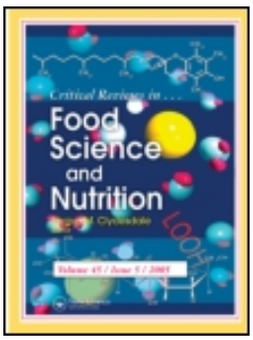

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A Review of Dietary Polyphenol-Plasma Protein Interactions: Characterization, Influence on the Bioactivity, and Structure-Affinity Relationship

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The interactions between polyphenols, especially flavonoids and plasma proteins, have attracted great interest among researchers. Few papers, however, have focused on the structure-affinity relationship of polyphenols on their affinities for plasma proteins. The aim of this review is to give an overview of the research reports on the characterization, influence on the bioactivity, and the structure-affinity relationship for studying the affinities between polyphenols and plasma proteins. The molecular properties that influence the affinities of polyphenols for plasma proteins are the following: 1) One or more hydroxyl groups in the B-ring (e.g., 3′,4′ dihydroxylated B ring catechol group) of flavonoids enhanced the binding affinities to proteins. However, the hydroxyl group in the C-ring will weaken the binding interaction. 2) The presence of an unsaturated 2,3-bond in conjugation with a 4-carbonyl group, characteristic of flavonols structure, has been associated with stronger binding affinity with plasma proteins; 3) The glycosylation of flavonoids decreases the affinities for plasma proteins by 1–3 orders of magnitude depending on the conjugation site and the class of sugar moiety; 4) The methylation of hydroxyl groups in flavonoids slightly enhanced the affinities for plasma proteins by 2–16 times; 5) The galloylated catechins have higher binding affinities for plasma proteins than do non-galloylated catechins and the pyrogallol-type catechins have higher affinities than do the catechol-type catechins. The affinity of the catechin with 2,3-trans structure was lower than those of the catechin with 2,3-cis structure; 6) The gallotannins with more gallol groups presented a much higher percentage of binding to plasma proteins. α-D-Gallotannin showed a greater affinity for plasma proteins than does the natural stereoisomer, β-D-gallotannin; 7) The binding degree of chlorogenic acid with only one caffeoyl group was lower than the binding degrees of caffeoyl quinic acids with more caffeoyl groups. The methylation of phenolic acid decreased the affinity for BSA.

Keywords polyphenols, plasma proteins, structure-affinity relationship, characterization

INTRODUCTION

Polyphenols are the most abundant antioxidants in human diet and are the most common and widespread constituents in plants (D'Archivio et al., 2007; Yang et al., 2008). They are considered to be secondary metabolites and have no specific metabolic function in plant cells (Bennick, 2002). Polyphenols contain at least one aromatic ring with one or more hydroxyl groups in addition to other substituents, and they can be divided into 15 major classes according to their chemical structures (Harborne, 1964). Among polyphenols are the compounds with one C_6 aromatic ring of hydroxybenzoic acids such as hydroxytyrosol, tanins, and gallic acid, those with a C_6-C_3 structure of hydroxycinnamic acids such as caffeic acid and coumaric acid, those with the C_6-C_2-C_6 structure of stilbenes such as resveratrol, those with the C_6-C_3-C_6 structure of flavonoids, and others with the C_6-C_4-C_6 structure of lignans such as secoisolariciresinol (Fig. 1).

Flavonoids are the major polyphenols present in a wide variety of plant sources (Aron and Kennedy, 2008; Krafczyk et al., 2009). Their structure is represented by a benzene ring (A), condensed with a heterocyclic six membered pyran or pyrone ring (C), which in the 2 or 3 position carries a phenyl ring (B) as a substituent (Fig. 2). Over 5,000 flavonoids have

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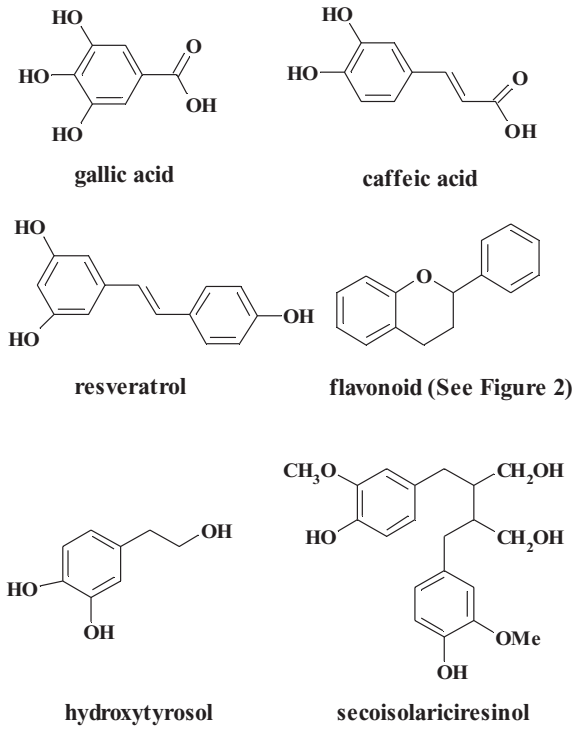

Figure 1 Chemical structures of polyphenols.

been separated and identified from plants, most of which are divided into subclasses, including anthocyanidins, flavanones, flavonols, flavones, and isoflavones (Fig. 2) (Bravo et al., 2006; Volpi and Bergonzini, 2006). Flavonols are the most widespread flavonoids in plants. The most prominent flavonols in foods are quercetin and kaempferol. Flavones are much less common than flavonols in fruits and vegetables. The prominent flavones in foods are luteolin and apigenin. Soybean and its related foods are the main sources of isoflavones in the diet. Isoflavones are naturally present in the soybean primarily in their β-glycoside forms such as genistein and daidzein (Erdman et al., 2007). The main active constituents of green tea are believed to be polyphenols, commonly known as the tea catechins. Polyphenols, especially flavonoids in plants foods, have attracted great interest since the 1990s due to growing evidence of their beneficial effect on human health (D'Archivio et al., 2010).

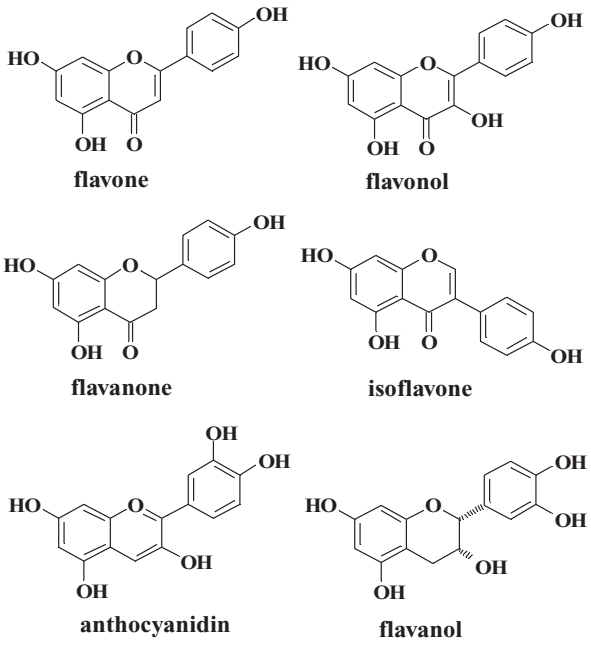

Figure 2 Chemical structures of flavonoids.

In vitro incubation of quercetin in normal human plasma showed that quercetin was extensively bound to plasma proteins (99% for concentrations up to 15 mol/L) (Boulton et al., 1998). Metabolites of quercetin are also extensively bound to plasma proteins in the plasma of rats fed with a quercetin-enriched diet (Manach et al., 1995; 2004). Albumins are the primary protein in blood responsible for the binding. The affinity of polyphenols for albumin varies according to their chemical structure. Manach et al. (2004) thought that the intrinsic affinity of circulating polyphenol conjugates for albumin might be much weaker than that of polyphenol itself. The degree of binding to albumin may have consequences for the rate of clearance of metabolites and for their delivery to cells and tissues. The conventional view is that the cellular uptake is proportional to the unbound concentration of metabolites. Yet, variations in local pH at specific sites may induce conformational changes in albumin, which lead to dissociation of the ligand-albumin complex. The interactions between polyphenols, especially flavonoids and plasma proteins, have attracted great interest among researchers. Most of the reports only focused on the binding process, such as the forces involved in the binding constant, binding distance, energy transfer, and molecular modeling. Few articles and reviews, however, have focused on the structure-affinity relationship of polyphenols on their affinities for proteins. The aim of this review is to give an overview of the characterization, influence on the bioactivity of polyphenols, and the structure-affinity relationship for studying the affinities between polyphenols and plasma proteins.

POLYPHENOL BIOLOGICAL FATES IN VIVO

The interest was stimulated mainly by epidemiological studies indicating an inverse association between the intake of foods rich in these compounds and the incidence of diseases, such as cardiovascular disease, diabetes mellitus, and cancer (see the review of their bioavailability by D'Archivio et al., 2007). The biological fates of flavonoids in herbs have been reviewed by Cao et al. (2002).

There are several factors affecting the bioavailability of polyphenols. One of the factors is the interaction with plasma proteins ((D'Archivio et al., 2007). According to the free drug hypothesis (Buxton, 2005), the polyphenol distribution within the body is generally held to be driven by the free concentration of unbound polyphenol in circulating plasma.

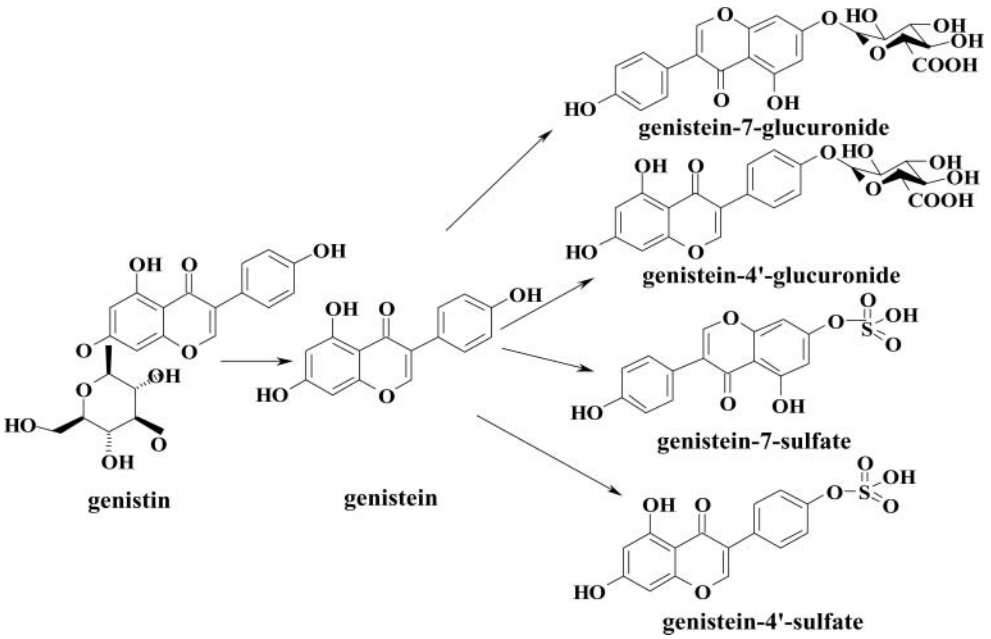

Figure 3 Structures of isoflavones and the conjugated metabolites (Hosoda et al., 2008).

Polyphenols and their metabolites rapidly exchange between free and bound forms within the circulation. The reversible binding to blood proteins, such as serum albumin, α_1-acid glycoprotein, and lipoproteins, may have consequences for the delivery of the polyphenols and their metabolites to cells and tissues. The sulfation and glucuronidation are particularly important for increasing the molecular weight and solubility of polyphenolic aglycones, which reduces their potential toxic effects and enhances their elimination ability (Day et al., 2002). According to the "free drug" hypothesis, only unbound drug exerts pharmacological activity and the disposition of drug is often altered by drug binding (Jansen, 1981; Shand et al., 1976). Prior to passage into the blood stream, the polyphenolic aglycones undergo the conjugation process to form methylation, sulfation, and glucuronidation compounds (Felgines et al., 2005). However, the methylation, glycosylation, sulfation, and glucuronidation of polyphenols in blood were unknown, but it probably depends greatly on the position of substitution. Burkon and Somoza (2008) investigated the *trans*-resveratrol (TRES) (Fig. 1) metabolites in plasma and urine by LC-MS/MS, NMR, and HPLC-DAD. The metabolites of TRES identified and quantified were TRES-3-sulfate, TRES-3,4′-disulfate, TRES-3,5-disulfate, TRES-3-glucuronide, and TRES-4′-glucuronide and upto 50% of the plasma TRES-3-sulfate, TRES-disulfates, and thenovel TRES-C/O-diglucuronides were bound to proteins. Shia et al. (2009) investigated the metabolism and pharmacokinetics of 3,3′,4′,7-tetrahydroxyflavone (fisetin), 5-hydroxyflavone, and 7-hydroxyflavone in rats and found that fisetin and 7-hydroxyflavone were rapidly and extensively transformed into their sulfate/glucuronide forms, whereas 5-hydroxyflavone was exclusively metabolized to its glucuronide. Hosoda et al. (2008) identified metabolites of daidzin and genistin in human plasma after administration of Kinako. The conjugation of the principal metabolites was at the 4′- and 7- position, such as daizein-7-glucuronide, daidzein-4′-glucuronide, genistein-7-glucuronide, genistein-4′-glucuronide, daidzein-7-sulfate, daidzein-4′-sulfate, genistein-7-sulfate, and genistein-4′-sulfate (Fig. 3).

In addition to other components, the human blood or plasma contains proteins (P) such as human serum albumin (HSA), a_1-acid glycoprotein (AAG), lipoproteins, and globulins (Table 1). Polyphenols (O) in plasma are bound to these proteins (P) to some degree. As shown in Fig. 4, the polyphenol-protein interaction is reversible in that the polyphenol-protein complex (PP) can dissociate and release the free polyphenol (P_f) (Mehvar, 2005).

INFLUENCE OF PROTEIN-BOUND ON THE BIOACTIVITY OF POLYPHENOLS

Polyphenols-protein interaction is expected to modulate the bio-availability of polyphenols. The bioactivities of

Table 1 Major drugs binding proteins in human plasma (Otagiri, 2005; Smith et al., 1992)

Protein	MW (g/mol)	Concentration (μM)	Type of drugs bound
Albumin	67,000	600	acidic
α_1-Acid glycoprotein	41,000	10–30	basic, neutral
Lipoproteins	200,000–2,400,000	Varies	lipophilic basic, lipophilic neutral

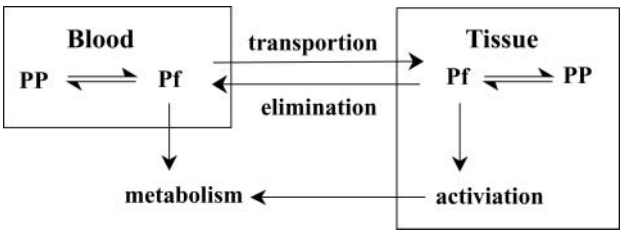

Figure 4 The pharmacokinetic model depicting the equilibrium between the free (P_f) and protein-bound polyphenol (PP) in blood and tissue. The model assumes that only the free polyphenol is subject to transport into the tissues (including the site of action).

polyphenols were often executed in complex biological systems such as in blood where various interactions take place (Otagiri, 2005; Zsila and Iwao, 2007). Smith et al. (1992) found that binding of polyphenols, such as quercetin, fisetin, myricetin, and morin, to albumin reduced their prooxidant activity. Arts et al. (2001) showed the antioxidant capacity of quercetin, rutin, and (+)-catechin in blood plasma was not additive. Arts et al. (2002) further checked the effect of the flavonoid-protein interaction on the antioxidant capacity of flavonoids with the Trolox equivalent antioxidant capacity assay. It was found that the effect of flavonoid-protein interaction on the bioavailability of flavonoids is not equivocal and the interaction of flavonoids with proteins will weaken the antioxidant capacity of the flavonoids both in products and in vivo.

The reversible and irreversible interactions of protein-polyphenol depend on the pH, the temperature, and the concentrations of protein and flavonoid (Luck et al., 1994). The biological fate of protein-polyphenol complexes in vivo is still unknown. Serafini et al. (1996) found that compared with tea consumed without milk, the antioxidant potential of the milk-tea system decreased. However, Hollman et al. (2001) showed that the addition of milk does not affect the absorption of flavonols from tea in man. The rate of the 2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonic acid) radical scavenging by procyanidin was decreased in the presence of protein because procyanidin and protein formed substantial amounts of perceptible complexes (Riedl and Hagerman, 2001). Rohn et al. (2004) showed that the quercetin-BSA interaction decreased the total antioxidant activity in comparison to an equivalent amount of free quercetin. Recently, Bae et al. (2009) indicated that EGCG is stable in human serum and HSA stabilized EGCG under aerobic condition and suggested that the antioxidant property and the binding capacity of HSA contribute to the stabilization of EGCG in human serum. Mikkelson et al. (2006) reported the biological activity of ophthalmic drugs was affected by drug-protein interaction in tissues and fluids of the eye. High concentration of protein in lacrimal fluid moved the drug solution away from the eye, which led to a considerable loss in drug activity for drugs that bind to protein.

We investigated the mechanism of the enhancing effect of (−)-epigallocatechin-3-gallate (EGCG) on huperzine A (Hup A) inhibiting acetylcholinesterase (AChE) activity in vivo by binding EGCG and Hup A to serum albumin (Xiao et al., 2008a).

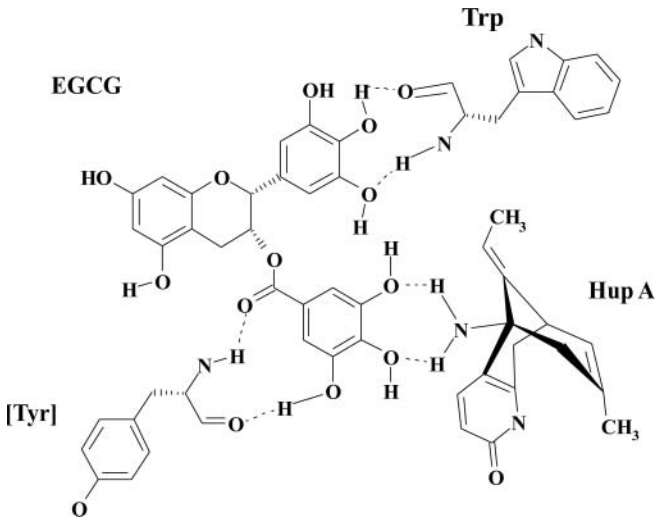

Figure 5 The suggested mechanism of enhanced effect of EGCG on Hup A inhibiting AChE (Xiao et al., 2008; Zhang et al., 2009).

Figure 5 showed the suggested mechanism of the enhanced effect of EGCG on Hup A inhibiting AChE. At first, the EGCG-BSA complex forms (Zhang et al., 2009). Hup A is suggested to be bound to the EGCG-BSA complex and locates in close proximity to the Tyr residues. The binding Hup A to the EGCG also increases the affinity of the EGCG for BSA through electron transfer. The enhanced transporting of Hup A in blood might be a cause of the enhanced effect of the EGCG on Hup A inhibiting AChE.

Takano-Ishikawa et al. (2006) reported the structure-activity relations of the inhibitory effects of various flavonoids on lipopolysaccharide-induced prostaglandin E2 production in rat peritoneal macrophages. The 50% inhibitory concentrations (IC_{50}) of 7-hydroxyflavone, chrysin, and baicalein for the inhibition of PGE2 biosynthesis were determined as follows: 7-hydroxyflavone (1 OH) > chrysin (2 OH) > baicalein (3 OH) > flavone (0 OH). In this work, we found that the IC_{50} values of flavones for the inhibition of PGE_2 biosynthesis are proportional to the binding constants with HSA (data were not shown here). Higher binding affinities with HSA are associated with lower inhibitory effect on PGE2 biosynthesis. Si et al. (2009) described the inhibition of CYP2C9 by a series of flavonoids. The apparent inhibitory constants (K_i, μM) for the inhibition of RECO® CYP2C9-mediated diclofenac 4′-hydroxylation activity by flavones were found to be proportional to the binding constants with HSA (data were not shown here). Higher binding affinities with HSA are associated with a higher inhibitory constant. Ko et al. (2003) studied the structure-activity relationships between flavonoids and their tracheal relaxant action. Flavones, flavonols, flavanones, isoflavones, and chalcones were tested. The IC_{50} values of these five classes indicated that flavones were more potent than flavonols. Flavones were also more potent than flavanones suggesting that the presence of a double bond between C-2 and C-3 is important. However, flavones showed a similar potency with isoflavones. Introduction of a

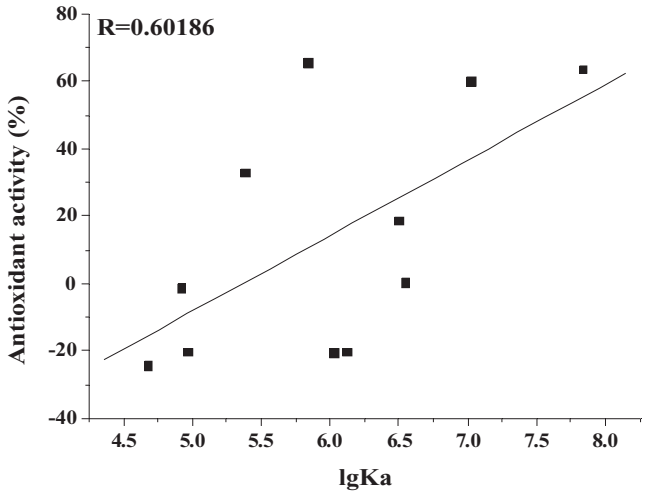

Figure 6 Relationship between binding constants and antioxidant activity of flavonoids tested. The antioxidant activities of flavonoids were from Farkas et al. (2004).

hydroxyl group at position C-6 or C-7 of flavones increases their relaxant activities. It appears that the optimal number of hydroxyl groups introduced to the A-ring of flavones is one. As more hydroxyl groups are introduced to positions at C-5, C-6, and/or C-7 of flavones, the IC_{50} values increase. These results are in accordance with the data obtained in this study. The IC_{50} values of flavones are inversely proportional to the binding constants to serum albumins. Kato et al. (2008) revealed that the presence of the 3′- and 4′-OH groups in the B-ring and the 2–3 double bond are the most important factors for inhibiting glycogen phosphorylase. Farkas et al. (2004) studied the quantitative structure–antioxidant activity relationships of flavonoids. A correlation (r = 0.60186) was found between binding constants value (lgKa) and value of antioxidant activity (%) of eleven flavonoids tested (Fig. 6). Higher affinities for serum albumins are associated with higher antioxidant activities for flavonoids. The binding of small molecules to plasma proteins is a very important parameter for drug metabolism and pharmacokinetic studies. If a molecule is highly bound to plasma proteins, the amount of drug available to diffuse into the target tissue maybe significantly reduced and the efficacy of the drug may consequently be poor. Determination of the level of binding, therefore, is critical and will directly correlate with in vivo efficacy of the molecule.

CHARACTERIZATION OF THE POLYPHENOL-PROTEIN INTERACTION

Polyphenol-protein interaction is a known phenomenon, such as polyphenols in food were bound to salivary protein to form an insoluble complex with astringent flavor (Baxter et al., 1997). Several techniques, such as the capillary electrophoresis, electrospray mass spectrum (ESI-MS), high-performance affinity chromatography (HPAC), NMR spectroscopy, fluorescence quenching, and multi-spectroscopic method (FL, RSL, IR, UV–vis, CD) have been developed to characterize the polyphenol-protein interactions. As shown in Table 2, the binding affinity, stoichiometry, kinetics, thermodynamics, and conformational changes were obtained by different methods. The multi-spectroscopic method consisting of FL, RSL, IR, UV–vis, and CD is a popular weapon to investigate the nature of polyphenol-protein interaction. Equilibrium dialysis, ultrafiltration, and ultracentrifugation are conventional separation methods to determine free drug concentration and protein-drug binding fraction in plasma. Equilibrium dialysis, ultrafiltration, and ultracentrifugation were compared by Barré et al. (1985) to determine their reliability and applicability in valproic acid-plasma proteins interaction. It was found that the results obtained from ultracentrifugation differed from those from equilibrium dialysis and ultrafiltration, which agreed reasonably well with each other. The development of an analytical apparatus has provided a number of new technologies for studying the polyphenol-protein interaction. A high-performance affinity chromatography column containing immobilized protein was useful and powerful tools in the study of drug–protein interactions. Chen et al. (2004a; 2004b) investigated the interaction between phosphated

Table 2 The characterization methods for polyphenol-protein interactions

Method	Information obtained[a]	References
CE	Ka, n, PB	El-Hady et al., 2010; He et al., 2010; Lu et al., 2008
Equilibrium dialysis	Ka, n	Barré et al., 1985
Ultrafiltration	Ka, n	Barré et al., 1985
Centrifugation	Ka, n	Barré et al., 1985; Qian et al., 2008
PCMB	K, PB	Marszałł and Buciski, 2010
ESI-MS	MW	Chen et al., 2004a, 2004b; Vergé et al., 2002
HPAC	K, Ka, PB	Chen et al., 2010; Ishii et al., 2010
NMR spectroscopy	high-affinity sites	Kenyon and Hamilton, 1994; Simard et al., 2005
x-ray crystallography	high-affinity sites	Simard et al., 2005
Raman spectroscopy	conformational changes	Fabriciova, et al., 2004a, 2004b
HPFA	Ka, n	Kimura et al., 2009
QCM	physical properties	Wang et al., 2007
Turbidimetric method	haze-forming activity	Siebert et al., 1996
Isothermal titration calorimetry	Ka, n, ΔG	Pripp et al., 2005
M-HPLC-DAD-MS	the binding degrees	Qian et al., 2008
IELC	nK	Hanai et al., 1999
DSC	Ka, n, ΔH	Ostojić et al., 2007
Fluorescence quenching	Ka, n	Xiao et al., 2007; 2007
Multi-spectroscopic method	Ka, n, r, conformational changes	Matei and Hillebrand, 2010; Mandeville et al., 2009

Ka, binding constant; n, the number of binding sites per one protein molecule; PB, the percentage binding of the compounds to proteins; association equilibrium constant, K; MW, molecular weight; BD, the binding degrees; r, binding distance; nK, drug-albumin binding affinity.

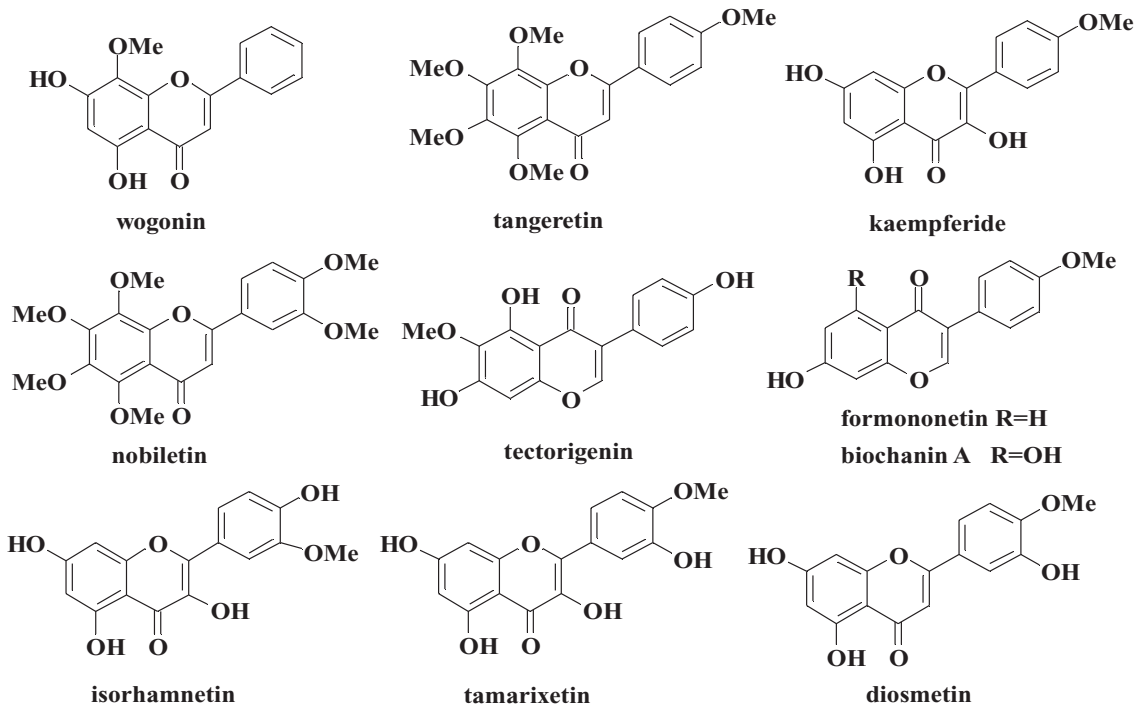

Figure 7 The structures of methyl flavonoids.

flavonoids and proteins by means of ESI-MS and found that the flavonoid-protein interactions are easier to form than the non-covalent complexes (Vergé et al., 2002). Simard et al. (2005) predict that if all binding sites were correlated with specific NMR peaks, ^{13}C NMR spectroscopic approach will become a powerful tool for solution studies of site-specific analyses of the competition between ligands for proteins. Papadopoulou and Frazier (2004) reviewed the development of capillary electrophoresis to investigate the physicochemical basis of protein–polyphenol interactions. Among these methods, the fluorescence quenching technique is an appropriate and simple tool to determine the binding constant and the number of binding sites between polyphenol and proteins. There are several reports about studies on fluorescence quenching of serum albumin induced by polyphenols.

STRUCTURE-AFFINITY RELATIONSHIP OF POLYPHENOLS FOR PLASMA PROTEINS

Methylation and Methoxylation of Flavonoids

Methylation of the free hydroxyl groups in the flavonoids dramatically increases their intestinal absorption and metabolic stability by preventing the formation of glucuronic acid and sulfate conjugates (Wen and Walle, 2006; Walle, 2009). Recently, Walle (2007a) reported that in vivo in the rat, oral administration of one methylated flavone resulted in high bioavailability and tissue distribution with no detectable levels of its unmethylated analogue and concluded that methylation appears to be a simple and effective way of increasing metabolic resistance and transport of the flavonoids (Walle, 2007a). Courts and Williamson (2009) found that flavonoid C-glycosides are methylated and glucuronidated in vivo in an intact form in humans.

Here, wogonin, nobiletin, kaempferide, formononetin, tectorigenin, biochanin A, and their original forms (chrysin, kaempferol, daidzein, genistein, tangeretin) (Fig. 7) were investigated to check the effect of methylation and methoxylation of the hydroxyl group of flavonoids on the affinities for HSA (Xiao et al., 2010). As shown in Fig. 8, the methylation and

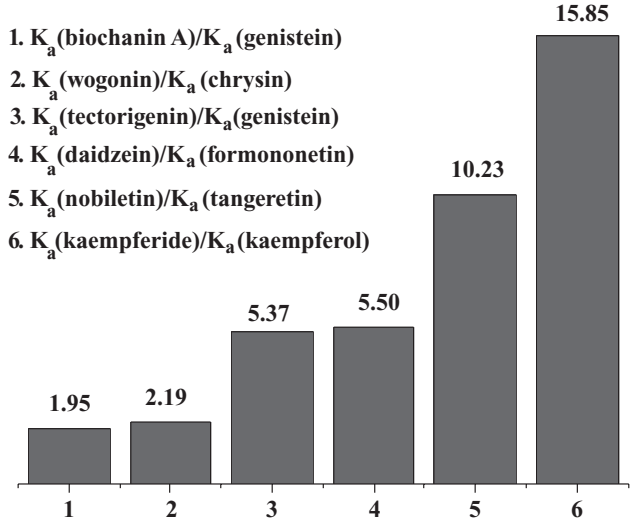

Figure 8 The methylation and methoxylation of flavonoids enhanced the affinities for HSA by 2–16 times (Xiao et al., 2010).

methoxylation of flavonoids improved the binding constants (K_a) and the number of binding sites (n) between flavonoids and HSA. In general, the methylation of flavoniods improved the affinities for proteins by 2–16 times. The affinity of kaempferide for HSA is about 16-times higher than that of kaempferol (Fig. 8). However, the affinity of biochanin A for HSA is only 2-times higher than that of genistein (Fig. 8). This result supports that the methylation of flavones enhanced the transporting ability, which leads to facilitated absorption and greatly increased bioavailability. The methylation increases the hydrophobicity of flavones and the hydrophobic interaction plays an important role in binding flavones to proteins.

The influence of methylation on the affinity for proteins was also assessed for quercetin and luteolin by Dufour and Dangles (2005). The methylation of 3′-OH (isorhamnetin) significantly lowers the affinity to BSA by 5.58 times. However, the methylation of 3′-OH (isorhamnetin) slightly increased the affinity to BSA. It showed the importance of a free OH at position 3′ for a strong binding to BSA.

The possible mechanism may be that the methylation and methoxylation of flavonoids increased the hydrophobicity and the hydrophobic interaction plays an important role in binding flavones to proteins. The methylation and methoxylation of flavonoids decreased the polarity and enhanced the capacity to penetrate into the tryptophan-rich hydrophobic regions of proteins, which are frequently buried in the interior of the folded proteins. The methylation of flavones also results in increased membrane transport, leading to facilitated absorption and greatly increased bioavailability (Walle, 2009) and results in derivatives with increased intrinsic ability to inhibit cancer cell proliferation (Walle, 2007b). An epidemiological study reported in 2006 provided strong support for the methylated flavones as potential cancer chemopreventive dietary agents (Maserejian et al., 2006). The potential therapeutic utility of the methylated flavones has also been advanced by finding that methylation reduces the possibility of toxic side-effects and confers increased solubility (Walle, 2009). The methylation of the hydroxyl group at position 4′ of flavonoid can substantially increase breast cancer resistance protein inhibition activity (Zhang et al., 2005). Recently Landis-Piwowar et al. (2008) reported that methylated flavonoids possess a different mechanism of action compared to unmethylated flavonoids for anti-cancer activity (Landis-Piwowar et al., 2008). The result in this paper supports that the methylation of flavonoids enhanced the transporting ability, which leads to facilitated absorption and greatly increased bioavailability. The methoxylation of stilbene also enhances stilbene bioactivity in *Caenorhabditis elegans* (Wilson et al., 2008).

The potential therapeutic utility of the methylated flavones has also been advanced by finding that methylation reduces the possibility of toxic side-effects and confers increased solubility (Walle, 2009). It has long been recognized that the aqueous solubility of flavonoids in general is quite low. Walle (2009) examined the effect of methylation on the aqueous solubility of flavone and found that the solubility of 5,7-dimethoxyflavone was 5-fold higher than its hydroxylation counterpart, chrysin.

Hydroxylation of Flavonoids

The presence of a C2-C3 double bond on the ring C, a dihydroxyl group (catechol-type) or three adjacent hydroxyl group (pyrogallol-type) on the ring B, and the presence of C-5, and C-7 hydroxyl group on the ring A are usually listed as requirements for antioxidant and anti-radical activity of flavonoids. The structural difference of flavonoids also strongly affects the binding process with HSA. Table 3 showed the effects of hydroxylation

Table 3 Effects of hydroxylation of flavonoids on the affinities for HSA in vitro

Class	Ring	Position	Example	Proteins	Effect	Ref
Flavone	A	7 H→OH	flavone→7ohflavone	HSA	↑	Xiao et al., 2010
		5 H→OH	7ohflavone→chrysin	HSA	↓	Xiao et al., 2010
		6 H→OH	chrysin→baicalein	HSA	No effect	Xiao et al., 2010
	B	4′ H→OH	chrysin→apigenin	HSA	↑	Xiao et al., 2010
		3′ H→OH	apigenin→luteolin	HSA	↑	Xiao et al., 2010
		3′ H→OH	apigenin→luteolin	BSA	↑	Qu et al., 2006
	C	3 H→OH	chrysin→glangin	HSA	↓	Xiao et al., 2010
			apigenin→kaempferol	HSA	↓	Xiao et al., 2010
			luteolin→quercetin	HSA	↓	Xiao et al., 2010
			luteolin→quercetin	BSA	↑	Dufour and Dangles, 2005
			diosmetin→tamarixetin	BSA/HSA	↓	Dufour and Dangles, 2005
Flavonol	A	5 H→OH	fisetin→kaempferol	HSA	↑	Xiao et al., 2010
	B	3′ H→OH	kaempferol→quercetin	HSA	↑	Xiao et al., 2010, Zhou et al., 2005
		3′ H→OH	kaempferol→quercetin	HSA	↓	Kanakis et al., 2006
		3′ H→OH	kaempferol→quercetin	BSA	↑	Dufour and Dangles, 2005
		4′ H→OH	glangin→kaempferol	HSA	No effect	Xiao et al., 2010
		5’ H→OH	quercetin→myricetin	HSA	No effect	Xiao et al., 2010
		4′ H→OH	glangin→kaempferol	BSA	↑	Xiao et al., 2008
		5’ H→OH	quercetin→myricetin	BSA	↑	Xiao et al., 2008
Isoflavone	A	5 H→OH	daidzein→genistein	HSA	↓	Xiao et al., 2010, Mahesha et al., 2006
		5 H→OH	daidzein→genistein	BSA	↑	Zhao and Ren, 2009; Xiao et al., 2009
			formononetin→biochanin A	HSA	↓	Xiao et al., 2010

of flavonoids on the affinities for HSA in vitro. As shown in Table 3, the hydroxylation on the rings A, B, and C of flavonoids significantly affected the binding affinities for HSA.

Hydroxylation on Ring A of Flavones

As shown in Table 3, the hydroxylation on position 7 of flavone significantly increased the binding affinity for HSA (Xiao et al., 2010; 2010). The affinity of 7-hydroxylflavone for HSA is about 42-times higher than that of flavone. However, the hydroxylation on position 5 of flavone weakened the binding affinity for HSA. The affinity of chrysin (5,7) for HSA is about 3-times lower than that of 7-hydroxylflavone for HSA. The hydroxylation on position 6 of flavone hardly affected the binding affinity for HSA. The affinities of chrysin (5,7) and baicalein (5,6,7) were similar. Therefore, it appears that the optimal number of hydroxyl groups introduced to the ring A of flavones is one, since the highest binding was observed with 7-hydroxyflavone containing only one hydroxyl group (Xiao et al., 2010; 2010). As more hydroxyl groups are introduced to positions at C-5, C-6, and/or C-7 of flavones, the affinities for serum albumins decreased.

Hydroxylation on Ring B of Flavones

As shown in Table 3, the apparent binding constants (K_a) between flavones and HSA increased with the increasing numbers of hydroxyl groups on the B-ring (Xiao et al., 2010). The hydroxylation on position 4′ or 3′ of flavone significantly improves the binding affinity for HSA. The affinities of apigenin (5,7,3′) and luteolin (5,7,3′,4′) for HSA were about 5.89 and 7.94-times higher than that of chrysin (5,7) and apigenin (5,7,3′). The similar results for BSA also were reported by Qu et al. (2006).

Hydroxylation on Ring C of Flavones

As shown in Table 3, the hydroxylation on the ring C of flavones decreased the binding affinities for HSA (Xiao et al., 2010). The affinities of chrysin (5,7), apigenin (5,7,3′), and luteolin (5,7,3′,4′) for HSA are about 1.38, 9.12, and 12.3-times higher than those of galangin (3,5,7), kaempferol (3,5,7,3′), and quercetin (3,5,7,3′,4′) for HSA. Dufour and Dangles (2005) also found that luteolin and diosmetin lacking the 3-OH group was bound to BSA slightly stronger than quercetin and tamarixetin respectively.

Hydroxylation on Rings A and B of Flavonols

In an earlier study we reported that the binding constants (K_a) and the number of binding sites (n) between flavonols and BSA increased with the increased hydroxyl groups on the ring B (Xiao et al., 2008). The addition of another hydroxyl group on the ring B of flavonols enhances the affinity for BSA by one order of magnitude (Xiao et al., 2008). It was found that the hydroxylation on position 3′ of flavonol significantly improves the binding affinity for HSA and the hydroxylation on positions 4′ and 5’ of flavonol hardly affected their binding affinities for HSA (Xiao et al., 2010). The affinity of quercetin (3′, 4′) for HSA is about 6-times higher than that of kaempferol (4′). However, Kanakis et al. (2006) found the spectroscopic results showed that quercetin and kaempferol are located along the polypeptide chains through H-bonding interactions with the affinity constants of 1.4×10^4 M^{-1} and 2.6×10^5 M^{-1}. The affinity of myricetin (3′, 4′, 5’) for HSA is almost the same as that of quercetin (3′, 4′) and the affinity of kaempferol (4′) for HSA is similar to that of galangin (There was no hydroxyl groups on ring B). The most prominent flavonols such as quercetin and kaempferol in foods exist with 5,7-dihydroxyl groups on ring A. Here, it was found that the hydroxylation on position 5 of flavonol slightly enhances the binding affinity for HSA by 2.39 times (Xiao et al., 2010). Zhou et al. (2005) determined binding constants between HSA with quercetin or kaempferol by the affinity capillary electrophoresis and found that the deletion of 3′-OH from quercetin to kaempferol significantly lower the affinity to HSA by 3.27 times.

The influence of deletion of 3′-OH on the affinity for proteins was assessed for quercetin and kaempferol by Dufour and Dangles (2005). According to the study by Dufour and Dangles (2005) the deletion of 3′-OH from quercetin to kaempferol significantly weakened the affinity for BSA by 11.26 times and the binding to HSA was much less affected.

Hydroxylation on Ring A of Isoflavones

As shown in Table 3, the hydroxylation on position 5 of isoflavones decreased the binding affinity for HSA. The affinities of daidzein and formononetin for HSA are about 5-times and 14-times higher than that of genistein and biochanin A (Xiao et al., 2010). Mahesha et al. measured the distance between ligands and HSA by Forster nonradioactive energy transfer (2006). It was found that genistein (3.68 nm) was much nearer to the tryptophan residues of HSA than daidzein (4.35 nm). However, Zhao and Ren (2009) reported the affinity of daidzein for BSA was about 16 times lower than does genistein for BSA. For daidzein and genistein, the binding constants for BSA were 5.2×10^4 and 8.40×10^5 L mol^{-1}, respectively (Xiao et al., 2009).

Glycosylation of Flavonoids

The dietary flavonoids in nature exist almost always as β-glycosides (Day et al., 1998). The flavonols are found mainly as the 3 and 7-O-glycoside, although the 4′ positions may also be glycosylated in some plants (Fossen et al., 1998). Other classes of flavonoids are mainly found glycosylated in the 7 position (Felgines et al., 2005). However, puerarin is an isoflavone-8-C-glucose.

Flavonoid glycosides in most cases are hydrolyzed to their aglycones to produce effects in the body (Walle et al., 2005). Flavonoids in general are absorbed as their aglycones after

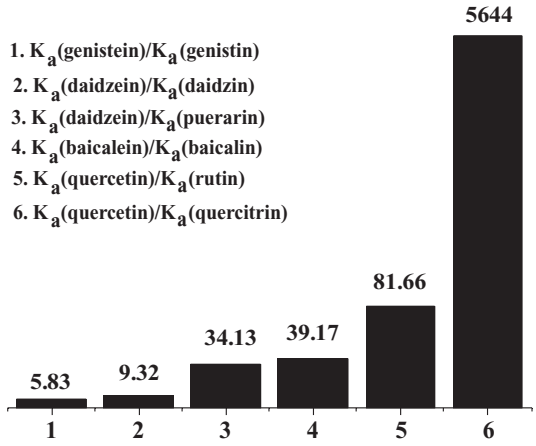

Figure 9 The glycosylation lowers the affinities of flavonoids for BSA by 1–3 orders of magnitude (Xiao et al., 2009).

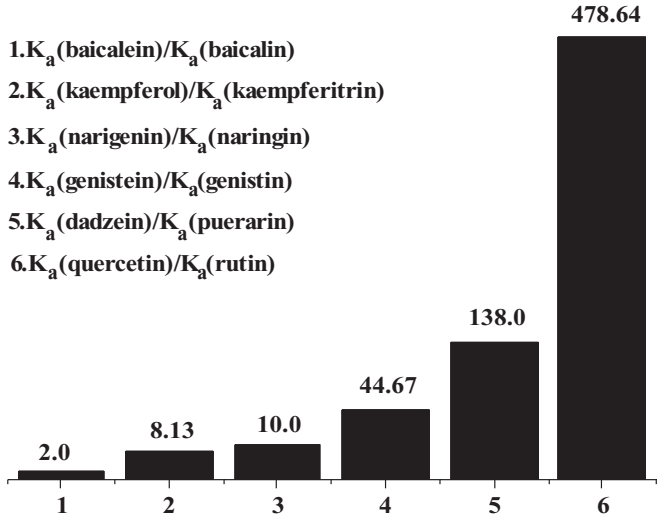

Figure 10 Glycosylation decreases the affinities of flavonoids for HSA by 1–2 orders of magnitude (Xiao et al., 2010).

prior hydrolysis of the glycosides along the aero digestive tract. Dufour and Dangles (1999; 2005) determined the binding constants between the flavonol (quercetin) and its 3-position glycosides with serum albumins and concluded that glycosylation of flavonoids could lower the affinity to albumins by one order of magnitude depending on the conjugation site. However, it is not reasonable to reach this conclusion because the structural differences between the different flavonoid classes were not taken into account. Recently, we have simply discussed the influence of glycosylation in ring A of soybean isoflavones on interaction with BSA in a short communication (Xiao et al., 2009). The sugar moieties are in 3,6,7,8-positions of flavonoids. As shown in Fig. 9, the binding affinities between flavonoids and BSA decreased after glycosylation. Glycosylation of flavonoids can lower the affinities for BSA by one to three orders of magnitude depending on the conjugation site and the class of sugar (Fig. 9). The affinity of quercetin for BSA is about 5600-fold and 82-fold higher than those of quercitrin and rutin, but the affinity of genistein for BSA is only 5 times higher than that of genistin (Xiao et al., 2009). The sugar moieties are glucopyranose, glucuronic acid, rhamnose, rutinose, and glucose-rhamnose. As shown in Fig. 9, the glucopyranosylation (daidzin and genistin) of isoflavones lowered the affinity for BSA by 5–10 times (Xiao et al., 2009). Rhamnosylation (quercitrin) of quercetin, however, lowered the affinity for BSA by 5600 times. Compared with the affinity of daidzein for BSA, the affinity of puerarin (daidzein-8-C-glucose) for BSA decreased 34 times (Xiao et al., 2009).

The effect of glycosylation on the affinities of flavonoids for HSA was investigated by our team (Xiao et al., 2010). The sugar moieties are in 3 or 7 -positions of flavonoids. As shown in Fig. 10, the glycosylation of flavonoids lowered the affinity for HSA by 1 to 2 orders of magnitude. The affinity of quercetin for HSA is about 478.64-fold higher than that of rutin, but the affinity of baicalein for HSA is only 2 times higher than that of baicalin. The glucopyranosylation (genistin) of genistein lowered the affinity for HSA by 44.67 times. Compared with the affinity of daidzein for HSA, the affinity of puerarin (daidzein-8-*C*-glucose) for HSA decreased 138 times.

Martini et al. (2008) applied NMR methodology to investigate the interaction between quercetin and quercetin 3-O-β-D-glucopyranoside with BSA. The calculated values of the affinity indexes and thermodynamic equilibrium constants suggested a much stronger capacity of quercetin to interact with BSA when compared with its glucosylated derivative.

Dangles et al. (1999) determined the binding constant and binding sites of the quercetin–BSA complex as 1.03×10^5 L/mol and 0.95 at 25°C by Scatchard analysis. In the same conditions, the binding affinities of the rutin–BSA complex and the isoquercitrin–BSA complex decreased 11.97 and 7.10 times. However, the binding sites of rutin–BSA complex and isoquercitrin–BSA complex improved to 2.66 and 3.18 times (Dangles et al., 1999). The presence of a sugar moiety on the 3-position obviously weakens the quercetin–BSA affinity and results in the rather loose binding of several flavonoid molecules to BSA. β-D-Rutinnoside and β-D-galactoside replace the proton (H) at C(3)–OH in the quercetin molecule, respectively, which results in the decreasing affinity of either rutin or hyperin for HSA (Bi et al., 2004). Diniz et al. (2008) estimated protein binding percentages for quercetin or rutin bound to HSA (PB_{HSA}), HSA + AGP ($PB_{HSA+AGP}$) and whole plasma proteins (Pb_{plasma}) by capillary electrophoresis. Quercetin presented a much higher percentage of binding to HSA (93%) than rutin (56%). However, quercetin and rutin showed similar percentage of binding to the whole plasma proteins (92%). The purified BSA and HSA are usually used as modeling proteins to investigate the interactions between flavonoids and plasma proteins. However, whole plasma proteins including albumin, α-acid glycoprotein, lipoproteins, and α, β and γ globulins play an important role in the pharmacokinetic and pharmacodynamic properties of drugs. And there are many metal ions existing in

blood which also affect the binding interaction between small molecules and proteins in blood.

Qian et al. (2008) determined the binding degrees of polyphenols in each single solution respectively, and compared with those in the Flos Lonicerae Japonicae extraction under the same conditions by microdialysis coupled with HPLC-DAD-MS. The results showed some differences of binding degrees. The binding degrees of chlorogenic acid, luteolin-7-*O*-glucoside, and 4,5-di-*O*-caffeoyl quinic acid decreased from each single solution to the extraction, which might be caused by competitive effect between constituents. However, the binding degrees of caffeic acid and rutin increased in extraction due to the synergistic effect.

The decreased affinity for plasma proteins after glycosylation may be caused by the increasing molecular size and polarity, and transfer to the non-planar structure. After the hydroxyl group is replaced by a glycoside, the steric hindrance may take place, which weakens the affinity for BSA. Another possible explanation is the glycosylation decreases the hydrophobicity of flavonoids. Many papers reported that the hydrophobic interaction plays an important role in binding small molecules to proteins.

These results give direct evidence to support the fact that the flavonoid aglycones are more easily absorbed than the flavonoid glycosides (Walle, 2004). The highly polar flavonoid glycosides cannot be absorbed after oral ingestion, but are hydrolyzed to their aglycones by bacterial enzymes in the lower part of the intestine (Walle, 2004). Quercetin-7-O-sulfate showed very strong binding affinities for HSA and BSA. However, the sulfation of 3-OH of quercetin significantly lower the affinity to BSA, the binding to HSA being much less affected. The quercetin-7,4′-O-disulfate markedly weakens the binding to both albumins (Dufour and Dangles, 2005).

The antioxidant properties of the quercetin conjugates in vivo and their binding to serum albumin were investigated by Janisch et al. (2004). In general, these compounds prolonged the lag time of copper-induced LDL oxidation in the order: quercetin-7-glucuronide > quercetin > quercetin-3-glucuronide = quercetin-3-glucoside > catechin > quercetin-4′-glucuronide > isorhamnetin-3-glucuronide > quercetin-3′-sulfate. Kq values (concentration required to achieve 50% quenching) for albumin binding, as assessed by fluorescence quenching of Trp214, were determined as follows: quercetin-3′-sulfate = quercetin ≥ quercetin-7-glucuronide > quercetin-3-glucuronide = quercetin-3-glucoside > isorhamnetin-3-glucuronide > quercetin-4′-glucuronide. The data show that flavonoid intestinal and hepatic metabolisms have profound effects on the ability to inhibit LDL oxidation and a lesser but significant effect on binding to serum albumin.

Hydrogenation of the C2 = C3 Double Bond of Flavonoids

We investigated the effect of hydrogenation of the C2 = C3 double bond in flavonoids on the affinities for HSA (Liu et al., 2010; Xiao et al., 2010). The C2 = C3 double bond in conjugation with a 4-oxo group plays a very important role in the affinity for HSA. Hydrogenation of the C2 = C3 double bond for many flavonoids decreased the binding affinity for HSA by 2–4 orders of magnitude. For myricetin and quercetin, the binding constants (Ka) for BSA were 1.84×10^8 and 3.83×10^7 L·mol^{-1}. For dihydromyricetin, the binding constant was 1.36×10^4 L·mol^{-1}, while dihydroquercetin hardly quenched the BSA intrinsic fluorescence (Xiao et al., 2010). The affinity of apigenin for HSA is about 250-times higher than that of naringenin.

Diniz et al. (2008) found that binding percentages of flavone for HSA (PB_{HSA}), HSA + AGP ($PB_{HSA+AGP}$) and whole plasma proteins (Pb_{plasma}) were almost the same as those of flavanone. It seems that the hydrogenation of the C2 = C3 double bond does not affect the affinities of flavonoids without any hydroxyl groups for proteins.

The planarity of the C ring in flavonoids may be important for binding interaction with proteins since the molecules with saturated C2-C3 bonds (flavanones and certain others) permit more twisting of the B ring with reference to the C ring. A C2 = C3 double bond increases the p-conjugation of the bond linking the B and C rings, which favors near-planarity of the two rings (Edenharder et al., 2003). The molecules with near-planar structure enter the hydrophobic pockets in proteins more easily.

Catechins

Catechins are the major polyphenols in green tea leaves. The major catechins of green tea extract are (-)-epicatechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin gallate (EGCG), and gallocatechin gallate (GCG) (Fig. 11). Recent studies have suggested that the catechins form complexes with HSA for transport in human blood. We determined the affinities between catechins and HSA by the fluorescence quenching method with a double logarithm regression curve (Xiao et al., 2010). The binding constants ($\log_{10}K_a$) between ECG, EGCG, and GCG for HSA were 4.32, 4.47, and 3.81, respectively. However, EC, EGC, and C hardly quench HSA fluorescence. We further investigated the apparent affinities of tea catechins for common rat plasma proteins (CRPP). The apparent binding constants were determined as: EGCG≈ GCG > ECG >> EC≈ EGC ≈ C. It illustrated that the galloylated catechins have higher binding affinities with HSA than non-galloylated catechins and the pyrogallol-type catechins had higher affinities than catechol-type catechins. The presence of galloyl moiety is the most decisive factor and the increasing hydroxyl groups on ring B increases the affinity for HSA.

Diniz et al. (2008) showed that polyphenols with large affinity towards HSA present a carbonyl group C = O in position C_4 indicating that this functional group could be important in the binding of the polyphenols to HSA. This fact could explain the observed relative low affinities of catechins for HSA due to the absence of this substitution in their structure.

Ishii et al. (2010) investigated the binding affinities of catechins and their analogs for HSA by high-performance

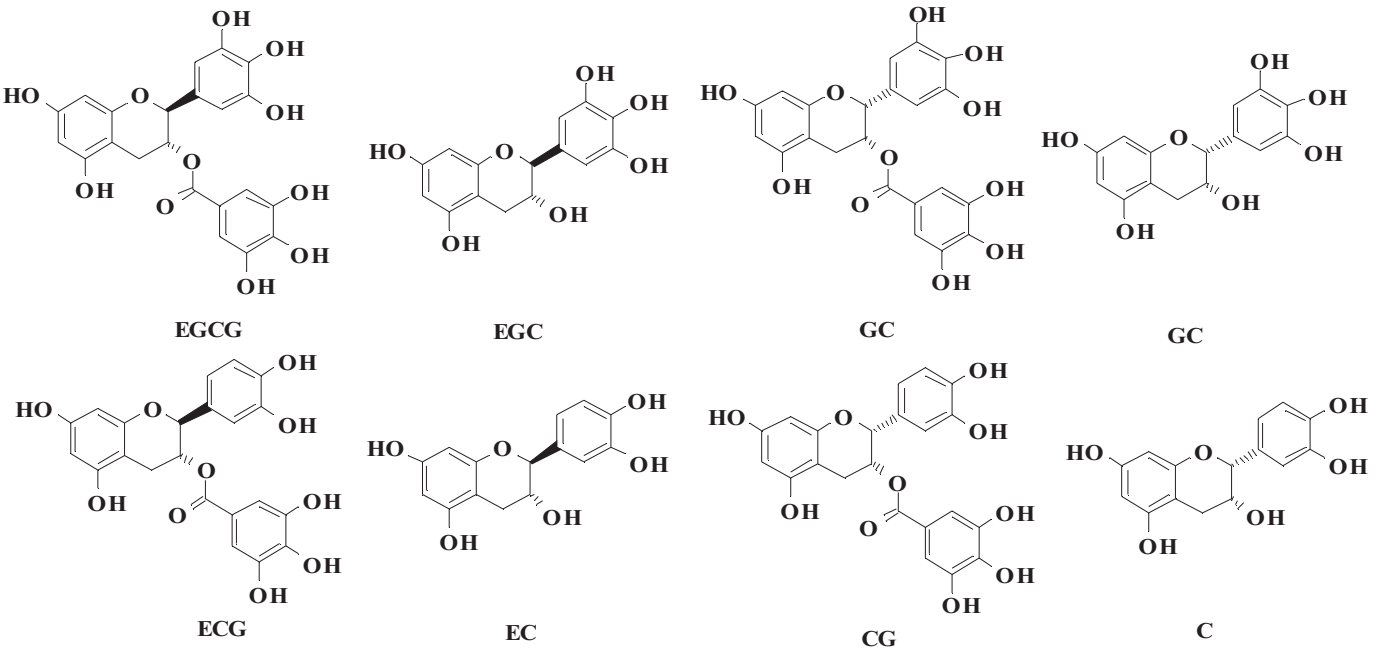

Figure 11 The structures of catechins.

affinity chromatography with an immobilized albumin column and found the similar results in some points. We found that the affinity of the catechin with 2,3-trans structure (GCG) was lower than those of the catechin with 2,3-cis structure (EGCG). However, Ishii et al. (2010) gave contrary results. They further examined the effect of methylation of hydroxyl groups on the interaction of ECCG with HSA. Diniz et al. (2008) also reported that binding percentages of C for HSA (PB_{HSA}), HSA + AGP ($PB_{HSA+AGP}$) and whole plasma proteins (Pb_{plasma}) were much higher than those of EC. Despite the positional difference of the methyl ether group between the three methylated EGCg derivatives (EGCg-3"OMe, EGCg-4"OMe, and EGCg-4′ OMe), the binding affinity value of each derivative was considerably lower than that of EGCG itself. These results indicate that the most important structural element contributing to HSA binding of tea catechins is the galloyl group, followed by the number of hydroxyl groups on the B-ring and the galloyl group or the configuration at C-2 (Ishii et al., 2010).

Hatano et al. (2003) investigated the water-soluble catechin–protein complexes by size-exclusion chromatography (SEC). The mixture of EGCG and BSA did not form a precipitate after the combination. The SEC peak size of the complex was varied with time, which suggested slow change of BSA conformation. The SEC profile varied with the combination of compounds. However, procyanidin B3 and (+)-C did not cause changes in the SEC profile of BSA.

Nozaki et al. (2009) studied the interaction of catechin metabolites for HSA with circular dichroism. Protocatechuic acid from EGC, together with its methylated compounds vanillic and isovanillic acids, were assigned to be bound to sites I and II of HSA, based on the competitive relationships with site-I-binding phenylbutazone and site-II binding diazepam.

Almajano et al. (2007) found albumin caused a synergistic increasing antioxidant activity of green tea catechins in oil-in-water emulsions. The results showed that the green tea catechins had moderate antioxidant activity in the emulsions containing BSA with the order of stability being ECG≈EGCG > EC > EGC.

Tannins

The most widely studied condensed tannins are based on the EC and C (Fig. 12). Frazier et al. (2010) compared the binding affinities of tea tannins and condensed tannins with proteins. The

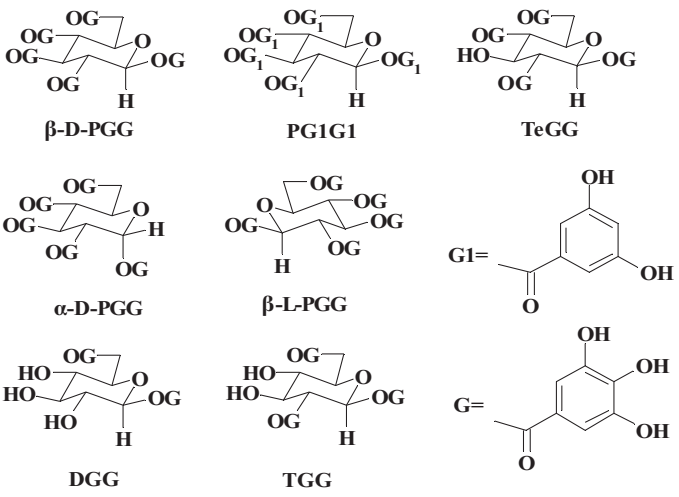

Figure 12 The structural model of gallotannins.

equilibrium binding constants of grape seed proanthocyanidins for BSA was 43 times higher than that of EGCG.

Gallotannins are naturally occurring polyphenols, which primarily consist of a glucose core esterified with gallic acid (GA) or its derivatives. The antioxidative and antibacterial activities of gallotannins have been described in several reports (Salah et al., 1995; Scalbert, 1991). The antioxidative capacities of gallotannins on lipid peroxidation were determined as: PGG > TeGG > TGG > DiGG > GA, which showed good prospects for use in the pharmaceutical industry (Kimura et al., 1984). The

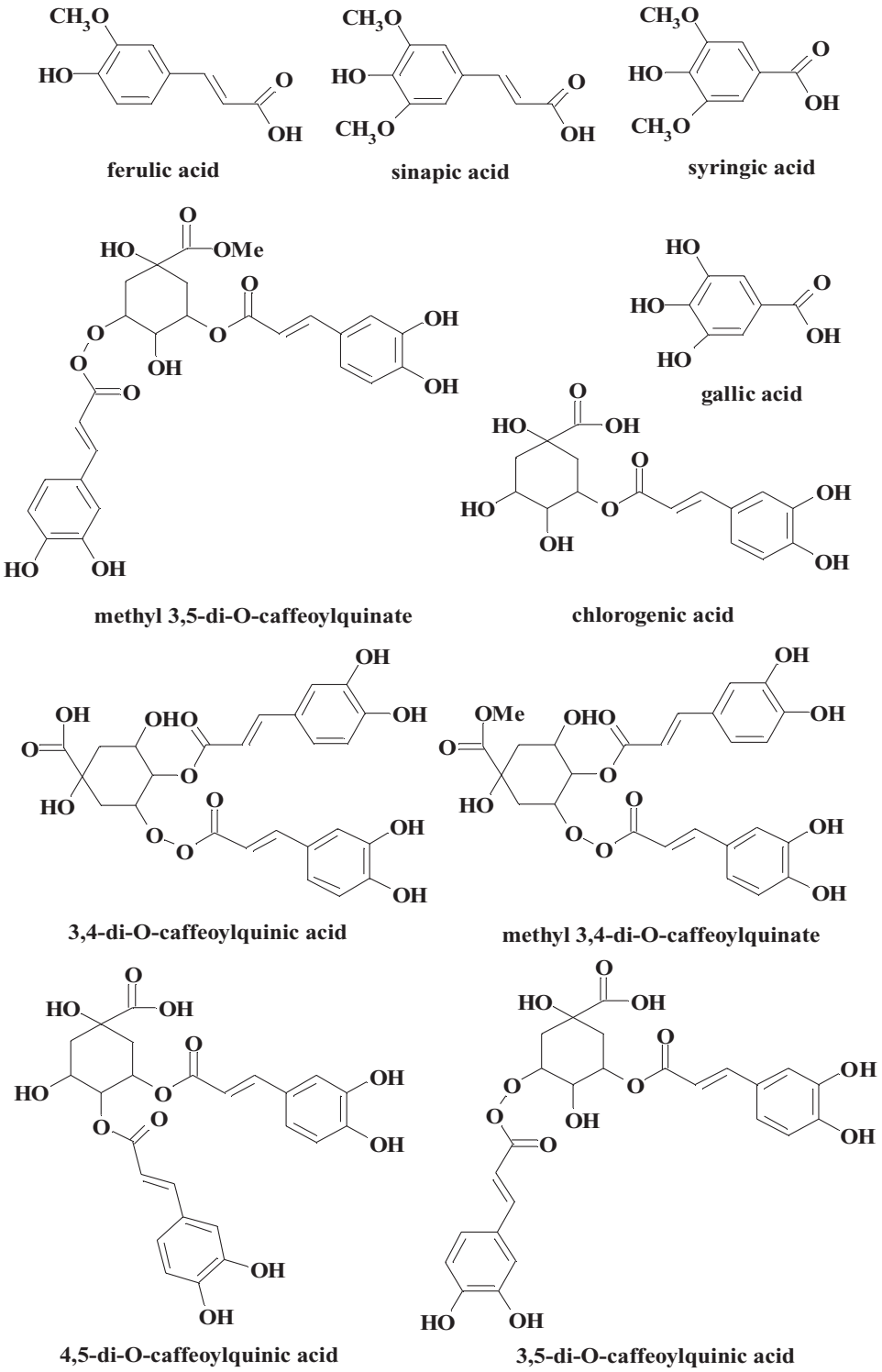

Figure 13 The structures of phenolic acids and caffeoylquinic acids.

structural basis for association between hydrolyzable tannins and select proteins has been probed in detail (Feldman et al., 1999). Feldman et al. (1999) and He et al. (2006) determined the binding affinities of gallotannin analogs with BSA.

The binding affinities of gallotannins to BSA were calculated based on the amount of gallotannin bound to BSA, expressed as percentage of the bound gallotannin. PGG presented a much higher percentage of binding to BSA (80%) than TGG (59%). PGG has more phenolic residues and can form more hydrogen bonds with proteins, which resulted in higher binding affinity to BSA than does TGG (He et al., 2006).

The free energy of transfer for the unnatural enantiomer β-L-PGG is indistinguishable from that of the natural stereoisomer β-D-PGG. The chiral "receptor" BSA has similar affinity for enantiomers of β-PGG, reinforcing the notion that a precise "lock-and-key" fit between tannin and protein is not required for complexation. α-D-PGG, a diastereomer of β-D-PGG showed a greater affinity for BSA than does the natural stereoisomer. β-D-PG1G1 exhibited a higher affinity for BSA than does β-D-PGG (Feldman et al., 1999).

Hydroxycinnamic Acids and Phenolic Acids

The typical hydroxycinnamic acids are caffeic acid, chlorogenic acid, and caffeoyl quinic acids (Fig. 13). Qian et al. (2008; 2008) analyzed the binding interaction of ferulic acid, chlorogenic acid, and caffeoyl quinic acids in Flos Lonicerae Japonicae with BSA by microdialysis coupled with HPLC-DAD-MS. The binding degrees of these compounds with BSA were defined as the following equation:

$$\text{Binding degree} = (C_b - C_a)/C_b$$

where C_b and C_a are the concentrations of each compound before and after the interaction with the BSA determined with HPLC.

The binding degree of chlorogenic acid with only one caffeoyl group was lower than the binding degrees of 3,5-di-O-caffeoyl quinic acid, 4,5-di-O-caffeoyl quinic acid, and 3,4-di-O-caffeoyl quinic acid, which had two caffeoyl groups (Qian et al., 2008; 2008). The result was in agreement with the previous reports (Xiang et al., 2001; Yoshimoto et al., 2002), which showed that the caffeoyl was the active group of caffeoyl quinic acid derivatives and it could increase the activity of this type of compound (Qian et al., 2008; 2008). The binding degrees of chlorogenic acid and 4,5-Di-O-caffeoyl quinic acid were 31.1% and 78.2% in each single solution while 22.6% and 66.8% in the extraction. The decrease might be caused by competitive effect between constituents (Qian et al., 2008).

Tang et al. (2008) investigated the interaction of caffeoyl quinic acid derivatives with BSA. The results showed that there were binding affinities for caffeoyl quinic acids with BSA, and the binding constants ranked in the following order: methyl 3,4-di-O-caffeoylquinate > methyl 3,5-di-O-caffeoylquinate > 3,4-di-O-caffeoyl quinic acid > 3,5-di-O-caffeoyl quinic acid > chlorogenic acid, under the physiological conditions. These results suggested that the numbers and the substituted positions of the caffeoyl group as well as the esterification of carboxyl group in the molecular structures appeared to contribute moderate effects to the interaction processes. In addition, once the carboxyl groups are being esterified, the binding affinities became stronger, which suggested that the esterification of the carboxyl group also affects the interaction processes. These results were in agreement with the conclusions of previous structure–activity relationship investigations by other researchers (Maruta et al., 1995). It was proved that the binding mechanism of the protein–ligands could be used as a mode to elucidate the biological and pharmacological properties of the ligands (Saito et al., 2005). Riihimäki et al. (2008) reported that the methylation of caffeic acid and gallic acid obviously improved their affinities for bovine and reindeer β-lactoglobulin.

Pulla Reddy et al. (1999) compared the binding constant of curcumin with HSA ($2.0 \times 10^5\ M^{-1}$) with that of caffeic acid ($2.8 \times 10^4\ M^{-1}$) and showed that the hydroxyl groups play a major role in binding hydrophenol to HSA. Curcumin may also react with the amino acids of proteins through its reactive carbonyl and phenolic hydroxyl groups. Gossypol binds to albumin with an association constant of $2.2 \times 10^6\ M^{-1}$ (Appu Rao, 1992).

CONCLUSIONS AND PERSPECTIVES

Some of the structural elements that influence the affinities of polyphenols for plasma proteins are the following: 1) One or more hydroxyl groups in the B-ring (e.g., 3′,4′ dihydroxylated B ring catechol group) of flavonoids enhanced the binding affinities to proteins. However, the hydroxyl group in the C-ring will weaken the binding interaction. The hydroxyl groups in the A-ring do not obviously affect the binding process to plasma proteins; 2) the presence or absence of an unsaturated 2,3-bond in conjugation with a 4-carbonyl group, characteristic of the structure of flavonals, has been associated with a stronger binding affinity with plasma proteins; 3) glycosylation of flavonoids decreases the affinities for plasma proteins by 1–3 orders of magnitude depending on the conjugation site and the class of sugar moiety; 4) methylation and methoxylation of flavonoids slightly enhanced the affinities for plasma proteins by 2–16 times; 5) the galloylated catechins have higher binding affinities for plasma proteins than do non-galloylated catechins and the pyrogallol-type catechins have higher affinities than do catechol-type catechins. The affinity of the catechin with 2,3-*trans* structure was lower than those of the catechin with 2,3-*cis* structure; 6) gallotannins with more gallol groups presenting a much higher percentage of binding to plasma proteins. α-D-Gallotannin showed a greater affinity for plasma proteins than does the natural stereoisomer, β-D-gallotannin; 7) the binding degree of chlorogenic acid with only one caffeoyl group was lower than the binding degrees of caffeoyl quinic acids with more caffeoyl groups. The methylation of phenolic acid

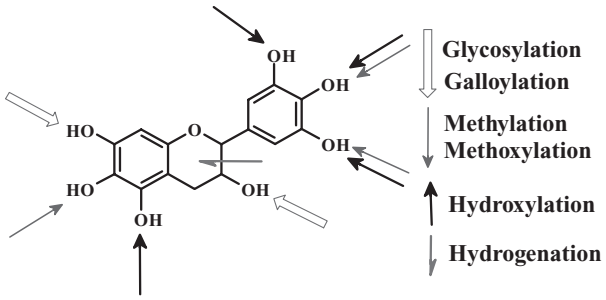

Figure 14 The potential sites of the polyphenols affecting the binding affinity for plasma proteins are schematically illustrated. The up arrows represent increasing the binding affinity; the down arrows represent decreasing the binding affinity.

decreased the affinity for BSA. The typical structure properties of the polyphenols affecting the affinity for plasma protein are shown in Fig. 14.

Polyphenol-protein interaction in blood is a complicated issue. There are many small molecules, such as metal ions, glucose, fatty acids, and metabolites in blood. Recently, it was found that the metal ions such as Al^{3+}, Zn^{2+}, Cu^{2+}, and Fe^{3+} significantly affect the interaction of several flavonoids for albumin in vitro (Mahesha et al., 2006; Cao et al., 2010; Tian et al., 2006). The concentrations of metal ions in normal human blood and patient blood are different from each other. The detailed reports on the difference between polyphenol-protein interaction in normal human blood and patient blood were few till now. Recently, the toxic interactions of serum albumins with nanoparticles were reported for the application of their drug nanocarriers (Xiao et al., 2010). Few reports, however, have focused on the effects of QDs nanocarriers on the interaction between small molecules and plasma proteins. Further work will focus on the following aspects: 1) Effects of metal ions and glucose in serum on the interaction between polyphenols and normal human/patient plasma proteins in vitro and in vivo; 2) Effect of nanoparticles in serum on the interaction between polyphenols and normal human/patient plasma proteins in vitro and in vivo.

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