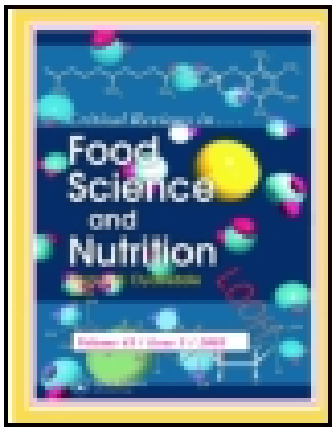

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Is There Consistency between the Binding Affinity and Inhibitory Potential of Natural Polyphenols as α-amylase Inhibitors?

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Is there consistency between the binding affinity and inhibitory potential of natural polyphenols as α-amylase inhibitors?

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Abstract

The inhibitory potential of natural polyphenols for Ŭ-amylases has attracted great interests among researchers. The structure-affinity properties of natural polyphenols binding to Ŭ-amylase and structure-activity relationship of dietary polyphenols inhibiting Ŭ-amylase were deeply investigated. There is lack of consistency between the structure-affinity relationship and the structure-activity relationship of natural polyphenols as Ŭ-amylase inhibitors. Is it consistent between the binding affinity and inhibitory potential of natural polyphenols as with Ŭ-amylase inhibitors? It was found that the consistency between the binding affinity and inhibitory potential

of natural polyphenols as with Ŭ-amylase inhibitors is not equivocal. For example, there is not consistency between the binding affinity and inhibitory potential of quercetin and its glycosides as Ŭ-amylase inhibitors. However, catechins with higher Ŭ-amylase inhibitory potential exhibited higher affinity with Ŭ-amylase.

Keywords: Structure-affinity relationship; structure-activity relationship; polyphenols; Ŭ-amylase

1. Polyphenols

Dietary polyphenols from natural sources are the most abundant antioxidants in human diets and are the most common and widespread phytochemicals in foods (1-5). Polyphenols are derived from an ñacidicò hydroxyl group that is directly attached to a phenyl ring. Polyphenols are characterized by having more than one phenol type unit per molecule in additional to other structural features. The most important polyphenol classes are phenolic acids, hydroxybenzoic acids, hydroxycinnamic acids, stilbenes, þavonoids and lignans (6-7). Polyphenols have displayed a diverse array of pharmacological activities, among which anti-protozoal, anti-inþammatory, immunomodulatory, nitric oxide inhibitory, anti-cancer, anti-HIV, and glycosidase inhibitors (8-10).

Polyphenols can be simple and low molecular compounds (e.g. gallic acid); however, they can be very large and complex polymeric molecules (e.g. condensed tannins). There are over 10000 polyphenolic structures known with half of these being flavonoids. Flavonoids are the major polyphenols present in wide variety of plant sources and represent a typical class of natural polyphenols bearing a common motif, the chromone moiety consisting of two rings (labeled with A and C) (11-12).

Flavonols are the most commonly found þavonoids in diets and the most prominent þavonols in foods are quercetin and kaempferol and their glycosides such rutin (2). Flavones are much less common than þavonols in fruits and vegetables and the prominent þavones in foods are illustrated to luteolin and apigenin (2). The dietary þavonoids in nature exist almost always as ß-glycosides. The þavonols are found mainly as the C-$_3$ and C-$_7$ glycosides, although the C-$_4$

position may also be glycosylated in some plants (2). Other classes of favonoids are found mainly glycosylated in the C-$_7$ position. A special example is puerarin, a isoþavone-8-C-glucose.

Catechins are the major polyphenols in tea leaves including the unfermented (green tea), semi-fermented (oolong tea), and fermented (black and puerh) forms. The major catechins in tea materials are (ï)-epicatechin (C), (ï)epicatechin (EC), (ï)-epigallocatechin (EGC), (ï)-epicatechin

gallate (ECG), (ï)-epigallocatechin gallate (EGCG), gallocatechingallate (GCG), theaþavin(TF1), theaþavin monogallate A (TF2A), theaþavin monogallate B (TF2B), and theaþavin digallate (TF3) (13). Dietary ellagitannins are the main dietary source of ellagic acid, which has been reported to have antiviral and anticarcinogenic properties. Ellagitannins from raspberry have also been found to exert potent vasodilatory properties (14-16).

Proanthocyanidins differ from other natural polyphenols by the polymeric nature. They are made of þavan-3-ol units with the average degree of polymerization from 3 to 11. Anthocyanins are the largest group of water-soluble pigments in the Plant Kingdom. They have been recently demonstrated to have potential health beneýts and disease prevention properties in animals and humans (17). Anthocyanins are included in the list of natural compounds known as potential antioxidants.

Stilbenes are phytoalexins that become activated when plants are stressed and are important polyphenols with the C_6-C_2-C_6 structure. The typical natural stilbenes are resveratrol and its 3-glucoside, polydatin. These compounds exist in foods and are widely consumed. Resveratrol is a grape-derived polyphenol, which possesses a wide range of bioactivities including antioxidant, anti-inþammatory, and anti-tumor effects (18).

Epidemiological research on the ñMediterranean dietò also illustrates the laws of a polyphenol-rich diet in reducing oxidative stress and preventing chronic diseases such as cancer, diabetes, hypertension, cardiovascular and neurodegenerative diseases, aging, and so on (19-21). Almost all natural polyphenols exhibit anti-oxidant potential which shall mediate their beneficial health effects associated. The presence of a C_2=C_3 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 antiradical activity of þavonoids. Although the molecular mechanisms of chemoprotection of dietary polyphenols are still unclear, it is generally believed that food polyphenols exert various beneficial effects on human health by anti-glycation, anti-inflammation, and anti-oxidation, and so on.

2. Dietary polyphenols as α-amylases inhibitors

Human Ŭ-amylases from both pancreatic and salivary origins have been widely studied for clinical and nutritional purposes because they are significantly crucial in the diagnosis of glands. Moreover, they are targets for drugs designed to treat some diseases, such as diabetes and hyperlipidemia. The inhibitory potential of natural polyphenols for Ŭ-amylases has attracted great interests among researchers (22-25).

Flavonoids

Methylation and Methoxylation

Recently, Walle reported that the oral administration of one methylated þavonoids to rats

resulted in high bioavailability and tissue distribution with no detectable levels of its unmethylated analog and it was concluded that the methylation appears to be a simple and effective way to improve metabolic resistance and transport of the þavonoids (26-27). However, the methylation and methoxylation of þavonoids obviously weakened the inhibitory effects for amylase *in vitro* by amount of reports (28-31). The methylation of 4¢-OH on apigenin and luteolin obviously lowered the inhibitory percentages for human Ŭ-amylase by (30-31). The methylation of 7-OH on 3,6-dimethoxyapigenin and 3-methoxyapigenin decreased the inhibitory effects on HPA (28). The methoxylation of 7-OH on 3-methoxyapigenin and 3,7-dimethoxyapigenin slightly reduced the inhibition (28).

Most recently, Manaharan et al isolated myricetin-3-O-rhamnoside, europetin-3-O-rhamnoside, myrigalone-G and myrigalone-B from *Syzygium aqueum* leaves (32). The methoxylation of 7-OH on myrigalone-G to form myrigalone-B significantly slightly reduced the inhibition against Ŭ-amylase. However, the methoxylation of 8-H on myricetin-3-O-rhamnoside to form europetin-3-O-rhamnoside also significantly enhanced the inhibition against Ŭ-amylase (32).

Hydroxylation

In most case, the hydroxylation of þavonoids improved the inhibitory effect on Ŭ-amylase. These data illustrated again that the hydroxyl group plays a very important role in inhibiting amylase (31, 33-34).

Glycosylation

Kim et al. compared the inhibitory potential of þavonoid glycosides on Ŭ-amylase (EC 3.2.1.1) (35). Baicalin, pectolinarin, and linarin (5 mg/mL) hardly inhibited Ŭ-amylase. The inhibitory percentage of luteolin was found to be similar with that of luteolin-7-O-glucoside. The

monoglycosides (quercitrin and hyperin) of quercetin are stronger than their polyglycoside form (rutin) as Ŭ-amylase inhibitors (35). Ye et al. also reported that the inhibitory potential of quercetin was much stronger than rutin against human pancreatic Ŭ-amylase (34). Wang et al. (31) isolated quercetin and its glycosides from guava leaves and compared their Ŭ-amylase inhibitory activity. It was found that Ŭ-amylase inhibitory activity of quercetin was better than that of its glycosides. Komaki et al. (36) separated and identiýed luteolin-7-O-ɓ-glucoside and luteolin-4ǫO-ɓ-glucoside as Ŭ-amylase inhibitors. Luteolin-7-O-ɓ-glucosid e and luteolin-4ǫO-ɓ-glucoside showed much weaker inhibition against Ŭ-amylase than that of luteolin (35). Ye et al. (34) investigated Ŭ-amylase inhibitory activity of common constituents from traditional Chinese medicine used for diabetes mellitus. The inhibitory percentage of 19µg/mL kaempferol was about 17.4% against Ŭ-amylase; however, the 180 inhibitory percentage of 400 µg/mL kaempferol-3-O-ɓ-Ddiglucoside was about only 8.8%, which indicated that the inhibitory effect of kaempferol is much higher than its glycoside forms (34).

Yang et al. isolated okanin, a chalcone, and its glycosides, okanin 4-methyl ether-3Ǹ-O-ɓ-d-glucoside and okanin-4Ǹ-ɓ-d-glc from *Bidens bipinnata* and found that okanin glycosides obviously exhibited weaker inhibition againstŬ-amylase than that of okanin (37).

Most recently, an exception was reported by Manaharan et al. (32). They isolated flavonoids from *Syzygium aqueum* leaves and found that myricetin-3-*O*-rhamnoside (EC_{50}=1.9 µM) showed stronger inhibition against Ŭ-amylase than that of myricetin (EC_{50}=17 µM).

In summary, the glycosylation of þavonoids decreased the inhibitory effect against Ŭ-amylase depending on the conjugation site and the class of sugar moiety. The decreasing inhibitory potential after glycosylation may due to the increasing molecular size and polarity, and transfer

to the nonplanar structure.

Hydrogenation of the C_2=C_3 double bond

Tadera et al. (2006) compared the inhibitory effect of apigenin and naringenin against HPA. It was found that the hydrogenation of the C_2=C_3 double bond on apigenin strongly decreased the inhibition from 21% to 5%. Planarity of the C ring maybe important for inhibiting amylases since the molecules with saturated C_2ï C_3 bonds (þavanones and certain others), which permits more twisting of the B-ring with reference to the C-ring. A C_2=C_3 double bond increases the p-conjugation of the bond linking the B- and C- rings, which favors near-planarity of the two rings (38). The molecules with near planar structure easier enter the hydrophobic pockets in enzymes.

Catechins

Recent catechins as Ŭ-amylase inhibitors have attracted great interests among researchers (30, 33, 39-40). The inhibition ratio of Ŭ-amylase was about 61%, when the concentration of tea polyphenols consisting of 5.1% C/ EGC, 40.9% EGCG, 30.4% ECG, 10.9% GCG, and 6.3% EC was 0.05 mg/mL. Tadera et al. (2006) determined the inhibitory effects as: EGCG> EC> EGC> C. Hara and Honda (1990) found the inhibitory effects were in the order of TF3> TF2A> TF2B> TF1> CG>GCG> ECG> EGCG. Yilmazer-Musa et al. determined the inhibitory effects of catechins on Ŭ-amylase activity as following order (IC_{50}): GCG ≈ EGCG ≈ ECG >>C >> EC≈ EGC (24). These data illustrated that the galloylated catechins have higher inhibition than nongalloylated catechins and the catechol-type catechins (CG and ECG) were twice more than pyrogallol-type catechins (GCG and EGCG).

It was found that the inhibition activities of the catechins with 2,3-trans structure (CG and GCG)

were 10 times higher than those of the catechins with 2,3-cis structure (ECG and EGCG) (39). C and cyanidin have similar hydroxyl groups including number and position. However, cyaniding showed much higher inhibition ratio than C (33).

Proanthocyanidins and Anthocyanidins

Polyphenol-rich extracts from a range of berries inhibited Ŭ-amylase *in vitro*, but the most effective were from raspberry and rowanberry (41). Extracts from yellow and red raspberries were equally able to inhibit Ŭ-amylase. Because the yellow raspberry extracts effectively lacked anthocyanins, this suggested that they were not crucial for amylase inhibition. Notably, however, higher levels of other phenolic components in yellow raspberries (particularly, ellagitannins) did not increase amylaseinhibition. Amylase inhibition in rowanberry was recovered in a fraction enriched in proanthocyanidins (PACs) (41).

Lee et al. (42) evaluated the anti-Ŭ-amylase effect of polymers and oligomers from proanthocyanidins of perisimmon peel. Polymers showed a stronger inhibitory activity than oligomers. Kawakami (40) separated water-soluble proanthocyanidins from perisimmon (*Diospyros kaki*) and investigated the Ŭ-amylase inhibitory activity. The major proanthocyanidins were unique proanthocyanidin oligmers, namely, EGC-4ƃBTE, EGCG-4ƃ-BTE, EC-4ƃ-BTE, and ECG-4ƃ-BTE, made of four heterogeneous extension units including EGCG, ECG, EC, and EGC (40). Akkarachiyasit et al. (43) investigated the inhibitory activities of cyanidin and its glycosides against HPA. Cyanidin 3-glucoside showed highest inhibition against pancreatic Ŭ-amylase in these cyanidins and cyanidin3-galactoside and cyanidin-3,5-diglucoside had no inhibitory activity against HPA.

Recent, Wang et al. reported the unripe chiku proanthocyanidins are thus good starting material for preparation of EGC derivative and was shown to inhibit Ŭ-amylase with an IC_{50} value of 4.2±0.2ε g/mL (44). The bark extract of *Acacia mearnsii* showed strong Ŭ-amylase inhibition activities. Kusano et al. fractionated the extract of *A. mearnsii*, which showed strong Ŭ-amylase inhibition activities and revealed that the active substances are proanthocyanidin oligomers mainly consisting of 5-deoxyþavan3-ol units (45). In addition, 4ęO-methylrobinetinidol 3ęO-ƀ-D-glucopyranoside, ýsetinidol-(4R,6)-gallocatechin, and epirobinetinidol(4ƀ,8)-catechin were isolated as new compounds.

Tannins

Straw berry and raspberry extracts showed stronger effect against Ŭ-amylase than does blueberry, blackcurrant, or red cabbage (46). The extracts most effective in inhibiting Ŭ-amylase (strawberry and raspberry) contain substantial amounts of soluble tannins. Other tannin-rich extracts (red grape, red wine, and green tea) were also effective inhibitors against Ŭ-amylase. It was also found that removing tannins from strawberry extracts with gelatin will weaken the inhibition (McDougall et al., 2005). The inhibitory components were identiýed as ellagitannins, such as sanguiin H6, sanguiin H10, nobatanin A, lambertianin C, and ellagic acid (46). Ellagitannins inhibit Ŭ-amylase activity and there is potential for synergistic effects on starch degradation after ingestion of berries containing appreciable amounts of ellagitannins and anthocyanins (47)

Procyanidins

Gonçalves et al studied the inhibition of a-amylase activity by the procyanidin fractions using an enzymatic assay based on the hydrolysis of *p*-nitrophenyl-Ŭ-D-maltopentaoside (PNPG5) (48). A relationship between the inhibition of Ŭ-amylase activity and the average degree of polymerization was found to be noticeable. Fractions II (MW=1513) and III (MW=2052) inhibit Ŭ-amylase more efficiently than Fraction I (MW=949). This may be explained as the result of a more extensive interaction of procyanidins with the enzyme since it is considered that more polymerised procyanidins have more interaction sites with proteins. These sites may result from the aromatic rings forming aromatic ring stacking or the hydroxyl groups forming hydrogen bonds, depending on the tested polyphenol and protein,.

Gu et al reported the inhibitory activity of cocoa procyanidins (DP = 2-10) and EC against pancreatic Ŭ-amylase (49). It was found that Lower molecular weight compounds with DP < 5 showed <15% inhibition against pancreatic Ŭ-amylase at a concentration of 10 0ε M, whereas the higher molecular weight procyanidins (DP=5-10) inhibited pancreatic Ŭ-amylase by 17-45.5% at 100 ε M. Compounds with DP ÓЬ inhibited pancreatic Ŭ-amylase by 37-53% at 20 ε M.

Hydroxycinnamic Acids and Phenolic Acids

Narita and Inouye (50) studied the mechanism on the inhibition of chlorogenic ccid, caffeic acid, and quinic acid against porcine pancreas Ŭ-amylase isozymes I and II. The inhibition potencies of the respective inhibitors against both Ŭ-amylase isozymes were almost the same and in the order of chlorogenic ccid > caffeic acid > quinic acid. Araki and Yamamoto (51) found that the inhibitory effect on pancreaticŬ-amylase in the order of caffeic acid> tannic acid> chlorogenic ccid = quinic acid. Recent, Narita and Inouye (52) compared the inhibitory effects of chlorogenic

acids from green coffee beans and cinnamate derivatives on porcine pancreas a-amylase isozyme I. The inhibition potential were determined as: dicaffeoylquinic acids (3,4-, 3,5-, and 4,5-diCQA) > caffeoylquinic acids (3-, 4-, and 5-CQA) > feruloylquinic acids (3-, 4-, and 5-FQA) > chlorogenic acids (CA). Caffeoylquinic acids with more caffeoyl moieties showed stronger inhibitory potential.

Structure-activity relationship

The structure-activity relationship of polyphenols inhibiting Ŭ-amylase are the following (53) (**Figure 2**): 1) The hydroxylation of flavonoids improved the inhibitory effect on Ŭ-amylase; 2) Presence of an unsaturated 2,3-bond in conjugation with a 4-carbonyl group has been associated with stronger inhibition; 3) The glycosylation of flavonoids decreased the inhibitory effect on Ŭ-amylase depending on the conjugation site and the class of sugar moiety; 4) The methylation and methoxylation of flavonoids obviously weakened the inhibitory effect; 5) The galloylated catechins have higher inhibition than nongalloylated catechins; 6) the catechol-type catechins were stronger than the pyrogallol-type catechins; 7) the inhibition activities of the catechins with 2,3-trans structure were higher than those of the catechins with 2,3-cis structure; 8) Cyanidin-3-glucoside showed higher inhibition against than cyanidin and cyanidin-3-galactoside and cyanidin-3,5-diglucoside had no inhibitory activity; 9) Ellagitannins with ɓ-galloyl groups at glucose C-1 positions have higher inhibitory effect than the Ŭ-galloyl and nongalloyl compounds and the molecular weight of ellagitannins is not an important element; 10) Procyanidins with higher degree of polymerization appear stronger inhibition against Ŭ-amylase; 11) Caffeoylquinic acids with more caffeoyl moieties showed stronger inhibitory potential.

In Silico Study.

Piparo studied the structure-activity relationships of flavoonids as Ŭ-amylase inhibitors by computational ligand docking (54) and found that the inhibitory activity of þavonols and þavones depends on: (i) hydrogen bonds between the hydroxyl groups of the polyphenol ligands and the catalytic residues of the binding site and (ii) formation of a conjugated ´-system that stabilizes the interaction with the active site.

The results revealed that OH groups present in position C-$_7$ of the ring A and the ones contained in the ring B, especially in position C-$_{4Nj}$ are particularly important for inhibition to occur, whereas less essential interactions involved positions C-$_3$ and C-$_{3Nj}$Overall, the inhibitory activity of þavonoids toward human salivary Ŭ-amylase depends on two parameters (54): (i) Canhydrogen bonds was formed between the OH groups in C-$_7$ and C-$_{4Nj}$of the þavonoid and the side chains of Asp197 and Glu233? (ii) Can a conjugated ´-system be formed between the indole Trp59 and the AC heterocyclic ring of the þavonoids?

For the þavones with a methoxyl in C-$_7$ and/or C-$_{4Nj}$(acacetin,genkwanin, and diosmetin), both the inhibition and the in silico score decreased as a result of fewer hydrogen bonds that may be formed with the amino acids of the binding site (54). This effect was most prominent for acacetin, which does not contain any hydroxyl group on ring B; its docking score was comparable to the ones for þavanols, þavanones, and isoþavones. This suggests that interactions between the side chains of Asp197, Glu233, and His305 and the hydroxyl groups in C-$_7$, C-$_{3Nj}$and C-$_{4Nj}$are of paramount importance for inhibition of salivary Ŭ-amylase by þavonoids (54).

In contrast to þavonols and þavones, þavanols and þavanones do not possess a carbon-carbon

double bond between C-$_2$ and C-$_3$ of ring C, and as a consequence their ring C is less electron rich compared to þavonols and þavanones (54). The missing electrons lead to weaker ´-´ interactions with the indole ring of Trp59 and, eventually, lead to a reduced inhibitory activity of these compounds toward human salivary Ŭ-amylase. Moreover, isoþavones did not form H-bonds with the catalytic residues of human salivary Ŭ-amylase, which is a likely consequence of the position of ring B; in isoþavones, as opposed to the other þavonoids studied, the B-ring is attached to carbon C-$_3$ rather than C-$_2$ of ring C (54).

3. Polyphenol-α-amylase interaction

We have determined the binding constants between polyphenols and Ŭ-amylase by fluorescence quenching method according to the double-logarithm equation (55):

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

where F_0 and F represent the fluorescence intensities of Ŭ-amylase in the absence and in the presence of polyphenols, K_a is the binding constant, n is the number of binding sites per Ŭ-amylase, and [Q] is the concentration of polyphenols.

Correlation of the binding constant with the number of sites

As it has been previously described, the quantitative analysis of the experimental spectral data leads to the estimation of K_a and n. The number of binding sites (n) was found to within the range of 0.7ï 1.2 and very good linear correlations were found between lgK_a and n. According to Berezhkovskiy (53,56), n reflects in fact the presence of both high affinity and low affinity sites and, therefore, a larger value for n can be correlated with a stronger affinity. Such linear

correlations with very high correlation coefficients were obtained for the polyphenol-Ŭ-amylase system (R = 0.9175) (55).

Flavonoids

The affinities of flavonoids to Ŭ-amylase are mainly correlated with structural motifs of the flavonoid molecules, frequently invoked as affecting their reactivity and activity in a variety of physiological processes. These structural motifs are as follows:

a) The number and position of the hydroxyl groups in the rings A, B, and C.

b) The methoxyl or methyl groups in the rings A and B.

c) The class and position of the glycosides.

d) The degree of saturation of the ring C (the presence or absence of a C2ï C3 double bond).

Scheme 1 showed the role of structure elements on the affinity and activity for Ŭ-amylase *via* their influence on some molecular properties.

Number/position of hydroxyl groups

It was found that there is a different effect of the number and position of the hydroxyl groups on the affinity for Ŭ-amylase., depending on the substituted rings, A, B and/or C (55).

Ring A. The values of K_a between flavones and Ŭ-amylase were found to increase with the increasing number of hydroxyl groups on the ring A. It appears that the optimal number of hydroxyl groups introduced to the ring A of flavones is three, as the highest binding was observed with baicalein (3 hydroxyl groups on A-ring). The hydroxylation on position 5 of ring A of fisetin significantly decreased the affinity for Ŭ-amylase. For isoflavones, the hydroxylation on position 5 obviously increased the binding affinities for Ŭ-amylase. The affinities of genistein

and biochanin A for Ŭ-amylase were about 8.91 and 61.98-times higher than those of daidzein and formononetin.

Ring B. For flavones, the hydroxylation on position 3' of chrysin significantly decreased the binding affinity for Ŭ-amylase. The affinity of luteolin (5,7,3',4') was almost the same as that of apigenin (5,7,3') for Ŭ-amylase. However, for flavonols, addition of another hydroxyl group on the ring B slightly decreased the affinity for Ŭ-amylase.

Ring C. It appears that the hydroxylation on the ring C of flavones significantly weakened the binding affinities for Ŭ-amylase. The affinities of chrysin (5,7), apigenin (5,7,3') and luteolin (5,7,3',4') for Ŭ-amylase are obviously higher than those of galangin (3,5,7), kaempferol (3,5,7,3') and quercetin (3,5,7,3',4'), respectively.

Number/position of methyl/methoxyl groups

The methylation of hydroxyl group in some flavonoids enhanced the binding affinities for Ŭ-amylase. In general, the methylation of hydroxyl group in flavonoids increased their binding affinities for Ŭ-amylase (55). However, the affinities of dadzein, chrysin and kaempferol for Ŭ-amylase were found to be similar to their methylated forms (formononetin, wogonin and kaempferide).

Class and position of glycosides in rings A and B.

The sugar moieties in flavonoid glycosides are usually glucopyranose, glucuronic acid, rhamnose, rutinose, and glucose-rhamnose. It was found that the glycosylation of flavonoids slightly affected the affinity for Ŭ-amylase depending on the conjugation site and the class of sugar moiety (55). The glycosylation of isoflavone and flavanone lowered their affinities for Ŭ-amylase and the glycosylation of flavone and flavonol enhanced their affinities for Ŭ-amylase.

The 7-glucosylation of genistein and daidzein, and 8-*C*-glucosylation of daidzein obviously weakened the affinities for Ŭ-amylase. However, the affinities of rutin, kaempferitrin, quercitrin, and baicalin for Ŭ-amylase were higher than their unglycosylated forms.

Hydrogenation of the C_2=C_3 double bond

It was found that hydrogenation of the C_2=C_3 double bond of flavonoids decreased the binding affinities for Ŭ-amylase (55). The hydrogenation of the C_2=C_3 double bond of apigenin decreased its affinity for Ŭ-amylase. However, the affinity of myricetin for Ŭ-amylase was almost similar to that of dihydromyricetin. Planarity of the C ring in flavonoids maybe important for binding interaction with proteins, as the molecules with saturated C_2=C_3 bonds (flavanones and certain others) permit more twisting of the B ring with reference to the C ring. A C_2=C_3 double bond increases the p-conjugation of the bond linking the rings B and C, which favors near-planarity of the two rings. Molecules with near-planar structure easier enter the hydrophobic pockets in proteins.

Catechins

The binding constants (lgK_a) between EGCG and GCG for Ŭ-amylase were 4.47 and 4.06, respectively. However, EC, EGC, GC, and C hardly quenched the fluorescence of Ŭ-amylase. It illustrates that the galloylated catechins have higher binding affinities with Ŭ-amylase than non-galloylated catechins and the pyrogallol-type catechin had higher affinities than the catechol-type catechin (55). The presence of the galloyl moiety is the most decisive factor. In our present study, the affinity of the catechin with 2,3-*trans* structure (GCG) for Ŭ-amylase was much lower than that of the catechin with 2,3-*cis* structure (EGCG) (55).

Stilbenes

The glycosylation of resveratrol obviously weakened the affinity for Ŭ-amylase (55). The affinity of resveratrol for Ŭ-amylase was slightly higher than that of polydatin. The decreasing affinity for protein after glycosylation may be caused by the non-planar structure. After the hydroxyl group is substituted by a glycoside, the steric hindrance may take place.

Gallic acids

The esters of gallic acid are termed as 'gallates'. The typical gallates are methyl gallate, ethyl gallate, and propyl gallate. The esterification of gallic acid significantly reduced the affinity for Ŭ-amylase (55). The affinities of gallic acid and its esters with Ŭ-amylase were determined as: gallic acid > methyl gallate > ethyl gallate > propyl gallate.

Anthocyanins

Wiese et al. (57) studied the non-covalent interaction between cyanidin-3-glucoside and Ŭ-amylase by means of þuorescence spectra and circular dichroism. Cyanidin-3-glucoside quenched the tryptophan þuorescence of Ŭ-amylase and upon ligand binding a change in protein structure was observed related to the corresponding decrease in the Ŭ-amylase activity.

Structure-affinity relationship

The relationship between the structural properties of natural polyphenols and their affinities for Ŭ-amylase were summarized (55) (**Figure 3**): 1) the methylation of hydroxyl group in flavonoids

increased their binding affinities for Ŭ-amylase; 2) The hydroxylation on rings A, B, and C of flavonoids also significantly affected their affinities for Ŭ-amylase; 3) The glycosylation of isoflavones and flavanones reduced their affinities for Ŭ-amylase and the glycosylation of flavones and flavonols enhanced their affinities for Ŭ-amylase. 4) Hydrogenation of the C_2=C_3 double bond on flavonoids decreased the binding affinities. 5) The galloylated catechins have higher binding affinities with Ŭ-amylase than non-galloylated catechins and the pyrogallol-type catechin had higher affinities than the catechol-type catechin. 6) The glycosylation of resveratrol decreased its affinity for Ŭ-amylase. 8) The esterification of gallic acid significantly reduced the affinity for Ŭ-amylase.

4. Relationship between the binding affinity and activity of natural polyphenols as α-amylase inhibitors

The structure-affinity relationship of natural polyphenols binding to Ŭ-amylase and structure-activity relationship of dietary polyphenols inhibiting Ŭ-amylase were deeply investigated. Is it consistent between the binding affinity and inhibitory potential of natural polyphenols as with Ŭ-amylase inhibitors?

As shown in **Figure 2** and **Figure 3**, there is lack of consistency between the structure-affinity relationship and the structure-activity relationship of natural polyphenols as Ŭ-amylase inhibitors. The binding interaction between polyphenols (mainly flavonoids) and Ŭ-amylase was mainly caused by hydrophobic forces, and the hydrogen bond force is not the main force to bind polyphenols to Ŭ-amylase (55, 58). This is mainly due to the polyphenols (mainly flavonoids) backbone structure and the hydrophobic tubby catalytic center of Ŭ-amylase. Therefore, the

scoring function should be concerned with this hydrophobic interaction in the flavonoid-enzyme system.

The most commonly occurring flavonols are those with dihydroxylation in the 3ôand 4ôpositions of the B ring. The preferred glycosylation site on the flavonoids is the 3 position (Figure 1). Kim et al investigated the interaction between quercetin and its glycosides with Ŭ-amylase by docking and scoring (59). It was found that the amino acid residues participated in hydrogen bonding and ´-´ interactions with flavonoid backbone. Although the sugar moieties of quercetin glycosides provide more opportunity to bind with receptors, including hydrogen bond donor/acceptors or inter-atomic interactions, it also cause steric hindrance. Steric hindrance is unfavorable to the binding of ligands against receptors (59).

The common interaction residues of isoquercetin and quercetin complex are TYR59 and GLN63 through ´-´ interaction and hydrogen bond, respectively (**Figure 4**) (59). Isoquercetin and quercetin have no direct interaction with GLU208, one of the catalytic-site residues of Ŭ-amylase. TYR59 interacts with A-, B-, and C-rings of isoquercetin and with A- and C-rings of quercetin, through ´-´ interaction (aromatic-aromatic interaction). Other residues are involved in hydrogen bonds, except TYR 62 in quercetin complex, which is involved in ´-´ interaction with B-ring of quercetin (59).

Li et al. studied the interaction between Ŭ-amylase with quercetin, isoquercetin, and rutin by fluorescence spectroscopy and enzymatic kinetics (58). It indicated that quercetin, isoquercetin, and rutin could bind to Ŭ-amylase to form a new complex and the number of binding sites (n) at 37 ºC were determined as rutin (1.390) > isoquercetin (1.226) > quercetin (1.195). It was also found that these 3 flavonoids are effective inhibitors against Ŭ-amylase and the inhibitory mode

was a competitive type (58).

Ye et al. also reported that the inhibitory effect of 200 ε g/mL quercetin with an inhibitory percentage of 65.4% was much stronger than does 200 ε g/mL rutin (9.6%) against human pancreatic Ŭ-amylase (34). The glycosylation of flavonoids commonly decreased the inhibitory effect on Ŭ-amylase, which may be caused by the increasing molecular size and polarity, and transfer to the non-planar structure. **As discussed above, there is not consistency between the binding affinity and inhibitory potential of quercetin and its glycosides as α-amylase inhibitors**.

Recent catechins, such as (ï)-epicatechin (C), (ï)-epicatechin (EC), (ï)-epigallocatechin (EGC), (ï)-epicatechin gallate (ECG), (ï)-epigallocatechin gallate (EGCG), gallocatechin gallate (GCG), theaflavin (TF1), theaflavin monogallate A (TF2A), theaflavin monogallate B (TF2B), and theaflavin digallate (TF3), as *α*-amylase inhibitors have attracted great interests among researchers (33, 40). Tadera et al. compared the inhibitory effect of C, EC, EGC, and EGCG against Ŭ-amylase. The inhibitory effects were determined as: EGCG > EC > EGC > C (33). Hara and Honda found the inhibitory effects were in the order of TF3 > TF2A > TF2B > TF1 > CG > GCG > ECG > EGCG (39). Yilmazer-Musa et al. determined the inhibitory effects of catechins on Ŭ-amylase activity as following order (IC_{50}): GCG ≈ EGCG ≈ ECG >>C >> EC≈ EGC (24). We reported that the binding affinities between catechins and Ŭ-amylase were determined as: GCG ≈ EGCG> >> C≈ EC≈ EGC (55). These data illustrated that **those catechins with higher α-amylase inhibitory potential exhibited higher affinities with α-amylase. It looks like that there is consistency between the binding affinity and inhibitory potential of catechins as α-amylase inhibitors.**

Lee et al. investigated the docking study for seven catechins and human salivary Ŭ-amylase (60). It was found that EGCG and ECG were bound to Ŭ-amylase to form complexes by the hydrogen bond interaction and other theaflavins, such as TF3, TF2A, TF2B, and TF1, were too large to dock into the active site of human salivary Ŭ-amylase (60).

The docking model of Ŭ-amylase-EGCG consisted of hydrogen bonds. The three hydroxyl groups of epigallo moiety formed complex by hydrogen bonding interactions with side chains of R195, H299, E231, E233, and D300, and hydrophobic interactions with W58 and Y62 (**Figure 5**). The two hydroxyl groups of catechin moiety formed complex by hydrogen bonds with side chain of H201 and the backbone carboxyl oxygen of G306. Catechin moiety was also bound to V234 and I235. The gallate moiety has three hydroxyl groups and two hydroxyl groups participated in hydrogen bondings with backbone carboxyl oxygen of Y62 and side chain of Q63. Y59, L162, L165, and T163 also formed hydrophobic interaction with the gallate moieties. The whole structure of ECG is equal to EGCG except that only one hydroxyl group is less than EGCG in epigallo moiety. Even though ECG has only two hydroxyl groups in epigallo moiety, the hydrogen bonding interactions with human salivary Ŭ-amylase were equal to those of Ŭ-amylase-EGCG (60).

5. Perspective

The natural polyphenols from plant sources as Ŭ-amylase inhibitors for diabetes have attracted great interests among researchers (61-64). However, the literatures on the Ŭ-amylase-polyphenol interaction are few. The molecular mechanism of polyphenols inhibiting Ŭ-amylase is still not clear.

Polyphenols exhibiting higher affinities with Ŭ-amylase may not show stronger inhibitory potential against Ŭ-amylase depending on the structure classes of polyphenols. The binding affinity of polyphenol-Ŭ-amylase interaction is not a key parameter for polyphenol inhibiting Ŭ-amylase, which maybe caused by follows: 1) the binding sites are different from the active sites of Ŭ-amylase; 2) Ŭ-amylase tends to be inactivated, so determination of the binding affinity does not reflect the real results and it is meaningless; 3) the methods for determination of the polyphenol-Ŭ-amylase interaction and studying of the docking model of Ŭ-amylase-polyphenol have shortcomings and should be improved. The further work on looking for natural Ŭ-amylase inhibitors should better understand the Ŭ-amylase-polyphenol interaction.

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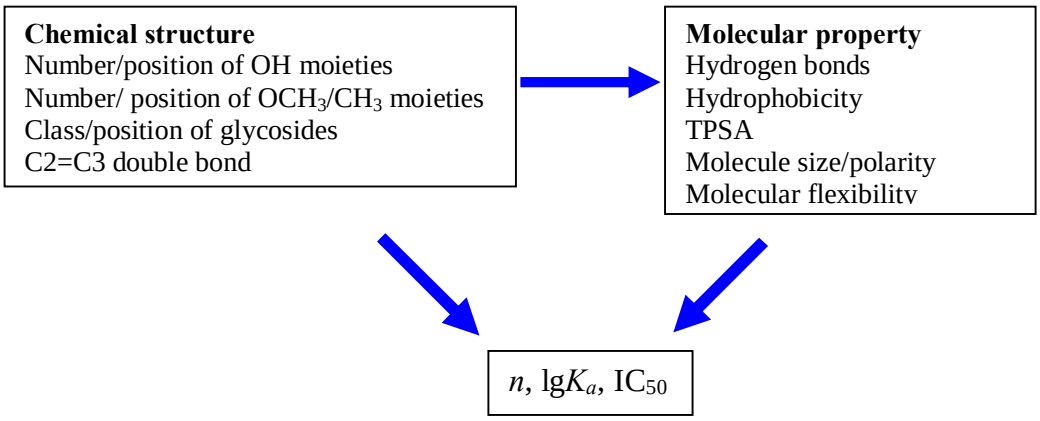


Scheme 1. Structural and molecular properties of flavonoids that influences the affinity and activity for α-amylase.

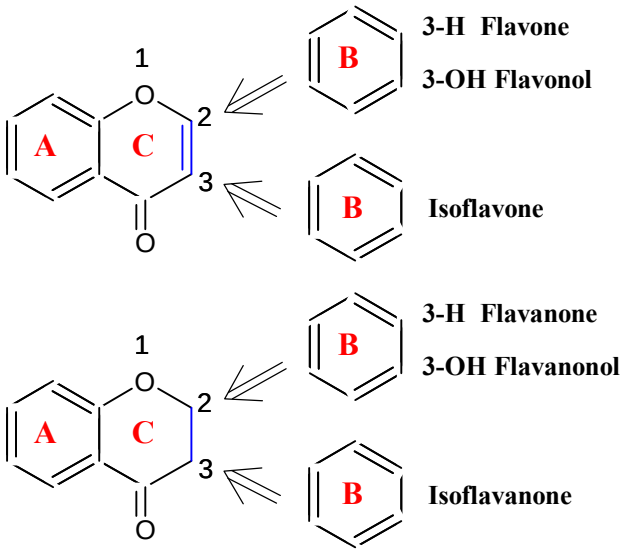


Figure 1. General structures of flavonoids.

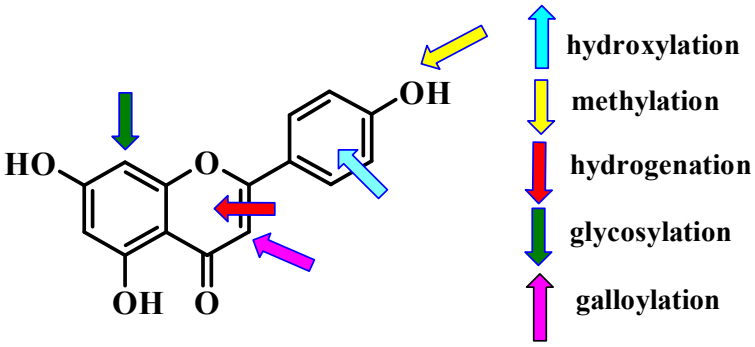


Figure 2. The potential sites of the flavonoids affecting the inhibitory effect against α-amylase are schematically illustrated. The up arrows represent increasing the inhibition; the down arrows represent decreasing the inhibition (53).

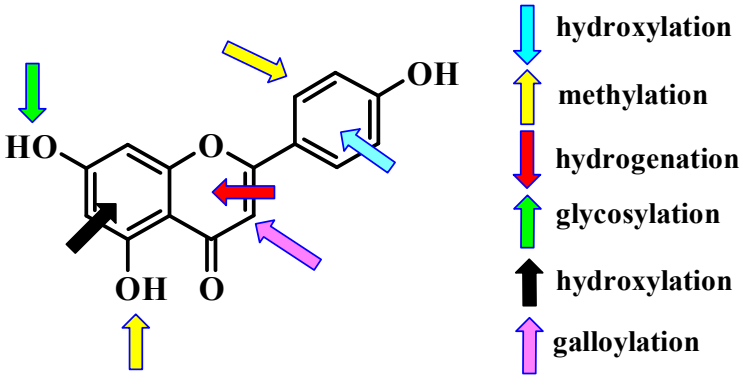


Figure 3. The potential sites of flavonoids affecting the binding affinities for α-amylase. The *up arrows* represent increasing the binding affinities; the *down arrows* represent decreasing the binding affinities (55).

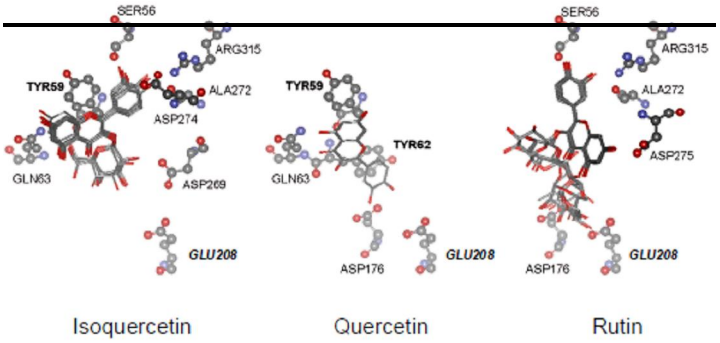


Figure 4. The interactions between flavonoids and residues of α-amylase nearby are indicated. GLU208 is a catalytic-site residue of α-amylase. Residues with normal letters are hydrogen bonded with each flavonoid. Residues shown in bold are involved in the aromatic interactions (π-π interaction; TYR59 and TYR62) with isoquercetin or quercetin flavonoids. Ten posed flavonoids (nine posed in rutin) with highest DOCK_SCORE are shown. GLU208 in isoquercetin and quercetin complex have no interaction with the flavonoids and are shown to compare with the rutin complex (59).

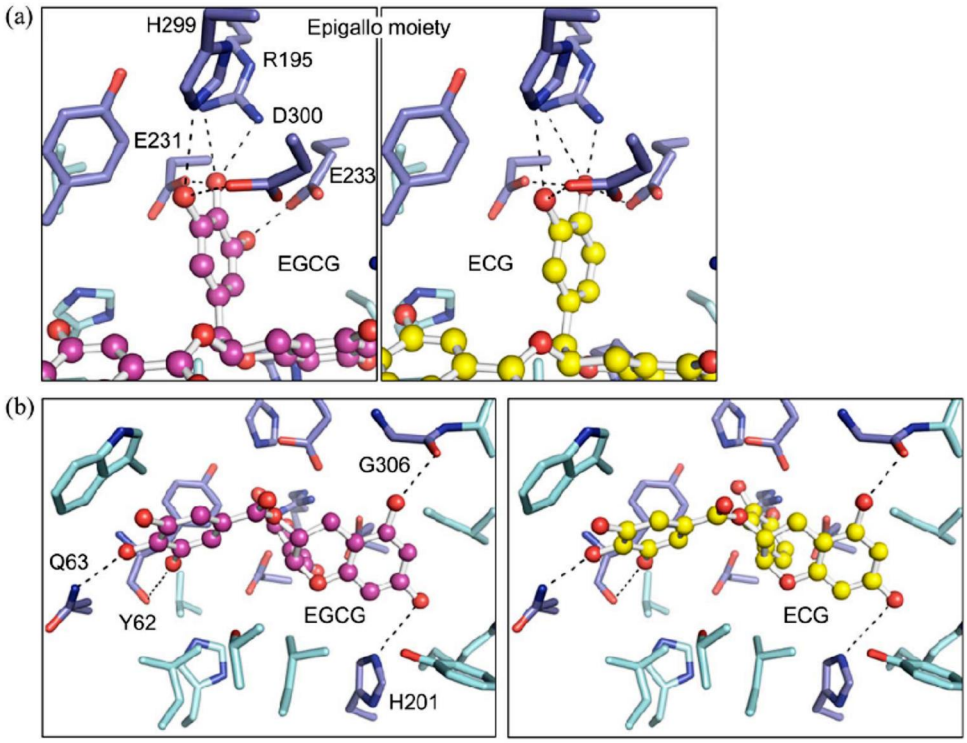
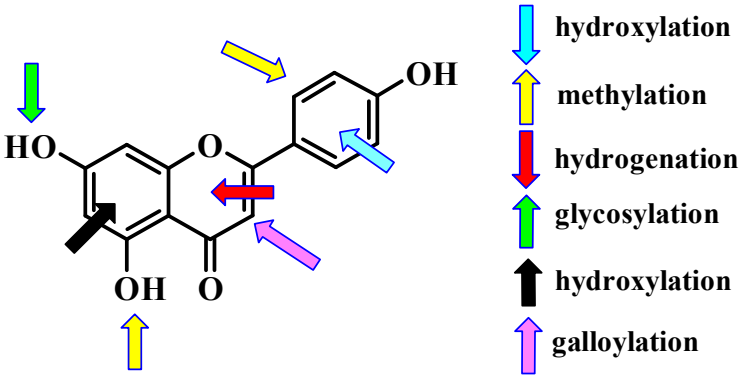


Figure 5. Docking model of catechines and human salivary α-amylase (60). (a) Interaction model of human salivary α-amylase and epigallo moiety of two catechins. (b) Interaction model of human salivary α-amylase and gallate moiety of catechins. EGCG and ECG formed identical hydrogen bonding interactions with human salivary α-amylase.

a TOC Graphic



The potential sites of flavonoids affecting the binding affinities for α-amylase.

