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HANNA CZECZOT and MAŁGORZATA BILBIN

EFFECT OF FLAVONES AND THEIR METABOLITES ON INDUCTION OF SOS REPAIR IN THE STRAIN PQ 37 — *E. COLI* K-12*

Department of Biochemistry, Institute of Biopharmacy, Medical School, Banacha 1; 02-097 Warszawa, Poland

The SOS-Chromotest was used to detect DNA damage induced by two flavones: apigenin and luteolin and/or their metabolites. It was found that the compounds tested weakly induced the SOS repair system in the strain studied.

Flavonoids (flavonoles, flavones) are interesting natural compounds which are regularly consumed by humans in the diet containing fruits and vegetables [1]. In several tests *in vitro* some flavonoids have been shown to possess mutagenic activity while feeding studies in animals did not confirm that flavonoids are potential carcinogens. In additions there is experimental evidence from both *in vitro* and *in vivo* tests, that flavonoids act as natural antimutagens or anticancerogens.

The data on genotoxic properties of flavonoids are fragmentary. The only compound studied in detail is flavonol-quercetin.

The aim of this work was to examine the effect of two flavones: apigenin and luteolin and their metabolites on the induction of the SOS system in *E. coli* K-12.

MATERIAL AND METHODS

The following chemicals were obtained from the sources listed below: Tris, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) were from Sigma; 4-nitroquinoline-*N*-oxide (NQO) was from Fluka AG; aflatoxin B_1 (AFB_1), *O*-nitrophenyl-β-*O*-galactopyranoside (ONPG) were from Calbiochem; *p*-nitrophenyl phosphate (PNPP) was from Merck; Aroclor 1254 was from Analabs Inc.; sodium dodecyl sulphate (SDS), Bacto trypton and Bacto yeast extract were from Difco; dimethyl sulfoxide (DMSO) was from Serva.

Apigenin (5,7,4'-trihydroxyflavone) and luteolin (5,7,3',4'-tetrahydroxyflavone) were isolated from medicinal herbs: *Erigeron canadensis* L. and

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All control mutagens and flavones were dissolved freshly in DMSO.

Fresh solutions of all chemicals were prepared immediately before use.

Bacterial strains. E. coli K-12 strains PQ37 (*sfiA::Mud*) AP *lac/cts, lacU169, mal*$^+$, *uvrA, galE, galY, PhoC, F*$^-$, *rfa, thr, leu, his, pyrD, thi, trp::Muc*$^+$, *sr1300::Tn10, rpoB* used in the SOS-Chromotest were a gift from P. Quillardet and M. Hofnung U.B.M.T.G. Institut Pasteur, Paris, France.

Bacterial test. The SOS-chromotest was applied under standard conditions [2, 3, 4]. This test is based on induction of the *sfiA* gene (belonging to the SOS system) by a DNA damaging factor. The PQ37 strain of *E. coli* K-12 has the gene of β-galactosidase linked to the promotor of the *sfiA* gene. The expression of the β-galactosidase gene observed in this strain depends, therefore, on the degree of the SOS system induction and, thus, on the number of DNA lesions introduced by the chemical compound tested [2, 3, 4]. Each assay was accompanied by positive controls: NQO (20 ng/assay) was used for estimations without metabolic activation and AFB_1 (20 ng/assay) for estimations with metabolic activation. The units of enzyme activity were calculated using a simplified version of the formula used for calculation of international units (U) [5].

$$\text{Enzyme unit (U)} = \frac{1\,000 \times A_{420}}{t}$$

where A_{420} is the absorbance at 420 nm and t is the time of incubation in the presence of the substrate (ONPG or PNPP) in minutes.

Liver microsome fraction (S9). This fraction was prepared by the method of Maron & Ames [6] using Aroclor 1254 treated male Wistar rats.

The activation mixture (S9 mix). The S9 mix used in the SOS-chromotest was prepared according to Quillardet & Hofnung [3].

RESULTS

The effect of apigenin and luteolin on the SOS system induction was assayed, with and without metabolic activation, on the bacterial strain PQ 37 *E. coli* K-12 (Tables 1 and 2). The compounds tested were used at concentrations (1-20 µg/sample) which were not toxic for the bacteria. 4-Nitroquinoline-*N*-oxide (NQO) and aflatoxin B_1 (AFB_1) were used as reference mutagens. The results obtained indicate that the two flavones tested and/or their metabolites are able to induce the SOS system but their effect is very low. The response of the SOS system to apigenin or luteolin was much weaker than to NQO or AFB_1, irrespective of the presence or absence of the S9 mix.

Table 1

Effect of apigenin and its metabolites on the SOS system induction in PQ 37 strain of E. coli K-12

Uβ, units of β-galactosidase activity; UP, units of alkaline phosphatase activity; I(F), induction factor is the ratio of the activities; β-galactosidase/alkaline phosphatase, divided by its value at concentration O of the compound tested. Each value is an average from 5 independent experiments ± SD; NQO, 4-nitroquinoline-*N*-oxide; AFB_1, aflatoxin B_1

Dose (μg/sample)	Uβ	UP	$\frac{U\beta}{UP}$	$I_{(F)}$
Apigenin				
0	2.65±0.50	12.04±0.90	0.22	1.00
0.1	1.89±0.62	12.74±1.23	0.17	0.72±0.38
0.5	2.70±0.31	12.48±0.70	0.22	0.98±0.12
1	1.64±0.16	12.55±1.50	0.13	0.61±0.10
1.25	1.96±0.11	15.66±4.40	0.13	0.59±0.13
2.5	2.90±0.70	13.14±1.40	0.17	0.75±0.19
5	1.90±0.51	10.62±0.40	0.18	0.83±0.18
10	4.34±1.80	11.44±0.78	0.38	1.74±0.60
20	4.71±0.18	13.03±2.20	0.36	1.68±0.13
NQO (20 ng/sample)	17.75±5.70	13.00±3.30	1.37	10.44±3.20
Apigenin +S9				
0	7.75±0.40	61.30±3.30	0.13	1.00
0.1	6.15±0.35	62.90±2.70	0.10	0.89±0.01
0.5	7.70±1.70	57.40±2.00	0.13	1.22±0.30
1	7.10±1.00	56.50±0.70	0.13	1.15±0.20
1.25	6.30±0.30	46.50±7.78	0.14	1.28±0.30
2.5	5.75±0.20	52.50±0.70	0.11	1.11±0.04
5	6.10±0.30	50.70±1.83	0.12	1.11±0.02
10	3.20±0.98	34.20±4.10	0.09	0.84±0.20
20	12.60±3.90	21.30±9.50	0.59	2.04±1.50
AFB_1 (20 ng/sample)	5.81±0.97	43.65±1.62	0.13	12.12±0.5

Table 2

Effect of luteolin and its metabolites on the SOS system induction in PQ 37 strain of E. coli K-12

Abbreviations as in Table 1

Dose (μg/sample)	Uβ	UP	$\frac{U\beta}{UP}$	$I_{(F)}$
Luteolin				
0	2.25±0.11	16.15±1.06	0.14	1.00
0.1	1.97±0.53	12.00±1.40	0.16	1.29±0.40
0.5	2.20±0.20	13.00±0.10	0.17	1.35±0.20
1	2.38±0.10	14.50±2.12	0.16	1.26±0.20
1.25	1.75±0.50	13.30±0.40	0.13	1.02±0.30
2.5	1.51±0.01	13.10±0.50	0.12	0.89±0.10
5	1.81±0.08	21.00±1.80	0.09	0.93±0.21
10	2.28±0.20	15.00±2.80	0.15	1.17±0.10
20	3.43±0.23	14.00±2.80	0.25	1.19±0.30
NQO (20 ng/sample)	17.75±5.70	13.00±3.30	1.37	10.44±3.20
Luteolin +S9				
0	2.26±0.10	9.90±1.01	0.22	1.00
0.1	2.06±0.08	9.76±1.12	0.21	0.96±0.03
0.5	1.92±0.07	12.20±0.60	0.16	0.72±0.01
1	1.80±0.04	8.05±1.64	0.22	1.01±0.11
1.25	1.67±0.16	9.60±2.04	0.17	0.81±0.25
2.5	2.30±0.35	7.99±1.30	0.29	1.31±0.15
5	2.35±0.49	10.85±1.20	0.22	0.99±0.20
10	2.48±0.14	11.91±0.70	0.21	0.94±0.10
20	3.47±0.19	8.79±2.60	0.39	1.78±0.02
AFB_1 (20 ng/sample)	5.82±0.97	43.65±1.62	0.13	12.12±0.50

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