JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Influences of Glucose on the Dietary Hydroxyflavonoid−Plasma Protein Interaction

Yali Wang,[†,‡] Yu Zhao,[†,‡] Fan Yang,[†,‡] Yongming Yuan,[§] Hui Wang,[§] and Jianbo Xiao*[,‡]

[‡]Department of Biology, College of Life and Environment Science, Shanghai Normal University, 100 Guilin Road, Shanghai 200234, People's Republic of China

[§]Shanghai Dahua Hospital, Shanghai 200237, People's Republic of China

Supporting Information

ABSTRACT: The influence of glucose on the interaction between flavonoids and plasma proteins from healthy humans (HPPs) was investigated. Glucose affected the flavonoid−protein interactions depending upon their structures. Glucose significantly reduced the affinities of HPPs for 6-hydroxyflavone by 10.72 times, slightly weakened the affinities of HPPs for quercetin, 7-hydroxyflavone, and kaempferol, and hardly affected the affinities of HPPs for myricetin, chrysin, and 3,7-dihydroxyflavone on the first day. However, glucose obviously enhanced the affinities of HPPs for 3-hydroxyflavone, luteolin, and apigenin. Glucose significantly weakened the binding affinities of HPPs for chrysin, kaempferol, quercetin, and myricetin by 6.17, 7.94, 14.12, and 112.2 times, when kept at 37 °C under air conditions for 14 days, and the binding affinities of HPPs for 7-hydroxyflavone, luteolin, 3,7-dihydroxyflavone, 3-hydroxyflavone, and 6-hydroxyflavone were slightly decreased by 1.35-, 1.58-, 1.58-, 1.9-, and 2.4-fold. The binding affinity between apigenin and HPP was hardly influenced. Glucose weakened the binding affinities of HPPs for hydroxyflavonoids. The differences between log K_a(absence) and log K_a(presence) were bigger for the more lipophilic hydroxyflavonoids, and more lipophilic hydroxyflavonoids are easily affected by glucose, when kept at 37 °C under air conditions for 14 days. These flavonoids with lower hydrogen donor/acceptor numbers prefer to stably interact with HPPs in the presence of glucose. However, other flavonoids with high hydrogen donor/acceptor numbers (multi-hydroxyl flavonoids) were apt to reduce their affinities with HPPs in the presence of glucose.

KEYWORDS: *human plasma proteins, multi-hydroxyl flavonoids, binding affinity, glucose, antioxidant*

INTRODUCTION

Polyphenols, especially flavonoids in dietary sources, have attracted great interest since the 1990s because of growing evidence of their beneficial effects on human health.[1−6] Over 10 000 flavonoids have been found from plants, and most of them always exist as β-glycosides.[7,8] The flavonoids are mainly found as 3- and 7-*O*-glycosides.[9] Flavonoid glycosides in most cases are hydrolyzed to their aglycones to produce *in vivo* effects.[10] Flavonoids in general are absorbed as their aglycones after prior hydrolysis of the glycosides along the aerodigestive tract. The structure−metabolite relationship of flavonoids in liver microsomes showed that the hydroxylation appears to be at the C-4′, C-3′, C-6, and C-8 positions on flavones, when there is a single or no hydroxy group on the B ring, and the hydroxylation appears at the 3′ and 4′ positions in flavonols.[11−14]

Diabetes is characterized as a high level of glucose (>6.10 mmol/L) in blood. The glucose can react with plasma proteins through a non-enzymatic process to form glycated hemoglobin and glycated serum albumin, which have been used to monitor the long- and short-term control of diabetes.[15]

Recently, the flavonoid−plasma protein interaction has obtained wide attention.[16−22] Flavonoids and their metabolites rapidly exchange between free and bound forms within the circulation. The reversible binding to plasma proteins may have consequences for the delivery of flavonoids and their metabolites to cells and tissues.[10] It has been suggested that the high level of glucose can influence the ability of plasma proteins to bind to small molecules.[23,24] However, how glucose influences the interaction between flavonoids and human plasma proteins (HPPs) is not clear. Herein, the influence of glucose on the interaction between flavonoids and HPPs was investigated. A total of 10 flavonoids (Figure 1) were tested.

MATERIALS AND METHODS

Apparatus and Reagents. The fluorescence spectra were recorded on a JASCO FP-6500 fluorometer (Tokyo, Japan). Chrysin (99.5%) was obtained commercially from Wako Pure Chemical Industries (Osaka, Japan). 3,7-Dihydroxyflavone, 7-hydroxyflavone, 6-hydroxyflavone, and 3-hydroxyflavone were purchased from TCI Chemical Industries (Tokyo, Japan). Apigenin, luteolin, quercetin, kaempferol, and myricetin were provided by Aladin (Shanghai, China). The working solutions of flavonoids (1.0×10^{-3} mol/L) were prepared by dissolving each flavonoid in methanol. All other reagents and solvents were of analytical grade, and all aqueous solutions were prepared using newly double-distilled water.

Collection of Plasma Protein Samples from Blood. Blood-derived serum was obtained using blood from four healthy adult volunteers. The levels of glucose and glycated hemoglobin were lower than 6.10 mmol/L and 6.3% for blood from healthy adult volunteers. The blood was taken and analyzed in Shanghai Dahua Hospital. We allowed blood to clot in glass centrifuge tubes for 2−4 h to obtain

Received: July 16, 2012
Revised: October 21, 2012
Accepted: November 19, 2012
Published: November 19, 2012

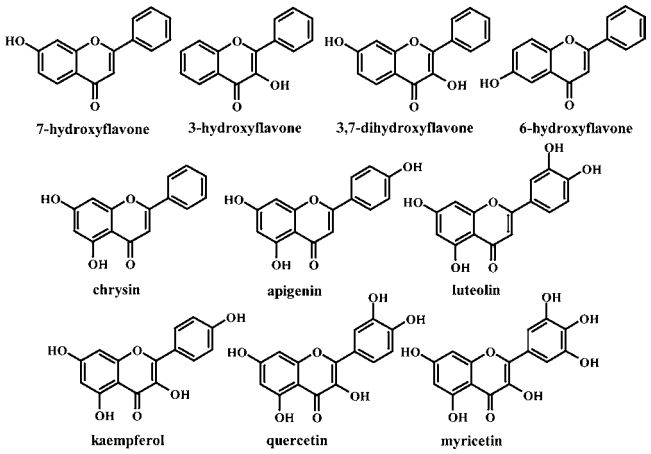

Figure 1. Chemical structures of flavones in this study.

serum. Blood-derived serum was clarified by centrifugation at 3000 rpm for 10 min to separate serum from the blood cells to obtain HPPs. The working solutions of HPPs (1:100) were prepared by directly diluting above plasma proteins with Milli-Q water. Fresh HPP (1:100) was mixed with glucose (50 mmol/L) for 0, 7, and 14 days at 37 °C.

Fluorescence Spectra. The working solutions of HPPs (1:100) were incubated with 50 mmol/L glucose at 37 °C under air conditions for 0−14 days. A total of 3.0 mL of working solution of HPPs or HPPs pre-incubated with glucose was transferred to a 1.0 cm quartz cell and then titrated with successive additions of 3.0 μL of flavonoid solution (1.0×10^{-3} mol/L). Titrations were performed manually by a trace syringe. In each titration, the fluorescence spectrum was collected with the working solution of HPPs or HPPs pre-incubated with glucose. The fluorescence spectra were recorded in the wavelength range of 310−450 nm upon excitation at 280 nm when HPPs or HPPs pre-incubated with glucose were titrated with flavonoids. Slit widths, scan speed, and excitation voltage were kept constant within each data set, and each spectrum was the average of three scans. The results of the time course experiments for the equilibration are not given here. Each fluorescence intensity determination was repeated and found to be reproducible within experimental error.

RESULTS AND DISCUSSION

Influence of Glucose on the Fluorescence Quenching of Flavonoids. The intensities of HPP fluorescence decreased remarkably with the addition of all flavonoids. Figure 2 shows the quenching effect of kaempferol on HPP fluorescence spectra in the absence and presence of glucose (data for other flavonoids were not shown here). Moreover, glucose hardly affects the HPP fluorescence spectrum (data were not shown here). When Figure 2A is compared to Figure 2B, the quenching effect on HPP fluorescence by kaempferol in the presence of glucose was obviously higher than that in the absence of glucose. The quenching percentages $[(F_0 - F)/F_0]$ of kaempferol (8 μmol/L) on HPP fluorescence spectra in the absence and presence of glucose were 46.15 and 47.95%, respectively (see Figure S1 of the Supporting Information). In the linear Stern−Volmer regression curve (see Figure S2 of the Supporting Information), the quenching constants (K_{SV}) for kaempferol in the absence and presence of glucose at 37 °C were 1.15×10^5 L/mol (R^2 = 0.9989) and 1.22×10^5 L/mol (R^2 = 0.9987), respectively, which illustrated that glucose influenced the quenching effect of flavonoids on HPP fluorescence.[25]

Binding Constants (K_a) and Number of Binding Sites (n). The binding constants were calculated according to the double-logarithm equation[26−28]

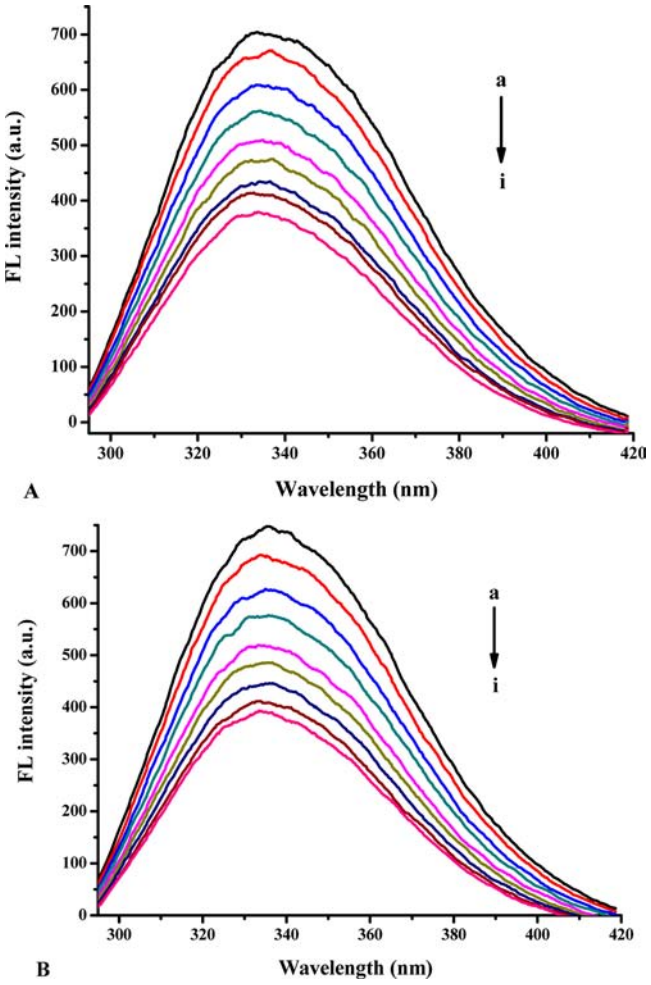

Figure 2. Quenching effect of kaempferol on HPP fluorescence intensity in the (A) absence and (B) presence of glucose (50 mM). λ_{ex} = 280 nm. (a−i) HPPs (1:100): 0.00, 1.00, 2.00, ..., 8.00 ($\times 10^{-6}$ mol/L) kaempferol.

$$\log[(F_0 - F)/F] = \log K_a + n \log[Q] \quad (1)$$

where F_0 and F represent the fluorescence intensities of HPPs or HPPs pre-incubated with glucose in the absence and presence of flavonoids, K_a is the binding constant, n is the number of binding sites, and $[Q]$ is the concentration of flavonoids. According to eq 1, the values of "$(F_0 - F)/F$" can be obtained in each "$[Q]$". Then, the linear regression equation between the "$\log[(F_0 - F)/F]$" values and "$\log [Q]$" values was obtained on the Origin 7.5 software. The slope factor means "n", and the intercept refers to "$\log K_a$".

The K_a and n values were summarized in Figure 3. The values of $\log K_a$ are proportional to n (Figure 3), which indicates that eq 1 used here is suitable to study the interaction between flavonoids and HPPs.[26−28] The linear regression equations using the Origin 7.5 software were n = 0.17301 + 0.16975 $\log K_a$ (R^2 = 0.96693; absence) and n = 0.14225 + 0.17555 $\log K_a$ (R^2 = 0.7361; presence). As seen from these data, the relationship of n−$\log K_a$ for HPPs in the absence of glucose exhibited almost the same model for HPPs in the presence of glucose.

Effect of Glucose on the Binding Affinities of HPPs for Flavonoids. As shown in Figure 4a, glucose obviously influenced the binding affinities of HPPs for flavonoids.

dx.doi.org/10.1021/jf303094e | J. Agric. Food Chem. 2012, 60, 12116−12121

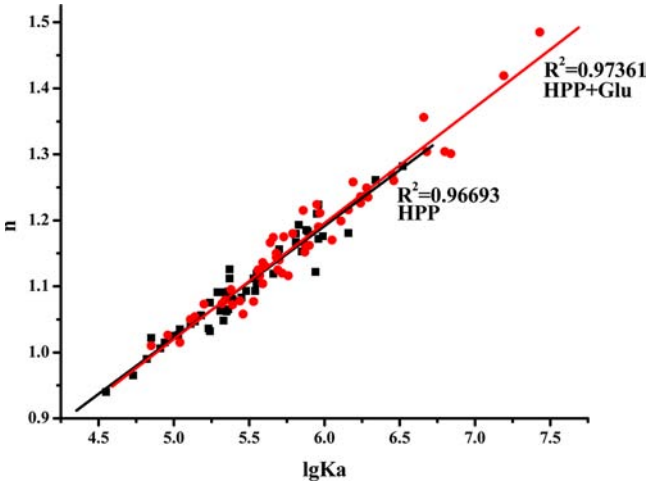

Figure 3. Relationship between the affinities (log K_a) and the number of binding sites (n) between flavonoids and HPPs in the absence and presence of glucose.

Glucose significantly reduced the binding affinities of HPPs for 6-hydroxyflavone by 10.72 times and slightly weakened the affinities of HPPs for quercetin, 7-hydroxyflavone, and kaempferol on the first day (Figure 4b). Moreover, glucose hardly affected the affinities of HPPs for myricetin, chrysin, and 3,7-dihydroxyflavone. However, glucose obviously enhanced the affinities of HPPs for 3-hydroxyflavone, luteolin, and apigenin (Figure 4b).

Time Course of the Flavonoid−HPP Interaction in the Presence of Glucose. The binding affinities of HPPs in the presence of glucose for flavonoids kept at 37 °C under air conditions from 1 to 14 days were determined (Figure 5). The

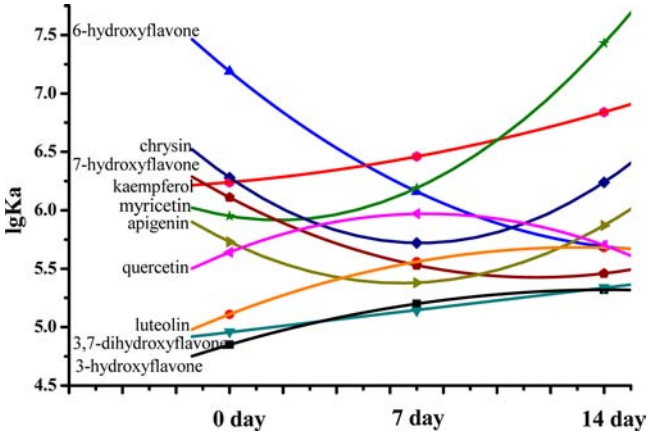

Figure 5. Time course of glucose influencing the flavonoid−HPP interaction.

binding affinities of HPPs for chrysin, apigenin, and quercetin were changed irregularly with an increasing incubation time. The binding affinities of HPPs for 3,7-dihydroxyflavone and 3-hydroxyflavone were significantly increased. The binding affinities of HPPs for kaempferol, myricetin, and luteolin

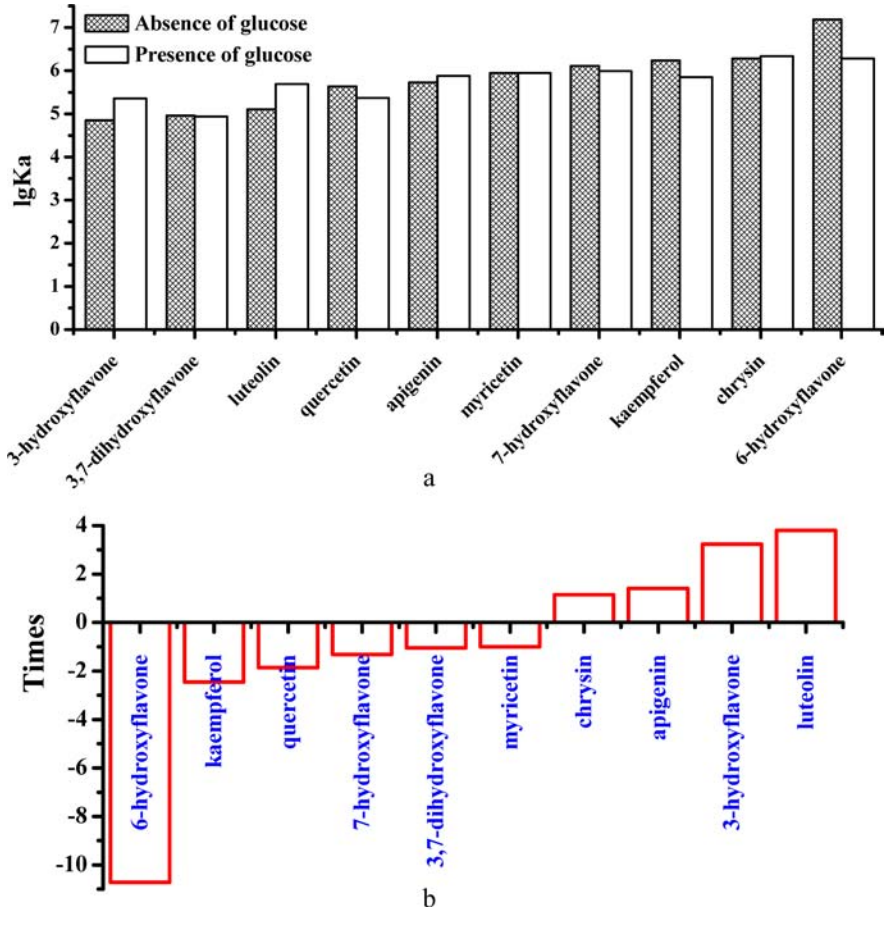

Figure 4. Glucose influences the affinities of HPPs for flavonoids on the first day.

were obviously improved when incubated from 1 to 14 days. However, the binding affinities of HPPs for 7-dihydroxyflavone and 3-hydroxyflavone were decreased with an increasing incubation time. As seen from Figure 5, with an increasing incubated time with glucose under air conditions (from 1 to 14 days), the binding affinities of HPPs for multi-hydroxyl flavoniods on ring B were obviously higher than those of non- or monohydroxyl flavones on ring B. The binding affinities of HPPs for flavonols after incubated 14 days with glucose were determined as myricetin > kaempferol > quercetin. We have reported that the hydroxylation on ring B of flavones increased the binding constants and the number of binding sites between flavonoids and serum albumins.[29]

Influence of Glucose on the Affinities of Flavonoids for HPPs Incubated for 14 Days. As shown in Figure 8, glucose significantly weakens the binding affinities of HPPs for chrysin, kaempferol, quercetin, and myricetin by 6.17, 7.94, 14.12, and 112.2 times, respectively, when kept at 37 °C under air conditions for 14 days. With an increasing incubation time at 37 °C under air conditions, the binding affinities of HPPs for 7-hydroxyflavone, luteolin, 3,7-dihydroxyflavone, 3-hydroxyflavone, and 6-hydroxyflavone were slightly decreased by 1.35-, 1.58-, 1.58-, 1.9-, and 2.4-fold, respectively. However, the binding affinity between apigenin and HPP was hardly affected by incubation with glucose at 37 °C under air conditions for 14 days.

As seen from Figure 6, after incubation with glucose at 37 °C under air conditions for 14 days, the affinities of HPPs for non-

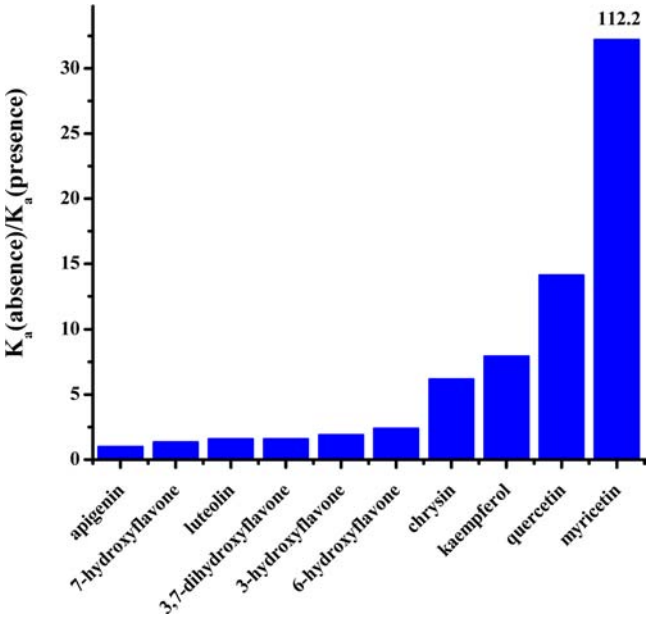

Figure 6. Glucose obviously reduces the binding affinities of HPPs for hydroxyflavonoids when kept at 37 °C under air conditions for 14 days.

or monohydroxyl flavonoids on ring B were slightly affected and the affinities of HPPs for multi-hydroxyl flavonoids on ring B prefer to be influenced. Moreover, flavonols were more easily affected, which illustrated that the hydroxyl moiety on the 3 position plays an important role when bound to HPPs in the presence of glucose. The reduced degree of HPP–flavonoid affinities were determined as myricetin > quercetin > kaempferol > chrysin ≫ 3-hydroxyflavone > 3,7-dihydroxyflavone > luteolin > 7-hydroxyflavone > apigenin.

Nature of Glucose Influencing HPP–Flavonoid Interactions. Human serum albumin (HSA) in HPP is the major target interacting with glucose in blood. Structural changes associated with the exposure of HSA to glucose were reported by Coussons et al.[30] Barzegar et al. found that partial denaturation in the structural integrity of HSA was caused by glycation at lower (1 mg/mL) and higher (5 mg/mL) concentrations of glucose.[31] Moreover, L-Trp has a lower affinity for the glycated form than for non-glycated HSA. The secondary structure of advanced glycation end product-modified human serum albumin (AGE-HSA) derived from glucose at 20 mmol/L contains higher α-helical content and elicits maximum expression of the receptor.[32]

The non-covalent interaction between small molecules and proteins is usually caused by four major interaction forces, namely, hydrogen-bonding force, van der Waals force, hydrophobic interaction, and electrostatic interaction. The nature of glucose affecting the flavonoid–HPP interactions was studied by investigating the molecular property–affinity relationship. The lipophilicity of flavones under study was assessed by their partition coefficient values (X log P_3) according to the PubChem public chemical database. There is a linear relationship between the X log P_3 values and log K_a values (R = 0.61246) of HPPs for flavonoids in the absence of glucose (Figure 7). Flavonoids with higher X log P_3 show lower

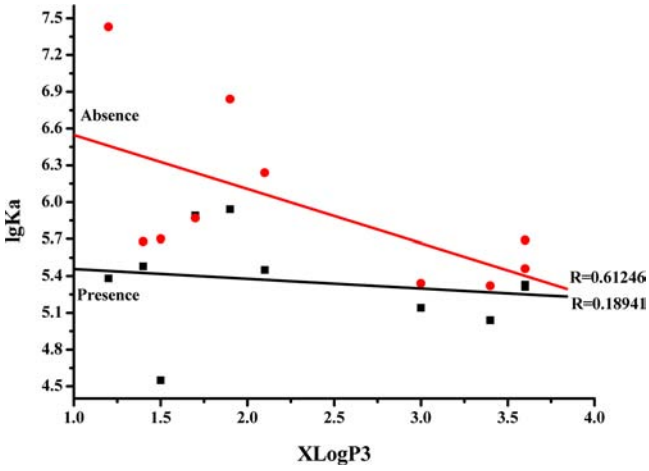

Figure 7. Relationship between X log P_3 values and log K_a values of the flavonoid–HPP complex when incubated with glucose at 37 °C under air conditions for 14 days.

affinities with HPPs. However, there is no relationship between the X log P_3 values and log K_a values (R = 0.18941) in the presence of glucose.

Moreover, hydroxyflavonoids with higher X log P_3 appear to have higher log K_a(absence)/log K_a(presence) values (Figure 8) of the flavonoid–HPP system when incubated with glucose at 37 °C under air conditions for 14 days, which illustrated that the differences between log K_a(absence) and log K_a(presence) were bigger for the more lipophilic hydroxyflavonoids and more lipophilic hydroxyflavonoids are easily affected by glucose.

To further investigate whether or not the hydrogen donor or acceptor of flavonoids plays an important role on the flavoniod–HPP interaction, the relationships of the hydrogen-bond donor/acceptor numbers (data were from PubChem

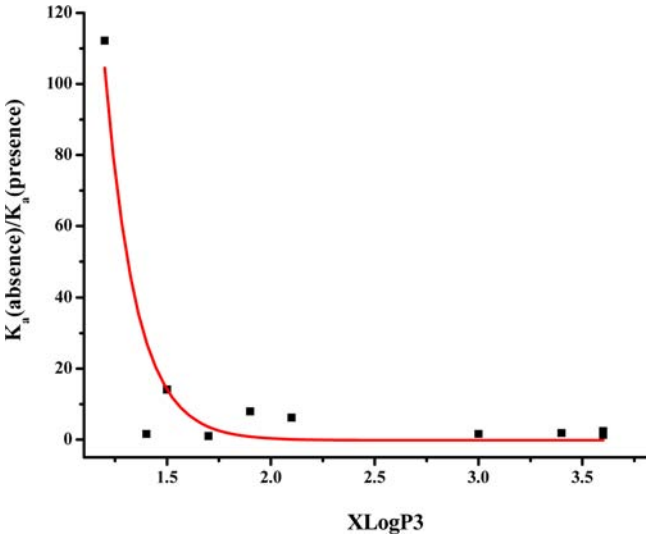

Figure 8. Relationship between X log P_3 values and K_a(absence)/K_a(presence) values of the flavonoid−HPP complex when incubated with glucose at 37 °C under air conditions for 14 days.

public chemical database) with K_a(absence)/K_a(presence) of the flavonoid−HPP system when incubated with glucose at 37 °C under air conditions for 14 days were studied. The K_a(absence)/K_a(presence) values of the flavonoid−HPP system increased with increasing the numbers of hydrogen-bond donor/acceptor numbers of flavoniods (Figure 9). These

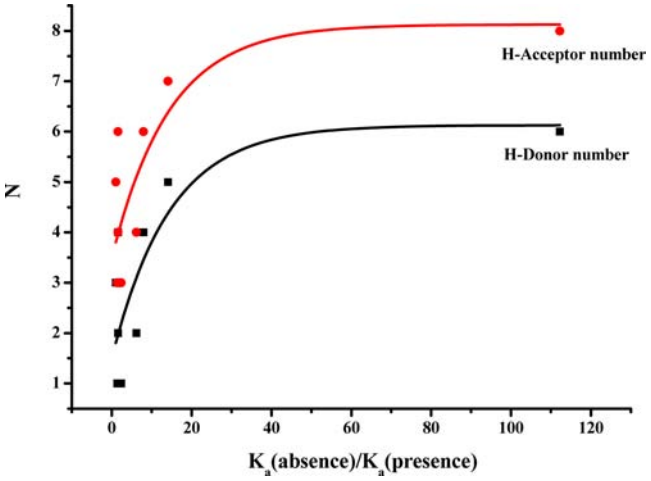

Figure 9. Relationship between hydrogen-bond donor numbers and log K_a values of the flavonoid−HPP complex when incubated with glucose at 37 °C under air conditions for 14 days.

flavonoids with lower hydrogen-bond donor/acceptor numbers prefer to stably interact with HPPs in the presence of glucose. However, other flavonoids with high hydrogen-bond donor/acceptor numbers (multi-hydroxyl) are apt to reduce their affinities with HPPs in the presence of glucose. These results indicate that multi-hydroxyflavonoids act as the donor/acceptor of hydrogen bonds for HPPs in the presence of glucose.

Glucose significantly weakened the binding affinities of HPPs for hydroxyflavonoids, especially for multi-hydroxyflavonoids, when incubated with glucose at 37 °C under air conditions for 14 days. The possible mechanism was shown in Figure 10, which illustrated that glucose competed to bind to plasma

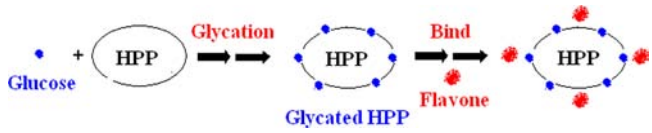

Figure 10. Glucose influences the interaction between HPPs with hydroxyflavonoids.

proteins with hydroxyflavonoids. With an increasing incubation time, the glycation of plasma proteins happened and returns to influence the binding affinities.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Tryptophan fluorescence quenching of HPPs (1:100) plotted as extinction of HPP tryptophans $[(F_0 - F)/F_0, \%]$ against concentration for kaempferol in the absence and presence of glucose, with fluorescence emission intensity recorded at λ_{ex} = 280 nm and λ_{em} = 335.6 nm (Figure S1) and Stem−Volmer curves of fluorescence quenching of HPPs by kaempferol in the absence and presence of glucose at 37 °C (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Telephone: +86-13611600163. Fax: +86-21-64321291. E-mail: jianboxiao@yahoo.com.

Author Contributions

†These authors contributed equally to this work.

Funding

The authors are grateful for financial support sponsored by the Shanghai Rising-Star Program (11QA1404700), the Shanghai Science and Technology Development Project (11440502300), the "China−African University 20+20" project by the China Ministry of Education, and the Program of Shanghai Normal University (SK201240).

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Bohin, M. C.; Vincken, J. P.; van der Hijden, H. T. W. M.; Gruppen, H. Efficacy of food proteins as carriers for flavonoids. *J. Agric. Food Chem.* **2012**, *60*, 4136−4143.

(2) Williamson, G.; Dionisi, F.; Renouf, M. Flavanols from green tea and phenolic acids from coffee: Critical quantitative evaluation of the pharmacokinetic data in humans after consumption of single doses of beverages. *Mol. Nutr. Food Res.* **2011**, *55*, 864−873.

(3) Yuan, J. M. Green tea and prevention of esophageal and lung cancers. *Mol. Nutr. Food Res.* **2011**, *55*, 886−904.

(4) Fletcher, J. N.; Kinghorn, A. D.; Slack, J. P.; McCluskey, T. S.; Odley, A.; Jia, Z. H. In vitro evaluation of flavonoids from *Eriodictyon californicum* for antagonist activity against the bitterness receptor hTAS2R31. *J. Agric. Food Chem.* **2011**, *59*, 13117−13121.

(5) Hostetler, G.; Riedl, K.; Cardenas, H.; Diosa-Toro, M.; Arango, D.; Schwartz, S.; Doseff, A. I. Flavone deglycosylation increases their anti-inflammatory activity and absorption. *Mol. Nutr. Food Res.* **2012**, *56*, 558−569.

(6) Claussnitzer, M.; Skurk, T.; Hauner, H.; Daniel, H.; Rist, M. J. Effect of flavonoids on basal and insulin-stimulated 2-deoxyglucose uptake in adipocytes. *Mol. Nutr. Food Res.* **2011**, *55*, S26−S34.

(7) Day, A. J.; DuPont, M. S.; Ridley, S.; Rhodes, M.; Rhodes, M. J. C.; Williamson, G.. Deglycosylation of favonoid and isofavonoid

glycosides by human small intestine and liver L-glucosidase activity. *FEBS Lett.* **1998**, *436*, 71−75.
(8) Xiao, J. B.; Cao, H.; Wang, Y. F.; Zhao, J. Y.; Wei, X. L. Glycosylation of dietary flavonoids decreases the affinities for plasma protein. *J. Agric. Food Chem.* **2009**, *57*, 6642−6648.
(9) Fossen, T.; Pedersen, A. T.; Anderson, Ø. M. Flavonoids from red onion (*Allium cepa*). *Phytochemistry* **1998**, *47*, 281−285.
(10) Xiao, J. B.; Kai, G. Y. A review of dietary polyphenol-plasma protein interactions: Characterization, influence on the bioactivity, and structure−affinity relationship. *Crit. Rev. Food Sci. Nutr.* **2012**, *52*, 85−101.
(11) Breinholt, V. M.; Offord, E. A.; Brouwer, C.; Nielsen, S. E.; Brøen, K.; Friedberg, T. *In vitro* investigation of cytochrome P450-mediated metabolism of dietary flavonoids. *Food Chem. Toxicol.* **2002**, *40*, 609−616.
(12) Gradolatto, A.; Canivenc-Lavier, M. C.; Basly, J. P.; Siess, M. H.; Teyssier, C. Metabolism of apigenin by rat liver phase I and phase II enzymes and by isolated perfused rat liver. *Drug Metab. Dispos.* **2004**, *32*, 58−65.
(13) Boersma, M. G.; van der Woude, H.; Bogaards, J.; Boeren, S.; Vervoort, J.; Cnubben, N. H.; van Iersel, M. L.; van Bladeren, P. J.; Rietjens, I. M. Regioselectivity of phase II metabolism of luteolin and quercetin by UDP-glucuronosyl transferases. *Chem. Res. Toxicol.* **2002**, *15*, 662−670.
(14) Cai, H.; Boocock, D. J.; Steward, W. P.; Gescher, A. J. Tissue distribution in mice and metabolism in murine and human liver of apigenin and tricin, flavones with putative cancer chemopreventive properties. *Cancer Chemother. Pharmacol.* **2007**, *60*, 257−266.
(15) Nakajou, K.; Watanabe, H.; Kragh-Hansen, U.; Maruyama, T.; Otagiri, M. The effect of glycation on the structure, function and biological fate of human serum albumin as revealed by recombinant mutants. *Biochim. Biophys. Acta* **2003**, *1623*, 88−97.
(16) Feroz, S. R.; Mohamad, S. B.; Bujang, N.; Malek, S. N. A.; Tayyab, S. Multispectroscopic and molecular modeling approach to investigate the interaction of flavokawain B with human serum albumin. *J. Agric. Food Chem.* **2012**, *60*, 5899−5908.
(17) Xiao, J. B.; Chen, T. T.; Cao, H.; Chen, L. S.; Yang, F. Molecular property−affinity relationship of flavanoids and flavonoids for human serum albumin *in vitro*. *Mol. Nutr. Food Res.* **2011**, *55*, 310−317.
(18) Kurlbaum, M.; Hoegger, P. Plasma protein binding of polyphenols from maritime pine bark extract (USP). *J. Pharm. Biomed. Anal.* **2011**, *54*, 127−132.
(19) Pan, X. R.; Qin, P. F.; Liu, R. T.; Wang, J. Characterizing the interaction between tartrazine and two serum albumins by a hybrid spectroscopic approach. *J. Agric. Food Chem.* **2011**, *59*, 6650−6656.
(20) Xiao, J. B.; Wu, M. X.; Kai, G. Y.; Wang, F. J.; Cao, H.; Yu, X. B. ZnO−ZnS QDs interfacial heterostructure for drug/food delivery application: Enhancement of the binding affinities of flavonoid aglycones to bovine serum albumin. *Nanomedicine* **2011**, *7*, 850−858.
(21) Zhang, G. W.; Wang, L.; Pan, J. H. Probing the binding of the flavonoid diosmetin to human serum albumin by multispectroscopic techniques. *J. Agric. Food Chem.* **2012**, *60*, 2721−2729.
(22) Xiao, J. B.; Zhao, Y. R.; Wang, H.; Yuan, Y. M.; Yang, F.; Zhang, C.; Yamamoto, K. Non-covalent interaction of dietary polyphenols with common human plasma proteins. *J. Agric. Food Chem.* **2011**, *59*, 10747−10754.
(23) Xiao, J. B.; Zhao, Y. R.; Wang, H.; Yuan, Y. M.; Yang, F.; Zhang, C.; Kai, G. Y. Non-covalent interaction of dietary polyphenols with total plasma proteins of type II diabetes: Molecular structure/property−affinity relationships. *Integr. Biol.* **2011**, *3*, 1087−1094.
(24) Xie, Y. X.; Xiao, J. B.; Kai, G. Y.; Chen, X. Q. Glycation of plasma proteins in type II diabetes lowers the non-covalent interaction affinities for dietary polyphenols. *Integr. Biol* **2012**, *4*, 502−507.
(25) Wei, X. L.; Xiao, J. B.; Wang, Y. F.; Bai, Y. L. Which model based on fluorescence quenching is suitable to study the interaction between *trans*-resveratrol and BSA? *Spectrochim. Acta, Part A* **2010**, *75*, 299−304.
(26) Xiao, J. B.; Cao, H.; Wang, Y. F.; Yamamoto, K.; Wei, X. L. Structure−affinity relationship of flavones on binding to serum albumins: Effect of hydroxyl groups on ring A. *Mol. Nutr. Food Res.* **2010**, *54*, S253−S260.
(27) Xiao, J. B.; Kai, G. Y.; Yang, F.; Liu, C. X.; Xu, X. C.; Yamamoto, K. Molecular structure−affinity relationship of natural polyphenols for bovine γ-globulin. *Mol. Nutr. Food Res.* **2011**, *55*, S86−S92.
(28) Xiao, J. B.; Mao, F. F.; Yang, F.; Zhao, Y. L.; Zhang, C.; Yamamoto, K. Interaction of dietary polyphenols with bovine milk proteins: Molecular structure−affinity relationship and influencing bioactivity aspects. *Mol. Nutr. Food Res.* **2011**, *55*, 1637−1645.
(29) Xiao, J. B.; Suzuki, M.; Jiang, X. Y.; Chen, X. Q.; Yamamoto, K.; Xu, M. Influence of B-ring hydroxylation on interactions of flavonols with bovine serum albumin. *J. Agric. Food Chem.* **2008**, *56*, 2350−2356.
(30) Coussons, P. J.; Jacoby, J.; McKay, A.; Kelly, S. M.; Price, N. C.; Hunt, J. V. Glucose modification of human serum albumin: A structural study. *Free Radical Biol. Med.* **1997**, *22*, 1217−1227.
(31) Barzegar, A.; Moosavi-Movahedi, A. A.; Sattarahmady, N.; Hosseinpour-Faizi, M. A.; Aminbakhsh, M.; Ahmad, F.; Saboury, A. A.; Ganjali, M. R.; Norouzi, P. Spectroscopic studies of the effects of glycation of human serum albumin on L-Trp binding. *Protein Pept. Lett.* **2007**, *14*, 13−18.
(32) Bala, K.; Gomes, J.; Gohil, N. K. Interaction of glycated human serum albumin with endothelial cells in a hemodynamic environment: Structural and functional correlates. *Mol. Biosyst.* **2011**, *7*, 3036−3041.