigelman, Israeli, & Livney, 2010).

Tannic acid (TA) is a glucoside of gallic acid polymer with multiple phenolic hydroxyl groups that are found in many plants (Gao & Zharov, 2014). TA has been widely employed as a functional

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food additive, exhibiting superior antimicrobial activity, excellent radical scavenging and hence antioxidative properties (Yogendra Kumar, Tirpude, Maheshwari, Bansal, & Misra, 2013). It is also used as nutraceuticals and/or pharmaceuticals in the prevention and treatment of diseases, due to their unique physiological activity and certain medicinal value, such as hemostasis, inhibition of microorganisms, anti-allergy, anti-mutation, anti-cancer, anti-tumor, anti-aging (Quideau, Deffieux, Douat-Casassus, & Pouységu, 2011; Schmidt, Halvorson, Gonzalez, & Hagerman, 2012; F. Zhang et al., 2017; Q. Zhang et al., 2017; Zhi-Jing et al., 2017). TA contains active functional groups such as phenolic hydroxyl group, hydroxyl group and carboxyl group which were able to form complex with proteins, polysaccharide, alkaloids and other substances (Balange & Benjakul, 2009; Kilic et al., 2017; Lau et al., 2017; Lomova et al., 2015; Quideau et al., 2011). Particularly, TA have a higher binding ability to protein, which will reduce the nutritional value of protein because high concentration of TA can interact with the protein, which consequently result in a non-digestible coagulation complexes, thereby affecting the absorption and utilization of protein in body (Hasni et al., 2011). Therefore, study and clarify the interaction model and type of force between tannic acid and protein from the molecular level, may help us to use and treat the relationship between TA and protein more rationally, thus adjusting and improving the dietary structure.

In this study, multi-spectroscopic and thermodynamics method were used to investigate the interaction between TA and SC. The formation of TA/SC complexes is characterized by turbidity measurements and dynamic light scattering (DLS). The interaction mechanism of protein and tannin acid was investigated by observing the fluorescence quenching of the protein. Fourier transform infrared spectroscopy (FTIR) and circular dichroism (CD) spectroscopy was utilized to estimate the change of secondary structure for protein. To confirm that the type of interaction between tannin acid and protein, the changes of thermodynamic parameters of TA bound to protein were detected by isothermal titration calorimetry (ITC).

2. Materials and methods

2.1. Materials

Sodium caseinate (soluble 50 mg/mL) from bovine milk was purchased from Sigma-Aldrich (St. Louis, MO). Tannic acid was purchased from Aladdin Chemical Co., China. All other chemicals used were of analytical grade, unless otherwise stated.

2.2. Sample preparation

Sodium caseinate (SC) and tannin acid (TA) aqueous solutions were prepared by dissolving proper amount of each powder in phosphate buffer solution (10 mM, pH 7.0) at room temperature and stirring for 2 h. Meanwhile, pH was adjusted with HCl (0.01 M) or NaOH (0.01 M). The TA/SC mixed systems were prepared by mixing appropriate volume of SC and TA solutions in order to achieve the desired final concentrations. The solutions were allowed to stand overnight at 4 °C to assure formation of complexes.

For DLS measurements, samples of single components (Sodium caseinate or Tannin acid) were filtered through 0.45 and 0.22 μm microfilters after pH adjustment and used immediately.

2.3. Methods

2.3.1. Turbidity

The phosphate buffer solution (used as the diluting solvent) and TA solution were respectively added into SC solution until the final mass ratio of TA/SC up to 0, 0.05, 0.1, 0.2, 0.3, 0.5, 0.8, 1.0 and 1.5 and the content of SC was fixed as 1% (w/v). After 1 h magnetic stirring at room temperature, the turbidity was measured using UV-vis spectrophotometer (UV-1100, MAPDA) at 600 nm. The turbidity of samples plotted as 100-T%, where T stands for transmittance curves at 600 nm of the sample (Chakraborty, Chakraborty, Moulik, & Ghosh, 2007; Liu & Guo, 2008). All measurements were conducted at 25 °C and repeated three times.

2.3.2. Particle size measurements

Particle size were carried out using a Dynamic Laser Light Scattering (DLS) instrument (Zetasizer Nano, Malvern Instruments Ltd, UK) provided with a He-Ne laser (633 nm). Monitoring was carried out at a fixed scattering angle of 173°. Investigated solutions were contained in a disposable polystyrene cuvette. All measurements were conducted at 25 °C and repeated three times.

2.3.3. Fluorescence spectroscopy

The binding of TA with SC was measured by fluorescence spectrophotometry. Intrinsic and synchronous fluorescence measurements were carried out by spectrofluorimeter (F-4600, HITACHI Ltd, Japan). The concentration of SC was fixing at 50 μg/mL in PBs (10 mM, pH 7.0) and the mass ratio of TA/SC was varied from 0 to 1.5. Emission spectra were recorded at an excitation wavelength of 280 and 295 nm. Quenching of protein fluorescence due to energy transfer from both tryptophan (Trp) and tyrosine (Tyr) residues to TA served to determine the binding of TA with SC. Synchronous fluorescence was recorded at $\Delta\lambda$ of 15 and 60 nm. Both excitation and emission slit widths were set at 10 nm.

2.3.4. Circular Dichroism (CD) measurements

The CD spectra were measured using a CD spectropolarimeter (J-1500, JASCO Ltd, Japan) under constant nitrogen flush at far-UV (190–250 nm) regions. The protein concentration was fixed to 0.5 mg/mL in phosphate buffer solution (10 mM, pH 7.0) and the mass ratio of TA/SC from 0 to 1.5. The parameters were set as follows: 0.2 nm step resolution, speed of 100 nm/min, 1 nm bandwidth and 3 accumulations. A quartz cell having a 0.1 cm path length was used for the measurements. CD spectra of 0.01 M phosphate buffer solution (pH 7.0) and solutions containing variable concentrations of TA were collected and subtracted from each sample spectra to baseline correction. The fractions of α-helix, β-sheet, β-turn, and random coil were calculated by CD spectropolarimeter. Each spectrum record was the average of three consecutive measurements.

2.3.5. FTIR spectra measurements

Fourier transform infrared spectrophotometer (NICOLET 470, PerkinElmer Ltd, US) was used to obtain the infrared spectra of samples to determine any interactions between SC and TA with the potassium bromide (KBr) pellet method. The samples of SC, TA and freeze-dried TA-SC complex (mass ratio of SC and TA were 1:0.1,1:0.5 and 1:1) were analyzed by spectrophotometer. All of the samples were sufficiently ground and uniformly mixed with KBr (dried at 120 °C for 3 h) at weight ratio of 1:50, and pressed into a transparent sheet. The spectra were acquired in the range of 500–4000 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans.

2.3.6. Isothermal titration calorimetry (ITC) determination

The thermodynamic parameters of TA/SC complexes in phosphate buffer solution (10 mM, pH 7.0) were investigated by using ITC200 microcalorimeter (MicroCal Inc., Northampton, UK) at 25 °C (Frazier, Papadopoulou, Mueller-Harvey, Kissoon, & Green, 2003). In a typical experiment, TA (0.29 mM) and SC (0.02 mM) solutions were placed in 200 μL of reaction cell and 40 μL syringe,

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