Effects of Casein, Ovalbumin, and Dextran on the Astringency of Tea Polyphenols Determined by Quartz Crystal Microbalance with Dissipation

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Tea polyphenols (TPPs) can bind with proteins and peptides through hydrophobic interaction and hydrogen bonding. Casein, ovalbumin, and dextran were used to investigate their influence on the interactions between TPP and gelatin and, therefore, to investigate their influence on TPP taste. Casein-*g*-dextran (CgD) and ovalbumin-*g*-dextran (OgD) grafting conjugates were prepared through the Maillard reaction. Dispersible CgD/OgD−TPP complexes formed in acidic pH solution even after a heating process. At the same weight ratio of protein to TPP, about 20−30% of TPP was bound to the proteins. TPP affinity for dextran is much lower. Gelatin, a model of the salivary proteins in buccal cavity, was immobilized on quartz crystal sensor surface through covalent bond. By use of a quartz crystal microbalance with dissipation, we found that the complexation of TPP with gelatin causes a dehydration and collapse of the gelatin layer on the sensor surface that is similar to the sensation of dryness and constriction on oral membranes caused by polyphenols. The complexation between TPP and casein/ovalbumin/dextran can decrease the interaction between TPP and gelatin by decreasing the free TPP molecules and shielding gelatin surface from TPP. Casein has stronger binding ability on the gelatin surface compared to ovalbumin and dextran, and therefore casein is more effective to decrease the sensation of astringency caused by TPP.

Introduction

Green tea is one of the most popular beverages in the world, especially in Asia. The typical polyphenols in green tea include (+)-catechin, (−)-epicatechin, (−)-epicatechin gallate, and (−)-epigallocatechin gallate.[1] It has been reported that tea polyphenols (TPPs) have biological and pharmacological effects, including antioxidant, antimutagenic, anticarcinogenic, antiviral, and antiinflammatory activities that are good for human health.[2−9] Polyphenols can bind with proteins and peptides forming soluble and insoluble complexes through hydrophobic interaction and hydrogen bonding.[1,10,11] The complexation affects the activities of TPP and the taste of tea.[11] The insoluble aggregates of polyphenols and salivary proteins in the buccal cavity prevent the polyphenols from becoming bioavailable[12] and induce the sensation of dryness and constriction, which is the origin of astringency of green tea.[13−15] Breslin et al. suggested that the astringent sensations are tactile sensations caused by the increase of friction between the oral membranes.[16] Polysaccharides can partly inhibit the precipitation of polyphenols with proteins by forming ternary protein−polyphenol−carbohydrate complexes and/or blocking the interactions between polyphenols and proteins.[13,17−19]

The interactions between polyphenols and proteins have been studied by various methods.[1,11,20−24] For example, Hagerman et al. reported the interactions of proanthocyanidin, condensed tannins, with different proteins by means of competitive binding.[25] At pH 4.9, the relative affinities range over more than 4 orders of magnitude, indicating that the tannins interact quite selectively with proteins and protein-like polymers. Proteins are precipitated by tannins most efficiently at pH values near their isoelectric points. Proline-rich proteins and polymers have very high affinities for tannins. Tightly coiled globular proteins have much lower affinities for tannins than conformationally loose proteins. The quartz crystal microbalance with dissipation (QCM-D) technique

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can simultaneously determine the changes of mass and viscoelastic properties of surface-bound molecules and is an ideal method for studying biological surface in situ and real time.[1,26−28] Huang and co-workers studied the binding of thearubigin and (−)-epigallocatechin gallate (EGCG) on bovine serum albumin (BSA) surface by QCM-D.[1,28] The thearubigin adsorption is dominated by electrostatic interaction and hydrogen bonding, while the adsorption of EGCG is dominated by hydrophobic interactions. Higher EGCG adsorption leads to the aggregation of BSA through EGCG bridges. Kaneda et al. reported the interaction of quercitrin/tannic acid on a gelatin surface[29] and Kamihira reported the adsorption of tea catechins on lipid bilayers with QCM without dissipation.[30] However, most of the studies of protein−polyphenol interactions focus on the precipitation and driving forces, and few works quantificationally or quasi-quantificationally investigate the effects of the interactions between TPP and proteins/polysaccharides on TPP activities and taste. Up to now, the mechanism of how proteins affect TPP activities and taste is still unknown.

Casein is the predominant protein in milk. Four casein constituents, αs1-, αs2-, β-, and κ-casein, exist in approximate proportions of 3:0.8:3:1 by weight; their molecular weights are 19−25 kDa. All four casein proteins are amphiphilic molecules and have no defined structure. Casein has an isoelectric point (pI) of 4.6 and proline content of 11.7%.[31,32] Jobstl et al. investigated the interactions of β-casein and EGCG using a variety of biophysical techniques. Their studies show that small soluble polydisperse particles formed at low EGCG ratios aggregate to larger particles with the further addition of EGCG.[33] The interaction of EGCG with a single casein molecule is multivalent and leads to a reduction in the persistence length and the radius of gyration of casein. It is the multivalent hydrophobic interaction between casein and EGCG that causes a compaction of the casein micelle.[11] Ovalbumin is a dominant component of egg white proteins with a pI of 4.8 and molecular weight of 47 kDa.[34−36] As a well-folded globular protein, ovalbumin has a low affinity for proanthocyanidin.[25]

Because of the substantial difference in the structures of casein and ovalbumin, in this paper we used these two proteins to investigate their interactions with TPP and their effects and mechanism on TPP activities and taste. Both casein and ovalbumin are acidic proteins. Casein is almost insoluble in the pH range of 4.0−5.0.[37] Ovalbumin is soluble at acidic pH, but precipitates when heating ovalbumin aqueous solution or mixing ovalbumin with TPP. Therefore, we employed casein-*g*-dextran grafting conjugates (CgD) and ovalbumin-*g*-dextran grafting conjugates (OgD) to suppress the precipitates of the proteins and protein−TPP complexes. The binding efficiency of TPP with the conjugates/dextran and the free total phenolic content in the mixture solutions were determined by ultrafiltration separation and the Folin−Ciocalteu method.

It has been reported that the open conformational proline-rich proteins found in saliva can interact with tannins through hydrophobic interaction and hydrogen bonds.[38,39] Gelatin is also a proline-rich protein with a proline molar content of 16.1% and an open coil conformation; therefore, it is usually used as a model of salivary proteins.[21,40] The study of Hagerman et al. revealed that the relative affinity of gelatin for tannins is about 3 orders of magnitude larger than the affinity of ovalbumin for tannins.[25] In this study, gelatin was immobilized on the surface of quartz crystal sensor through covalent bond. By use of QCM-D, we can detect the changes in both mass and conformation of gelatin layer induced by TPP and the complexes of the protein/dextran–TPP, which provides comprehensive information of the complexation between TPP and gelatin, a model of the complexation of TPP with salivary proteins.

Experimental Section

Materials. Casein (from bovine milk, technical grade, Sigma), ovalbumin (OVA, from egg white, grade V, Sigma), gelatin (from bovine skin, tape B, molecular weight about 50 kDa, pI of 4.7−5.2, Sigma), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, Sigma), *N*-hydroxysuccinimide (NHS, Sigma), mercaptoacetic acid (98%, Acros), dextran (62 kDa, Amersco) and Folin−Ciocalteu reagent (10%, v/v, Fluka) were used as received. Tea polyphenol (TPP) powder extracted from green tea was from Unilever, Shanghai, China, which contains 74.6% total polyphenols and 69.1% total catechins. All solutions were prepared with deionized water.

Preparation of Protein-*g*-dextran Grafting Conjugates and TPP Complexes. The Maillard reaction can conjugate polysaccharide and protein by linking the reducing end carbonyl group in the former to the amino group in the latter. Details for the Maillard reaction and the analysis of conjugation degree of CgD and OgD conjugates can be found elsewhere.[37,41] The conjugates used in this study were prepared from dextran (62 kDa) and casein/OVA with the weight ratio of dextran to protein 10:1 and a 48 h Maillard reaction. The conjugation degree was analyzed through OPA (*o*-phthaldialdehyde) assay based on the loss of free amino groups of casein or OVA after the Maillard reaction. The conjugation degree of dextran to casein and OVA is 16.4 ± 2.0% and 11.8 ± 2.8%, respectively, which means that 2.3 ± 0.3 dextran molecules were conjugated to casein and 2.5 ± 0.6 dextran molecules were conjugated to OVA on average.

The conjugates, proteins, dextran, and TPP were dissolved in water separately with mild stirring. The pH of the solutions was adjusted with HCl or NaOH solution. The concentration of the stock solutions of TPP, protein was 10 mg/mL separately, conjugate 110 mg/mL, and dextran 100 mg/mL. In the mixture solutions of TPP with the proteins, dextran, or conjugates, the final concentration of TPP was 5 mg/mL, protein 5 mg/mL, dextran 50 mg/mL, and conjugate 55 mg/mL. TPP and the mixture solutions were heated at 90 °C for 30 min and then cooled naturally to mimic the preparation process of tea soup.

Turbidity Measurement. The turbidities of the mixture solutions of TPP with the conjugates or proteins and dextran at pH range of 4.0−7.0 were recorded at 500 nm absorbance on a spectrophotometer (Lambda 35, Perkin-Elmer). The absorbance of individual TPP solution with the same pH and heating process was subtracted from the absorbance of the mixtures.

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Polyphenol Analysis. The total phenolic content of TPP was assayed according to Folin−Ciocalteu method with gallic acid as a standard.[42] Typically, 1 mL of the diluted sample was mixed with 5 mL of Folin−Ciocalteu reagent (10%, v/v). After 3 min of incubation at room temperature, 4 mL of a 7.5% Na_2CO_3 solution was added to the mixture. The absorbance at 765 nm was recorded (Lambda 35, Perkin-Elmer) after 1 h of incubation at room temperature.

Free polyphenols in the mixture solutions were separated by ultrafiltration (cutoff molecular weight 10 kDa; MicroconYM-10, Millipore). The ultrafiltrates were analyzed by the Folin−Ciocalteu method. The amount of bound polyphenols on the conjugates or dextran was obtained by subtracting the free polyphenols in the ultrafiltrate from the polyphenols in feed in the mixture. The adsorption of the polyphenols on the ultrafiltration membrane was calibrated with individual TPP solution at the same condition.

Preparation of Gelatin Surface on Quartz Crystal Sensor. A quartz crystal sensor coated with gold was first cleaned by dipping into a 1:1:5 (volume ratio) mixture of ammonium hydroxide (NH_4OH, 25%), hydrogen peroxide (H_2O_2, 30%), and deionied water at 75 °C for 10 min and then rinsing with deionized water and drying with nitrogen gas. The carboxyl-modified surface was obtained by immersing the fresh cleaning crystal into a 5.0 mM mercaptoacetic acid ethanol solution for 10 h, rinsing with adequate ethanol and water to remove mercaptoacetic acid, and drying with nitrogen gas in succession. The quartz crystal sensor with modified surface was mounted in a QCM module. The carboxyls on the crystal surface were activated by 1.0 mL of aqueous solution containing 0.2 M EDC and 0.05 M NHS for 10 min.[43] After the excess reagents were rinsed off with water of pH 6.7, 1.0 mL of a 1.0 mg/mL pH 6.7 gelatin solution was loaded to react with the activated carboxyls for 1 h. The surface was rinsed with water of pH 6.7. The gelatin loading and water rinsing process was repeated several times to ensure that all activated sites were coupled with gelatin.

QCM-D Measurement. A QCM-D instrument (E4, Q-sense AB) was used to determine the interactions of gelatin with TPP, proteins, dextran, conjugates, or their mixtures. The crystal sensor is an AT-cut quartz crystal with a fundamental resonant frequency of 5 MHz.[44] When a quartz crystal is excited to oscillate in the thickness shear mode at its fundamental resonant frequency, a small change of the mass added on the crystal surface induces a decrease of the resonant frequency. If the added layer is rigid, evenly distributed, and much thinner than the crystal, the change of resonant frequency (Δf) is related to the mass of the added layer and overtone number (n = 1, 3, 5,. . .).[45] If the added layer is viscoelastic, the layer will not fully couple to the oscillation of the crystal but undergo a deformation under shear oscillatory motion which dampens the crystal's oscillation and influences the change of the resonant frequency.[46] The dissipation factor (D) of the crystal's oscillation is related to the film's viscoelasticity and thickness, which is defined by $D = E_d/2\pi E_s$, where E_d is the energy dissipated during one oscillation and E_s is the energy stored in the oscillating system. The measurement of D is based on that the voltage over the crystal decays exponentially when the driving voltage of a piezoelectric oscillator is switched off.[44] By switching the driving voltage on and off periodically, we can simultaneously obtain a series of the changes of the resonant frequency and the dissipation factor.

A measurement was initiated by switching the liquid on a freshly modified gelatin surface from pH 6.7 water to a solution of TPP, protein, dextran, conjugate, or the complex. The solvent effect of the sample on the frequency and dissipation responses can be removed by using the same solvent as a reference.[47,48] The tendency of the frequency and dissipation change is quite uniform in different overtones, and therefore both Δf and ΔD values were recorded in the ninth overtone ($n = 9$) in this study. All QCM-D measurements were performed at 25 ± 0.02 °C at least two times. TPP concentration was 1 mg/mL, protein concentration was 1 mg/mL, dextran concentration was 10 mg/mL, and conjugate concentration was 11 mg/mL in the QCM-D measurements.

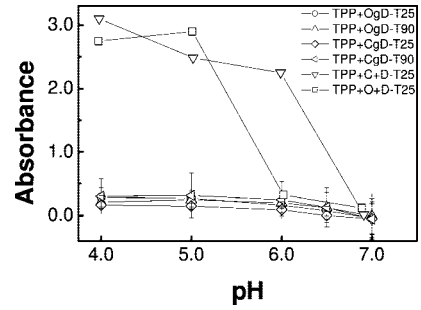

Figure 1. Absorbance of the mixture solutions of TPP with CgD, OgD, casein and dextran (C + D), or OVA and dextran (O + D) at pH range of 4−7 with and without a 90 °C heat treatment.

Results and Discussion

Interactions of TPP with CgD, OgD, and Dextran. The original pH value of the TPP solution we studied is about 3.9. Considering TPP stability, in this paper, we only investigated the interactions of TPP with the proteins/dextran at acidic pH. Figure 1 shows 500 nm absorbance of the TPP solutions containing the protein/dextran or the conjugates at pH 4−7. The mixture of TPP and dextran is clear, but precipitates form when mixing TPP with casein or OVA at pH around 5, which is about the pI of casein and OVA; i.e., the proteins carry about zero net charges. The precipitation also happens in the physical mixture of TPP and dextran and the proteins. This result is consistent with the report of Freitas et al.[18] Their study demonstrates that dextran is not effective to induce a solubilization of polyphenol−BSA complexes. The data in Figure 1 show that the precipitation can be suppressed and dispersible complexes form in the mixtures of TPP with CgD or OgD; precipitates do not form even after heating the mixtures at 90 °C for 30 min. These phenomena suggest that the highly hydrated dextran covalently conjugated to the proteins stabilizes the complexes.

The total phenolic content of TPP was analyzed with the Folin−Ciocalteu method.[42] Our analysis reveals that the TPP solutions are quite stable. The pH 4 TPP solution decreases only 2%, 5%, and 6%, while pH 6.7 TPP solution decreases 2%, 6%, and 10% of the total phenolic content after 2, 5, and 10 days of storage, respectively. After a heat treatment at 90 °C for 30 min, the total phenolic content of the TPP solution decreases 6.5% at pH 4 and 9.9% at pH 6.7. These results may not conflict with the report of Zhu et al.[49] Their HPLC analysis shows the green tea catechins degrade rapidly at pH 6.5 or higher. It is possible that the degraded polyphenols also contain phenolic groups that can react with the Folin−Ciocalteu reagent, and therefore the total phenolic content does not decrease very much. This speculation implies that the degraded polyphenols in intestine (pH 4.8−8.2) and blood (pH 7.35−7.45) may still have phenolic groups and, therefore, may still have bioactivity.

Table 1 shows the percent of the polyphenols bound with CgD, OgD, dextran, casein, and OVA, based on the analysis of total phenolic content. The polyphenols bound with the conjugates or dextran were obtained by subtracting the free polyphenols in

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Table 1. Percent of Polyphenols Bound with CgD, OgD, Dextran, Casein, and OVA in the Conditions of pH 4.0 and 6.7 with and without a Heat Treatment at 90 °C for 30 min

heat treatment	pH	bound polyphenols (%) CgD	OgD	dextran	casein[a]	OVA[b]
no	4.0	48.8 ± 4.3	34.4 ± 0.4	16.7 ± 5.9	32.1	17.7
	6.7	51.9 ± 6.8	34.6 ± 5.5	22.5 ± 0.4	29.4	12.1
yes	4.0	30.6 ± 2.7	28.0 ± 2.1	3.5 ± 5.9	27.1	24.5
	6.7	37.1 ± 0.6	24.4 ± 5.2	8.0 ± 4.4	29.1	16.4

[a] Obtained from the difference between CgD and dextran bound polyphenols. [b] Obtained from the difference between OgD and dextran bound polyphenols.

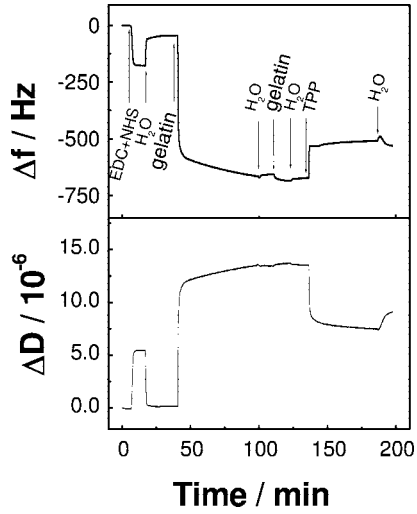

Figure 2. Time course of the changes of frequency (Δ*f*) and dissipation (Δ*D*) during the process of activation, gelatin conjugation, and TPP binding on the gelatin layer.

the ultrafiltrate from the polyphenols in feed in the mixture. Dextran shows an affinity for the polyphenols. However, considering that the weight ratio of dextran to protein in the solutions is 10:1, the binding ability of dextran to the polyphenols is about 1 order of magnitude lower than the ability of the proteins. The binding ability of dextran with the polyphenols decreases significantly after heating the mixture at 90 °C for 30 min. The reason is unknown at this stage. The data in Table 1 indicate that casein has a stronger binding ability with polyphenols than does OVA. As mentioned above, casein is linear amphiphilic proteins[32] that can interact with polyphenols through hydrophobic interaction,[11] whereas OVA is a well-folded globular protein whose hydrophobic residues are embedded. After a heat treatment at 90 °C for 30 min, OVA denatures and its hydrophobic residues are exposed to the surface,[34] which leads to an increase of hydrophobic interaction between OVA and the polyphenols. So the binding ability of OVA with the polyphenols increases after the heat treatment.

Gelatin Modification on the Surface of Quartz Crystal Sensor. Figure 2 shows the changes of frequency (Δ*f*) and dissipation (Δ*D*) during the processes of activation and gelatin conjugation on the sensor surface. NHS and EDC were used to activate the carboxyl groups and promote the formation of amide bonds between the carboxyl on the surface and amine of the gelatin. The value of Δ*f* decreases and Δ*D* increases markedly after NHS and EDC loading; but they almost come back to the baseline after rinsing with water. This phenomenon indicates that the activation does not significantly change the mass and the conformation of the molecules on the sensor surface. The changes of Δ*f* and Δ*D* before rinsing may attribute to the difference of

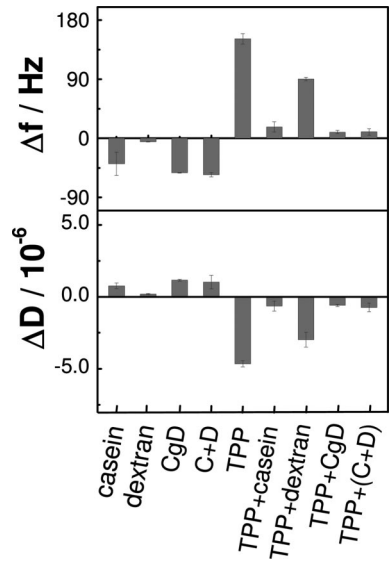

Figure 3. Changes of frequency (Δ*f*) and dissipation (Δ*D*) after separately loading TPP, casein, dextran, CgD, the mixture of casein and dextran (C+D), and TPP complexes at pH 6.7 on the gelatin surface of the sensor, followed by rinsing with pH 6.7 water for 10 min.

the density and viscosity between the solution and water,[47,48] as well as the physical adsorption of NHS and EDC. The subsequent loading of gelatin aqueous solution of pH 6.7 results in a significant decrease of the frequency and an increase of the dissipation, which implies the binding of gelatin and the formation of a loose layer on the surface. After 1 h of binding reaction, the surface was rinsed with pH 6.7 water. Then, the loading of gelatin solution and rinsing process was repeated. The slight changes of Δ*f* and Δ*D* suggest that almost all activated sites are coupled with gelatin.

Interaction of TPP or the Complexes with the Gelatin Surface. After the formation of gelatin layer on the sensor surface, the analytes, i.e., TPP, dextran, proteins, conjugates, mixtures of dextran and proteins, as well as the TPP complexes, were separately loaded on the gelatin surface to mimic their interactions with the salivary proteins. The loading experiments were performed at pH 6.7 where all of the solutions were homogeneous. In the loading, the gelatin-modified sensor surface was exposed to a solution containing the analyte for about 40 min to ensure a complete adsorption and binding. Then, the sensor was exposed to a flowing water of pH 6.7 for 10 min to eliminate the difference of the density and viscosity in the different solutions as well as physical adsorption.

Figure 3 shows the changes of Δ*f* and Δ*D* after separately loading TPP, casein, dextran, CgD, the mixture of casein and dextran, and TPP complexes on the gelatin surface. The Δ*f* value decreases and the Δ*D* value increases after loading casein solution and rinsing, indicating a binding of casein on the gelatin layer. At pH 6.7, both gelatin and casein carry net negative charges, but the asymmetric charge distribution on the protein surface can cause electrostatic attraction.[50] Furthermore, casein proteins are linear amphiphilic molecules[32] that can interact with gelatin through hydrophobic interaction. Therefore, casein can be bound significantly on the gelatin surface even after rinsing with water for 10 min. In the case of dextran, the values of Δ*f* and Δ*D* are very small, suggesting few dextran molecules were bound on the gelatin surface after washing. Dextran is a family of 1→6-α-D-glucans. Unlike casein, the electrostatic and hydrophobic interactions between dextran and gelatin are not significant. The

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changes of Δf and ΔD caused by CgD or the physical mixture of casein and dextran are similar to the sum of the changes caused by individual casein and dextran, which implies that the cooperative adsorption or obstructive adsorption of casein and dextran on the gelatin surface do not exist significantly.

Gelatin is a proline-rich protein with an open coil conformation.[21,40] Polyphenols can strongly associate with gelatin through hydrogen bonding and hydrophobic interaction.[25] Figure 3 shows Δf value increases and ΔD value decreases remarkably after loading TPP solution on the gelatin surface and then rinsing with pH 6.7 water for 10 min. These results indicate a reduction of the mass and the thickness of the gelatin layer, i.e., a release of the water molecules bound to gelatin originally and a collapse of the gelatin layer, which is very similar to the sensation of dryness and constriction in the buccal cavity caused by polyphenols.[13−15] When TPP solution was induced into the gelatin layer, the formation of gelatin−polyphenol complex aggregates results in a more compact sensor surface. In the TPP loading process, the decrease of mass from the dehydration exceeds the increase of mass from TPP binding on the sensor surface, causing the increase of Δf and the decrease of ΔD. The responses of Δf and ΔD indicate that the astringency of TPP can be measured by QCM-D. Kaneda et al. reported that the gelatin immobilized quartz crystal sensors specifically responded to quercitrin and tannic acid.[29] The frequency decreased when the gelatin sensor was exposed to quercitrin or tannic acid solution, which is different from our result that the frequency increased when the gelatin sensor was exposed to TPP solution. As they did not use dissipation to investigate the structure of gelatin, we speculate three factors that may cause the difference between their result and ours. One factor is that in their gelatin modification process most of the gelatin chains may be immobilized on the sensor surface in a multivalent mode, which produces a relatively compact gelatin layer. The second factor is that their sensor was soaked in pH 4.3 acetate buffer before loading; pH 4.3 is close to the pI of gelatin, and therefore the gelatin layer has a relatively compact structure before loading. The third is that there was no rinsing process after their sensor was exposed to quercitrin or tannic acid solution. These factors may cause that the increase of mass from quercitrin/tannic acid binding and adsorption exceeds the decrease of mass from the dehydration in their system.

In the cases of the TPP and casein mixture, the TPP and CgD mixture, as well as the TPP and casein and dextran mixture, the changes of their Δf values are much smaller than the sum of the Δf values caused by individual TPP, casein, and dextran and so are the ΔD. These results suggest that casein can effectively reduce the interaction between TPP and gelatin and, therefore, can effectively decrease the dehydration of the gelatin layer and effectively decrease the changes of Δf and ΔD. In other words, casein can effectively decrease the sensation of astringency caused by TPP. In the case of the TPP and dextran mixture, the data in Table 1 show casein and dextran having somewhat similar polyphenol binding amounts at the concentrations investigated. However, Figure 3 reveals that dextran is much less effective to decrease the interaction between TPP and gelatin. This result may be explained by the binding difference of casein and dextran on the gelatin layer. Two factors may decrease the interaction of TPP with the gelatin layer. One is the decrease of free polyphenols in the mixture because of the formation of TPP−casein and TPP−dextran complexes. The other is the binding of casein and dextran on the gelatin layer. The stronger binding of casein on the gelatin may block the binding of the free polyphenols with the gelatin more effectively. To verify this speculation, we loaded casein solution on the gelatin surface first and then loaded pH 6.7 water, TPP solution, and pH 6.7 water on casein-bound gelatin surface in succession. Compared with TPP binding on the gelatin surface, the Δf values caused by TPP decrease about 24% on the casein-bound gelatin surface, which confirms that casein bound on gelatin surface can significantly decrease TPP interaction with gelatin.

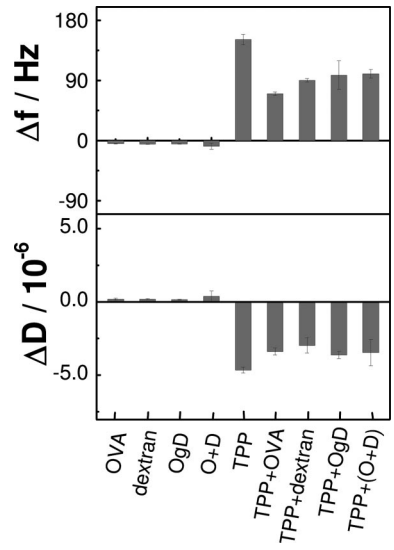

Figure 4. Changes of frequency (Δf) and dissipation (ΔD) after separately loading TPP, OVA, dextran, OgD, the mixture of OVA and dextran (O + D), and TPP complexes at pH 6.7 on the gelatin surface of the sensor, followed by rinsing with pH 6.7 water for 10 min.

Figure 4 shows the changes of Δf and ΔD after separately loading TPP, OVA, dextran, OgD, the mixture of OVA and dextran, and TPP complexes on the gelatin surface. Unlike casein, OVA is a well-folded protein and with fewer proline residues. As shown in Table 1 and Figure 4, OVA cannot effectively bind TPP at pH 6.7 and cannot be effectively bound on the gelatin surface through hydrophobic interaction. Therefore, it is reasonable that OVA, OgD, and the mixture of OVA and dextran have much less influence on the interaction of TPP with gelatin layer compared to casein. That is to say, OVA can decrease the sensation of astringency caused by TPP, but the effect of OVA is less compared to that of casein.

Conclusions

Casein-*g*-dextran (CgD) and ovalbumin-*g*-dextran (OgD) grafting conjugates were prepared by the Maillard reaction. On average, 2.3 ± 0.3 dextran molecules were conjugated to casein and 2.5 ± 0.6 dextran molecules were conjugated to ovalbumin (OVA). Turbidity measurement shows the conjugates form dispersible complexes with tea polyphenols (TPP) in the pH range of 4.0−7.0 even after a heat treatment at 90 °C for 30 min. The binding ability of TPP with the conjugates and dextran was characterized by ultrafiltration separation and the Folin−Ciocalteu method. The affinity of TPP for the proteins is about 1 order of magnitude higher than the affinity for dextran. Casein has a stronger binding ability with TPP than OVA because of the linear amphiphilic structure. After a heat treatment at 90 °C for 30 min, OVA denatures, which leads to an increase of hydrophobic interaction with TPP. The binding ability of dextran with TPP decreases significantly after the heat treatment.

Gelatin, a model of the salivary proteins in buccal cavity, was immobilized on a quartz crystal sensor surface through covalent bonding. By use of a quartz crystal microbalance with dissipation (QCM-D), we can detect the changes in both mass and

conformation of gelatin layer induced by TPP and the complexes of the protein/dextran–TPP. Casein can be bound significantly on the gelatin surface through hydrophobic interaction, while few dextran or OVA molecules can be bound on the gelatin surface. The binding of TPP causes a dehydration and collapse of the gelatin layer on the sensor surface, which is very similar to the sensation of dryness and constriction in the buccal cavity caused by polyphenols. The mixture of TPP and casein/ovalbumin/dextran can decrease the interaction of TPP with the gelatin by decreasing the free TPP molecules and shielding the gelatin surface from TPP. Compared to OVA and dextran, the stronger binding ability of casein on the gelatin surface results in that casein is more effective to decrease the binding of the free polyphenols on the gelatin surface. That is to say, casein is more effective to decrease the sensation of astringency caused by TPP.

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