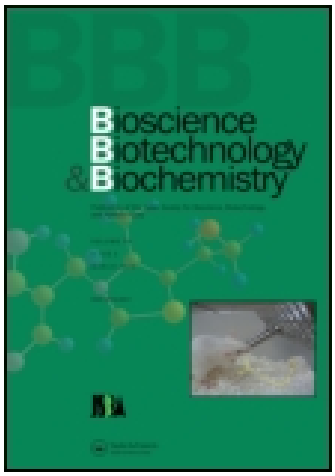

Bioscience, Biotechnology, and Biochemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbbb20

Effects of Polyphenols, Including Flavonoids, on Glutathione S-Transferase and Glutathione Reductase

Masayoshi Iio[a], Hiromi Kawaguchi[a], Yumiko Sakota[a], Junko Otonari[a] & Hanako Nitahara[a]
[a] Department of Food Sciences and Nutrition, Faculty of Life Sciences, Kumamoto Women's University Kenguncho,, Kumamoto 862, Japan
Published online: 12 Jun 2014.

To cite this article: Masayoshi Iio, Hiromi Kawaguchi, Yumiko Sakota, Junko Otonari & Hanako Nitahara (1993) Effects of Polyphenols, Including Flavonoids, on Glutathione S-Transferase and Glutathione Reductase, Bioscience, Biotechnology, and Biochemistry, 57:10, 1678-1680, DOI: 10.1271/bbb.57.1678

To link to this article: http://dx.doi.org/10.1271/bbb.57.1678

Biosci. Biotech. Biochem., **57** (10), 1678–1680, 1993

Effects of Polyphenols, Including Flavonoids, on Glutathione *S*-Transferase and Glutathione Reductase

Masayoshi IIO, Hiromi KAWAGUCHI, Yumiko SAKOTA, Junko OTONARI, and Hanako NITAHARA

Department of Food Sciences and Nutrition, Faculty of Life Sciences, Kumamoto Women's University, Kenguncho, Kumamoto 862, Japan

Received April 9, 1993

Effects of twelve flavonoids and five catechins as well as gallic acid on two kinds of glutathione-related enzymes were investigated. Glutathione *S*-transferase (EC 2.5.1.18) activity was measured by *S*-2,4-dinitrophenyl glutathione formation from 1-chloro-2,4-dinitrobenzene and reduced glutathione. Glutathione reductase (EC 1.6.4.2) activity was followed by NADPH dehydrogenation. Fisetin and myricetin were potent inhibitors of glutathione *S*-transferase, while kaempferol, quercetin, baicalein, and quercitrin were medium inhibitors. Epicatechin gallate and epigallocatechin gallate also showed medium inhibition. Kinetic analyses indicated that fisetin was a mixed type inhibitor of glutathione *S*-transferase with respect to both substrates, while myricetin was a competitive inhibitor of the same enzyme with both substrates. Fisetin and myricetin were noncompetive inhibitors of glutathione reductase with both NADPH and oxidized glutathione. The inhibition patterns of GT and GR as well as the results of kinetic analyses indicated a possibility that inhibitory flavonoids might have some influence on the glutathione recognition sites of the two enzymes.

Flavonoids and other polyphenolic substances have been studied from plant chemical, biochemical, and physiological standpoints as well as food science.[1] Our laboratory has reported on biochemical and physiological effects of flavonoids including their inhibitory activities on various enzymes of interest, for example, glyoxalase I,[2] dextran sucrase,[3] xanthine oxidase,[4] α-glucosidase,[5] and lipoxygenase, which are or might be related to inflammation, dental caries, gout, digestion, and lipid rancidity, respectively. We found that certain glutathione derivatives known to inhibit glutathione *S*-transferase (GT)[6] inhibit glyoxalase I also.[2] The latter enzyme was also inhibited by certain flavonoids such as baicalein and quercetin in a competitive manner. The two enzymes have a common point in that both use glutathione as a substrate. This suggested that flavonoids could suppress glutathione *S*-transferase activity as well by binding to glutathione recognition sites competitively. This hypothesis prompted us to study the effects of flavonoids and other polyphenols like catechins on glutathione-related enzymes; glutathione *S*-transferase and glutathione reductase (GR). The purpose of this study is to add more information to our data base of the inhibition spectra of various enzymes shown by flavonoids and other polyphenols. This paper also describes the inhibition patterns and mechanisms of GT and GR by flavonoids as well as discussion about structure-activity relationship.

Materials and Methods

Chemicals were purchased from the commercial sources indicated and used without further purification. *Flavonoids*: Morin, rutin, baicalein, and baicalin (Wako Pure Chemicals), fisetin, flavone, kaempferol, and quercitrin (Tokyo Kasei), myricetin, chrysin, and 7-hydroxyflavone (Aldrich), quercetin and apigenin (Sigma Chemical Company), and catechins (Kurita/Funakoshi). Flavonoids were dissolved in dimethyl sulfoxide, and catechins in ethanol. *Substrates*: 1-Chloro-2,4-dinitrobenzene (CDNB), glutathione (GSH), oxidized glutathione (GSSG), and NADPH were purchased from Wako Pure Chemicals. *Enzymes*: Equine liver GT and bovine intestinal mucosa GR were products of Sigma Chemical Company (Activity; 57 and 70 units/mg protein, Catalogue Number; G6511 and G1762, respectively). Both enzyme preparations showed no side reactions with substrate mixtures in the absence of GSH or GSSG either with or without flavonoids.

Glutathione S-transferase activity assay. The activity was measured by the increment of absorbance at 340 nm due to 2,4-dinitrophenyl *S*-glutathione formation from CDNB and GSH as Harbig *et al.* reported.[7] The reaction was done in a pair of cuvettes with a 1-cm light path and 1.7-ml inner volume. The cells were held in a temperature-controlled cuvette holder kept at 37°C and attached to a Shimadzu spectrophotometer UV 240. The reaction mixture contained 1.44 ml of 0.1 M potassium phosphate buffer, pH 7.0, containing 1 mM EDTA, 10 μl of 150 mM CDNB, 50 μl of 75 mM GSH, 10 μl of a test compound solution, and 10 μl of an enzyme solution (0.03 units). The control cuvette contained no enzyme solution and the difference in mixture volume was ignored. The reaction was started by adding a CDNB solution and the absorbance increase in the first 30 seconds was recorded. The overall control was take by using solvent for test compounds (=vehicle only) instead of a test compound solution.

Glutathione reductase activity assay. This enzyme assay was done quite similarly to that of GT. The absorbance decrease at 340 nm due to NADPH dehydrogenation was followed. The reaction mixture contained 750 μl of 0.2 M phosphate buffer, pH 7.0, 75 μl of 2 mM NADPH, 75 μl of 20 mM GSSG, 565 μl of water, 15 μl of a test compounds solution, and 20 μl of an enzyme solution (0.012 units) in a total volume of 1.5 ml. The control contained water in place of an enzyme solution. The reaction was started by adding an enzyme solution. The absorbance change was followed for the first 30 seconds. The overall control was taken as in GT experiments.

Results and Discussion

Of 12 flavonoids tested, fisetin and myricetin were potent inhibitors of GT (Table I). Their IC_{50}'s were 14 and 24 μM, respectively. Kaempferol, quercetin, baicalein, and quercitrin were medium, and chrysin, flavone, baicalin, morin, and rutin were weak inhibitors of GT. The effects of these flavonoids on GR were somewhat smaller than on GT. In general, flavones were less potent than flavonols as Merlos

Abbreviations: GR, glutathione reductase; GT, glutathione *S*-transferase.

Table I. Effects of Flavonoids and Catechins on GT and GR Activities

GT activity was measured by increment of the reaction product, *S*-2,4-dinitrophenyl glutathione; GR activity was followed by decrease of NADPH. For experimental details, see the text. The concentrations of test compounds were 100 μM. Numbers indicated in the classification item are those of phenolic OH groups.

Compound	Classification		Remaining activity (%)	
			GT	GR
(Flavonols and flavones)				
Fisetin	Flavonol	4	0	42
Myricetin	Flavonol	6	0	55
Kaempferol	Flavonol	4	24	72
Quercetin	Flavonol	5	27	61
Baicalein	Flavone	3	32	61
Quercitrin	Flavonol, glycoside	4	33	67
Chrysin	Flavone	2	41	75
Flavone	Flavone	0	44	91
Baicalin	Flavone, glycoside	2	44	79
Morin	Flavonol	5	52	70
Rutin	Flavonol, glycoside	4	55	78
Apigenin	Flavone	3	—*	67
(Catechins)				
Epigallocatechin gallate		8	16	63
Epicatechin gallate		7	27	71
Epigallocatechin		6	54	72
Epicatechin		5	65	76
Catechin		5	80	76
Gallic acid		3	65	87

* Not soluble at 100 μM.

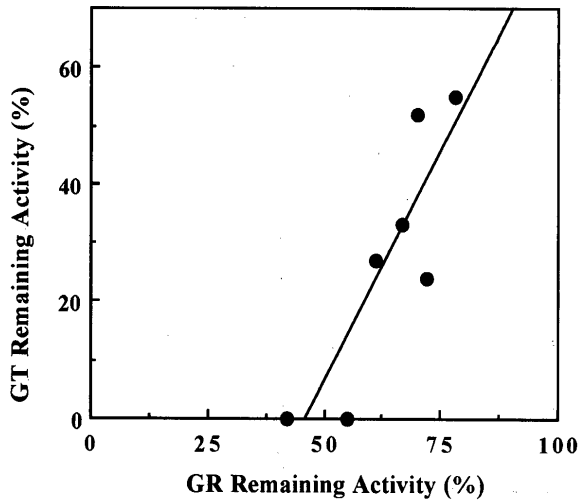

Fig. 1. Correlation of GT- and GR-Inhibition by Flavonols at 100 μM.

GT activity was measured by increment of *S*-2,4-dinitrophenyl glutathione and GR activity was measured by NADPH decrease. The correlation was calculated by the least squares method.

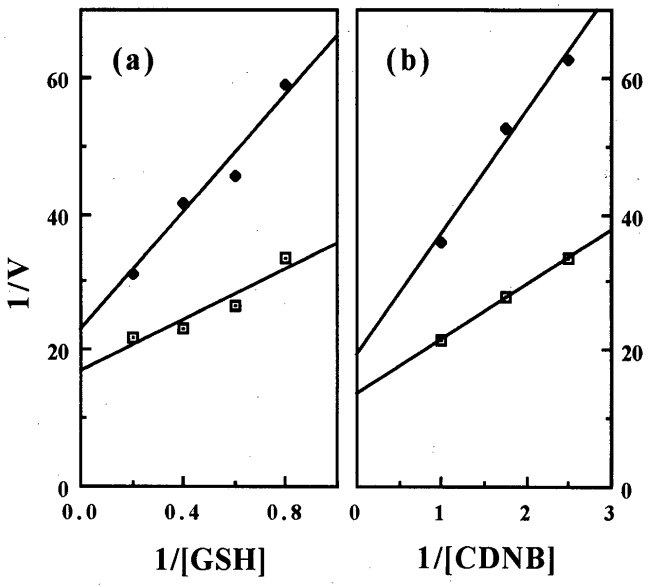

Fig. 2. Lineweaver–Burk Plots of Glutathione *S*-Transferase in the Presence and Absence of Fisetin.

GT activity was measured by increment of *S*-2,4-dinitrophenyl glutathione. The fisetin concentration was 4.0 μM. The unit of *x*-axis is $10^3\,M^{-1}$ and values of *y*-axis are the reciprocals of absorbance increments at 340 nm for 30 s. Closed squares are with and open squares without fisetin. The concentrations of CDNB (a) and GSH (b) were 1 and 2.5 mM, respectively.

et al. reported with rat liver cytosolic enzyme.[8] Aglycons were stronger inhibitors than their corresponding glycosides (for example, quercetin > quercitrin > rutin; baicalein > baicalin, *etc.*). The orders of inhibitions by the flavonoids were similar for each enzyme with a few exceptions. To compare the degree of similarity, we calculated the correlation coefficients between inhibitory activities of a pair of enzymes among GT, GR, and glyoxalase I (G-I) in the presence of various flavonoids at 100 μM. The coefficients were 0.621 between GT and GR, 0.915 between GT and G-I, and 0.771 between GR and G-I, suggesting that three kinds of enzyme inhibition pattern by flavonoids were, to some extent, similar or homologous to each other, especially with GT and G-I. When we divided the flavonoids into two subgroups, that is, flavonols and flavones, the coefficients between GT and GR became greater from 0.621 to 0.859 (flavonols, Fig. 1) and 0.904 (flavones).

From kinetic analysis, fisetin was found to show mixed type inhibition against GT with respect to both GSH and CDNB (Fig. 2), while myricetin was shown to be a competitive inhibitor of GT with respect to both substrates, GSH and CDNB (Fig. 3). In the fisetin inhibition, K_I and K_I' were 3 and 11 μM, respectively, with GSH, while K_I and K_I' were 3 and 10 μM, respectively, with CDNB, where K_I and K_I' are dissociation constants of enzyme–inhibitor complex and enzyme–substrate-inhibitor complex, respectively. In the myricetin inhibition, K_I's were 6 and 37 μM with GSH and CDNB, respectively. Hence some flavonoids were certainly shown to compete for GT with GSH.

Lee[9] reported that GT from a phytophagous insect, *Papilio polyxenes*, was inhibited by quercetin; it was competitive with CDNB, but noncompetitive with respect to GSH. Merlos *et al.*[8] showed that quercetin was a potent inhibitor of rat liver cytosolic GT; quercetin had a noncompetitive profile *versus* both GSH and CDNB. The orders of inhibitory activities were a little different from each other with the two enzymes, as the order was quercetin > morin > kaempferol with the murine enzyme, while it was kaempferol > quercetin > morin with the equine enzyme studied by us. Thus the patterns and modes of GT inhibition were a little different depending on the origins of GT. The mode of GR inhibition by fisetin was uncompetitive with respect to both GSSG and NADPH, and so was that by myricetin (data not shown).

In a flavonoid structure-activity relationship study,

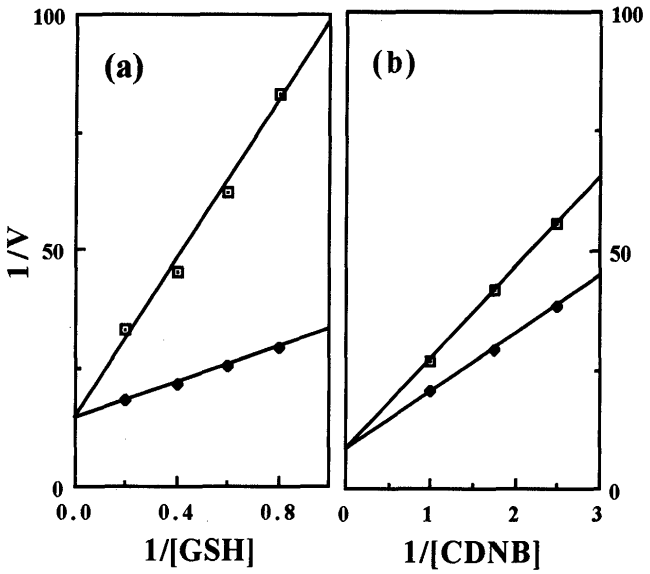

Fig. 3. Lineweaver–Burk Plots of Glutathione *S*-Transferase in the Presence and Absence of Myricetin.

GT activity was measured by increment of *S*-2,4-dinitrophenyl glutathione. The myricetin concentration was 23 μM. The unit of *x*-axis is $10^3\,M^{-1}$ and values of *y*-axis are the reciprocals of absorbance increments at 340 nm for 30 s. Closed squares are with and open squares without myricetin. The concentrations of CDNB (a) and GSH (b) were 1 and 2.5 mM, respectively.

Table II. Correlation between Number of Phenolic OH's and Inhibitory Activity.

Remaining activities of each enzyme in the presence of various flavonoids were plotted against the numbers of free phenolic OH's in the flavonoids and correlation coefficients were calculated by the least squares method.

Enzyme	Correlation coefficient		
	Flavonol	Flavone	Catechin
GT	0.290	0.735	0.972
GR	0.233	0.957	0.959

Hagiwara *et al.*[10] reported that tyrosine kinase activities of an oncogene product (pp 130^{fps}) and insulin receptor were inhibited by certain flavonoids and that the degrees of inhibition were linearly related to the numbers of phenolic hydroxyl groups in the flavonoids (correlation coefficients, $r=0.974$ and 0.926, respectively). Hence we investigated the correlation between the inhibition degrees of glutathione-related enzymes and the number of phenolic hydroxyl groups in the flavonoids. As shown in Table II, the coefficients for GT were 0.290 with flavonols, 0.735 with flavones, and 0.972 with catechins; those for GR were 0.233 with flavonols, 0.957 with flavones, and 0.959 with catechins (Fig. 4). Thus, good correlations were observed with catechins for both GT and GR (as well as for G-I[11]: $r=0.923$), and with flavones for GR. We could say that the greater the number of free phenolic hydroxyl groups is, the more potent the inhibition is, with flavones and catechins. With flavonols, however, it was not their numbers, but the sites of hydroxyl groups that decided the degree of inhibition. The followings were observed: (1) *o*-diphenol on the B ring was more potent than *m*-diphenol (quercetin > morin), (2) *o*-triphenol was still more potent than *o*-diphenol (myricetin > quercetin), (3) the absence of a hydroxyl group at the 5-position enhanced the inhibitory activity (fisetin > quercetin).

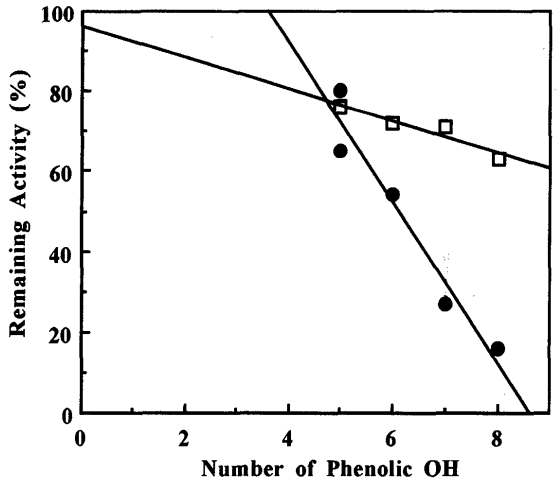

Fig. 4. Correlation between Numbers of Polyphenolic Hydroxyl Groups and Remaining Activities of GT and GR with Catechins at 100 μM.

The correlation was calculated by the least squares method. Closed circles, GT; open squares, GR.

As shown in Table I, the inhibition pattern of GT and GR by catechins was like that by flavonols and flavones. The inhibitory activities of catechins were strengthened by esterification with gallic acid (epicatechin < epicatechin gallate; epigallocatechin < epigallocatechin gallate); interestingly, the degree of activity increments coincided with the inhibitory activity of gallic acid itself, indicating that this strengthening might be an additive effect of gallic acid and the original catechins. However, it was not clear whether the inhibition by gallate-type catechins was specific to GT or not, because gallate-type catechins are known to be non-specific protein-precipitating agents.

Studies are now under consideration about effects of flavonoids and other polyphenols on various kinds of glutathione-related enzymes including glutathione peroxidase.

Acknowledgment. A part of this study was supported by a special grant of Kumamoto Women's University.

References

1) "Plant Flavonoids in Biology and Medicine II," ed. by V. Cody, E. Middleton, Jr., J. B. Harborne, and A. Beretz, Alan R. Liss, New York, 1988.
2) M. Iio, K. Sadakane, T. Shiotsuki, and M. Eto, *Biosci. Biotech. Biochem.*, **56**, 1461–1462 (1991).
3) M. Iio, M. Uyeda, T. Iwanami, and Y. Nakagawa, *Agric. Biol. Chem.*, **48**, 2143–2145 (1984).
4) M. Iio, Y. Ono, S. Kai, and M. Fukumoto, *J. Nutr. Sci. Vitaminol.*, **32**, 635–642 (1986).
5) M. Iio, A. Yoshioka, Y. Imayoshi, C. Koriyama, and A. Moriyama, *Agric. Biol. Chem.*, **48**, 1559–1563 (1984).
6) T. Shiotsuki, A. Koiso, and M. Eto, *Pesticide Biochem. Physiol.*, **37**, 121–129 (1990).
7) W. H. Habig, M. J. Pabst, and W. B. Jacoby, *J. Biol. Chem.*, **22**, 7130–7139 (1974).
8) M. Merlos, R. M. Sanchez, J. Camarasa, and T. Adzet, *Experientia*, **47**, 616–619 (1991).
9) K. Lee, *Insect. Biochem.*, **21**, 353–361 (1991).
10) M. Hagiwara, S. Inoue, T. Tanaka, K. Nunoki, M. Ito, and H. Hidaka, *Biochem. Pharmacol.*, **37**, 2987–2992 (1988).
11) M. Iio, A. Moriyama, Y. Matsumoto, N. Takaki, and M. Fukumoto, *Agric. Biol. Chem.*, **49**, 2173–2176 (1985).