

Dietary quercetin glycosides: antioxidant activity and induction of the anticarcinogenic phase II marker enzyme quinone reductase in Hepalcl7 cells

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It has recently been shown by Hollman *et al.* (*Am. J. Clin. Nutr.*, 62, 1276–1282) that flavonoid glycosides are preferentially absorbed from dietary onions compared to the flavonoid aglycone. In the light of this, we have compared the bioactivities of the two most abundant flavonoid glycosides that we have purified from onions (quercetin-3,4'-diglucoside and quercetin-4'-glucoside) to the quercetin aglycone, and also to the more commonly studied commercially-available flavonoid glycosides, rutin (quercetin-3-rutinoside) and isoquercitrin (quercetin-3-glucoside). Quercetin aglycone was the most effective inducer of the anticarcinogenic phase II marker enzyme, quinone reductase (QR), in mouse Hepalcl7 cells. Of the glycosides, only quercetin-4'-glucoside was able to induce QR activity in this assay. Inhibition of NADPH/iron- and ascorbate/iron-induced lipid peroxidation of human liver microsomes, and the Trolox C-equivalent antioxidant capacity (TEAC), were also measured. The 4'-glycosylation dramatically decreased activity in the 'antioxidant' assays, whereas 3-substitutions produced much smaller changes. These results show that the preferentially-absorbed quercetin glycosides in onions have markedly different biological properties compared with the aglycone.

Introduction

Flavonoids are found in many fruits and vegetables, and may play a role in reducing the risk of heart disease (1). Of the flavonols, quercetin derivatives are present in high concentration in onions, apples, broccoli, wine and tea (2) and increased consumption of onions resulted in a reduced risk of stomach carcinoma (3). Many studies have examined the biological effects of quercetin aglycone, and these effects have been reviewed (4,5). However, the forms of quercetin that are most abundant in onions are the 4'-substituted glycosides, quercetin-4'-glucoside and quercetin-3,4'-diglucoside (6,7). These glycosidic derivatives are preferentially absorbed in humans (8) compared with the quercetin aglycone. Onions are always a major source of flavonoids, and sometimes the major source. In the onion, the 4'-glycosides contribute >85% to the total flavonoids (6,7), and so these derivatives are probably more relevant in a dietary context than the widely studied quercetin aglycone. We have therefore examined the properties of these two important major dietary flavonoids. The assays chosen indicate: (i) the ability to induce phase II enzymes, as one indicator of potential anticarcinogenic activity (9); (ii) aqueous

phase antioxidant activity, by measuring the ability of the test compounds to scavenge the radical cation of 2,2'-azinobis(3-ethyl-benzothiazoline-6-sulphonate) (ABTS) relative to the water-soluble vitamin E analogue, Trolox C (expressed as Trolox C equivalent antioxidant capacity, TEAC) (10); (iii) lipid phase antioxidant activity, by determining the inhibition of NADPH/iron- or ascorbate/iron-induced peroxidation of human liver microsomes (11).

Materials and methods

Quercetin (aglycone), rutin (quercetin-3-rutinoside) and isoquercitrin (quercetin-3-glucoside) were obtained from Apin Chemicals Ltd. The purity was checked by HPLC analysis, and was >95% for each compound. Quercetin-4'-glucoside and quercetin-3,4'-diglucoside were purified from onions as previously described (6). HPLC analysis (6) demonstrated that they were 91–95% pure. All quercetin derivatives used in this study were shown by HPLC to be free of the aglycone. The glycosides were very stable even after a variety of treatments and storage conditions (Price and Rhodes, unpublished data).

Human liver microsomes were obtained as trimmed pieces from liver transplants. Hepalcl7 cells were a gift from Professor P.Talalay, Johns Hopkins University, USA. Trolox C, BHT, NADPH and metmyoglobin were from Sigma Chemical Co. ABTS was from Aldrich.

Induction of quinone reductase was measured according to published methods (9,12), with the following modifications. Heat-treated fetal calf serum (from Life Technologies Ltd., Paisley, UK) was pre-treated with charcoal (activated, Sigma Chemical Co., cat. no. C-9157, 1 g per 100 ml serum) for 2 h at 20°C, a modification of the procedure reported in (9). Protein content of cell extracts was measured using bicinchoninic acid using bovine serum albumin as a protein standard (13). The assay was conducted using six or eight replicates, with a positive control each time using β -naphthoflavone at a concentration of 0.1–0.8 μ M. Typically, the latter compound induced quinone reductase by over four-fold at a concentration of 0.8 μ M, in agreement with previous results using this assay (14).

Iron/ascorbate- and NADPH/iron-induced peroxidation were performed according to Plumb *et al.* (11). Flavonoid derivatives were dissolved in dimethyl sulphoxide at a concentration of up to 5 mM, and added to the assays up to a maximum concentration of 0.1% dimethyl sulphoxide. Control samples containing the equivalent amount of dimethyl sulphoxide were used in all cases. Positive controls using BHT, a potent inhibitor of lipid peroxidation, were also performed, and showed 50% inhibition at 1.8 μ M and 0.18 μ M in ascorbate/iron- and NADPH/iron-induced peroxidation, respectively.

The total antioxidant activity was measured by the method of Salah *et al.* (10). Values are expressed relative to a standard of Trolox C, the water soluble analogue of vitamin E. Values for quercetin aglycone and for rutin measured in our laboratory (see Table I) are in good agreement with published values (10). The assay is based on the relative ability of antioxidants to scavenge the radical cation of 2,2'-azinobis(3-ethyl-benzothiazoline-6-sulphonate) generated by interaction with activated metmyoglobin and H₂O₂. The extent of quenching at 734 nm was compared with standard amounts (1 mM) of Trolox C.

Quercetin-4'-glucoside, quercetin-3,4'-diglucoside, quercetin-3-glucoside, quercetin-3-rutinoside and quercetin aglycone were dissolved in dimethyl sulphoxide, and were added to the culture media of Hepalcl7 cells to a final concentration of dimethyl sulphoxide of <0.1%.

Results

The induction of quinone reductase was measured after 24 h (Figure 1). Quercetin aglycone was the most effective and doubled quinone reductase at ~13 μ M (= CD), compared to a value of 10–30 μ M reported by Talalay and co-workers in this assay (14). Quercetin-4'-glucoside also induced quinone reductase activity (CD = 30 μ M) but none of the other

Table 1. Trolox C equivalent antioxidant capacities (TEAC) of quercetin derivatives

Flavonoid	Trolox equivalent antioxidant activity (TEAC) ^a
Quercetin aglycone	4.43 ± 0.02 ^b
Q-3-rutinoside	2.09 ± 0.07 ^b
Q-3-glucoside	2.15 ± 0.09
Q-4'-glucoside	0.91 ± 0.07
Q-3,4'-diglucoside	0.58 ± 0.01
Trolox C	1.00

^aThis value is relative to antioxidant activity given by Trolox C (1 mM) (16).

^bThis compares with a value of 4.7 ± 0.1 for quercetin and 2.42 ± 0.06 for rutin (10,16). The values shown are the mean and standard deviation of at least three determinations.

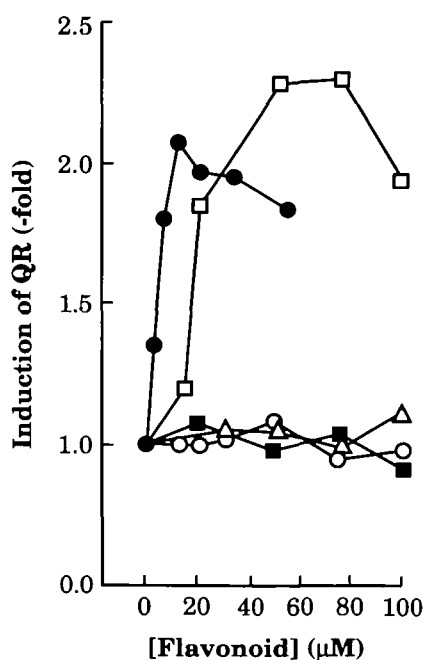


Fig. 1. Effect of flavonoids on induction of quinone reductase activity in mouse hepatoma Hepalcl7 cells. Cells were treated with quercetin (●), quercetin-3-rutinoside (Δ), quercetin-3-glucoside (○), quercetin-4'-glucoside (□) or quercetin-3,4'-diglucoside (■) for 24 h and the change in the levels of quinone reductase measured. Each point represents the mean of six determinations. Standard deviations are smaller than the symbol size.

compounds induced quinone reductase (QR). These results show that whereas 4'-glycosylation affects induction of phase II enzymes by a relatively small amount, 3-glycosylation completely abolishes this activity.

TEAC values for the flavonoids are shown in Table I. The values for quercetin and quercetin-3-rutinoside are in good agreement with values published in other laboratories. The results show that 3-glycosylation decreases the TEAC values by two-fold whereas glucosylation at the 4'-position reduces the TEAC values by 4- to 5-fold, and so the 3,4'-diglucoside is by far the poorest antioxidant.

Glucosylation at the 4' position of quercetin abolishes inhibition of either ascorbate/iron- (Figure 2a) or NADPH/iron- (Figure 2b) induced peroxidation. In contrast, 3-substitution has only a small effect on the activity of quercetin against NADPH/iron-induced peroxidation. Although the exact partition coefficients are not known, it would be expected that the monoglucos-

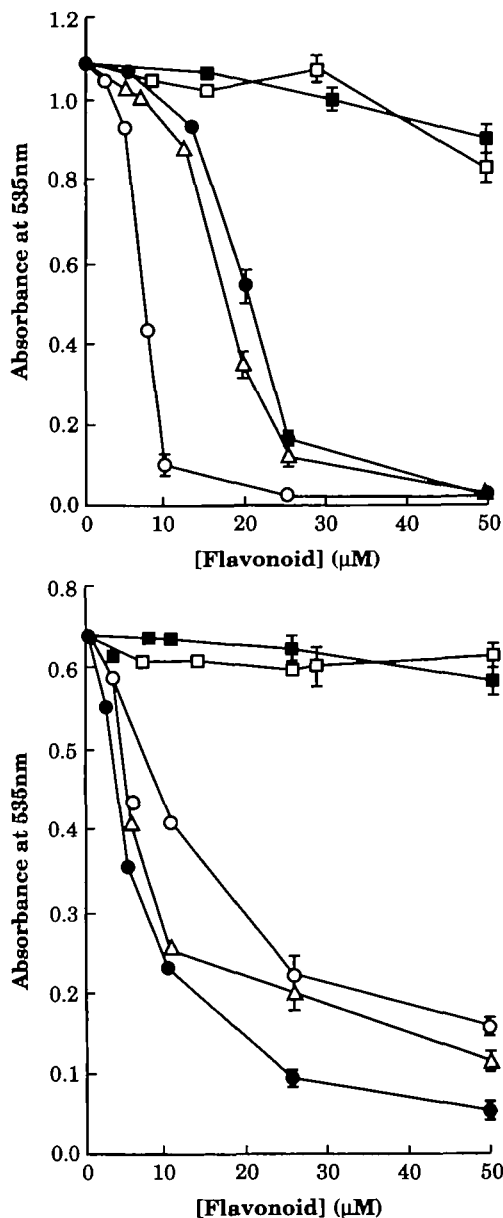


Fig. 2. Inhibition of (a) ascorbate/iron- and (b) NADPH/iron-induced lipid peroxidation of human liver microsomes. The peroxidation reaction was performed in the presence of quercetin (●), quercetin-3-rutinoside (Δ), quercetin-3-glucoside (○), quercetin-4'-glucoside (□) and quercetin-3,4'-diglucoside (■). Values represent the mean of three separate determinations. Typical standard deviations are shown for some values.

ides (3-glucoside and 4'-glucoside) would partition into the membrane to similar extent. Therefore, the differences between the 3-glucoside and the 4'-glucoside is unlikely to be due to membrane partitioning. This parameter has a large influence on the properties of certain other polyphenolics in this system (15). The differences between the 3- and 4'-glucosides are even more pronounced in ascorbate/iron-induced peroxidation. Again, 4'-substitution renders quercetin inactive in this assay. However, 3-substitution slightly increases the activity, with the 3-glucoside being significantly more active than the unsubstituted form.

Conclusions

The relationship between flavonoid structure and antioxidant activity has been studied (10,16,17). A 3',4'-dihydroxy substi-

tution in the B ring, as in quercetin, increases the antioxidant activity substantially compared with a mono-hydroxy substituent, as in kaempferol (16,18). From the work reported here, we can conclude that 4'-glucosylation also reduces the antioxidant action substantially, as determined by three assay methods. This does not appear to be related to partitioning between the aqueous and lipid phase, since the 3-glucoside is a relatively effective antioxidant. In contrast, 4'-glucosylation does not greatly influence the ability of quercetin to induce the anticarcinogenic marker enzyme, quinone reductase. Quercetin aglycone and kaempferol both induce quinone reductase in Hepa1c1c7 cells (14, and Uda *et al.*, unpublished results). The ability to induce quinone reductase does not correlate with the antioxidant activity of these glycosides.

3-glycosylation in the C ring does not eliminate the conjugation between the A and B rings, which has been shown to be important with respect to antioxidant activity. The latter is affected by 3-glycosylation, but to a much lesser extent compared with 4'-glycosylation. The effect of 3-glycosylation, as in rutin, on antioxidant activity has been observed in both lipid and aqueous phases (16,19).

Quercetin glycosides are the most abundant flavonoids in onions, and are preferentially absorbed (8), which shows that the bioactive properties of the glycosides are more relevant in dietary terms than the aglycones. Most work has been reported in rutin and quercetin simply because these materials are readily available, and there is a need for future studies to examine the derivatives that are actually present in the diet. We have addressed this by studying the quercetin derivatives that are actually absorbed and metabolised *in vivo*, and it is quite clear that the antioxidant and potential anticarcinogenic activities of the naturally-occurring and preferentially absorbed quercetin derivatives are very different to quercetin aglycone.

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