Quercetin introduces strand breaks into bacterial DNA

Hanna Czeczot, Iwonna Rahden-Staroń and Małgorzata Bilbin

Department of Biochemistry, Institute of Biopharmacy, Medical School, S. Banacha 1; 02-097 Warsaw, Poland

Quercetin (3,3′,4′,5,7-pentahydroxyflavone, CAS No. 117-39-5) conjugated with sugars is widely distributed in plant kingdom (Scheme 1).

Scheme 1

![Structure of Quercetin](image)

It is the main component of diet flavonoids, consumed daily in about 1 g amount mostly in vegetables [1]. In human lower intestinal tract, quercetin glycosides are hydrolysed by bacteria and free quercetin is released [2]. In contrast to glycosides, free quercetin is mutagenic in the Ames assay [3-5]. Some tests performed in vitro and in vivo in mammalian cell systems suggest that quercetin is genotoxic [6-8]. It also was reported to induce bladder and intestinal tumors in rats [9]. However, the mechanism of genotoxic activity of quercetin still remains obscure.

The aim of this work was to check whether quercetin and its metabolites formed in the presence of microsomal enzymes of fraction S9 introduce the SOS-inducing DNA lesions and, if they do, whether the induction results from single-strand breaks in DNA.

Induction of the SOS response was monitored in bacterial test as the expression of one of SOS genes, sfiA gene [10]. In the bacterial strain used, E. coli K-12 PQ37 uvrA, sfiA gene is fused with lacZ, a structural gene for β-galactosidase [11, 12]. The effects of quercetin were studied both without and with metabolic activation. Quercetin was used at the concentration range of 2.5 - 60 μg/ml, which was far from being toxic to bacteria (i.e. 100 μg/ml).

The results presented in Fig. 1 indicated that quercetin and probably its metabolites were able to induce the SOS system in PQ37 uvrA, strain although the effects were poorly expressed.

To study the ability of quercetin and its metabolites to produce single strand breaks in DNA we used E. coli strain MD322-PQ37 serB, leu+, dnaC 28. Strain MD322 is derivative of PQ37 uvrA strain with termosensitive mutation in gene dnaC. In double mutant dnaC (Ts) uvrA the SOS response at nonpermissive temperature (42°C) may be induced only by agents producing DNA breaks, since neither excision repair or reinitiation of DNA replication is possible [13, 14].

We found that both quercetin and its metabolism products induced the SOS response at permissive temperature in the strain MD322-PQ37, but the induction factor (IF) for metabolites of quercetin was much higher than that measured in the SOS-Chromotest (see Figs. 1 and 2). At nonpermissive temperature IF remained higher only for metabolites (Fig. 2).

We suggest that the above phenomenon could result from oxidation of quercetin metabolites, which is known to lead to formation of H₂O₂.
Fig. 1. Induction of the SOS-system in E. coli strain PQ37 by quercetin and its metabolites.
The recipe was prepared according to original protocol
[11, 12]. Briefly, bacteria at the concentration of $5 \times 10^7$ /ml
were incubated till $A_{600}$ reached about 0.2, then diluted
with 10 vols. of fresh L medium and treated with
increasing concentrations of quercetin with ($\bullet$) or without
(□) 59 mix (10% 59) for 2 h at 37°C. The induction factor
(IF) is defined as the ratio of the β-galactosidase activity
in the samples to which quercetin was added to that in the
samples devoid of this compound.

and free radicals [7, 15] and, eventually, to DNA
breaking.

REFERENCES

191.
2. Tamura, G., Cold, C., Ferro-Luzzi, A. & Ames,
4965.
297 - 309.
58, 231 - 239.
5. Czeczot, H., Tudor, B., Kusztelak, J., Szymczyk,
T., Dobrowolska, B., Glinkowska, G., Malinowski,
6. Carver, J.H., Carrano, A.V. & MacGregor, J.T.
and Medicine Biochemical. 5, pp. 33 - 43, Alan R.
Liss Inc.

Fig. 2. Induction of β-galactosidase activity in E. coli
strain MD332 by quercetin and its metabolites at
permissive or nonpermissive temperature.
MD332Δlac, ΔaraA, sfiA::Mud (Aplic) was a gift from B.
Salles, Toulouse (France). The strain was grown in M9
minimal medium supplemented with 0.5% casamino
acids and 1 μg/ml thiamine [16]. Bacteria at the
concentration of $5 \times 10^7$ /ml were incubated for 70 min at
30°C or 42°C, then treated with 50 μg/ml of quercetin
(with or without 59 mix) for 1 h and kept at either
temperature throughout the experiment. The results
represent the average of at least 3 experiments. Quercetin
without 59 mix at 30°C (□) or 42°C (●). Quercetin with 59
mix at 30°C (△) or 42°C (▲).

8. van der Hoeven, J.M., Burggeman, F.M.H. &
9. Morino, K., Matsukura, N., Kawachi, T.,
Carcinogenesis 3, 93 - 97.
799.
11. Quillardet, P., Huisman, O., d’Ari, R. & Hof-
5971 - 5975.
147, 65 - 78.
- 59.
Mason, R.P. (1990) Free Radical Biology and
Medicine 9, 441 - 449.
Genetics, pp. 352 - 355, Cold Spring Harbor Lab.,
Cold Spring Harbor, New York.