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Mutagenic Activity of Quercetin and Related Compounds

Abstract. *The mutagenic activities of several flavonoids and flavonoid metabolites were examined by means of* Salmonella typhimurium *mutants that reveal base-pair substitution and frameshift mutagens. Of the compounds tested (naringin, rutin, neohesperetin, hesperetin, dihydroquercetin, quercetin, quercetin pentaacetate, permethylquercetin,* m-*hydroxyphenylacetic acid, and* m,p-*dihydroxyphenylacetic acid), only quercetin was mutagenic without microsomal activation. With activation, however, the mutagenic activity of quercetin was increased significantly and that of quercetin pentaacetate was revealed. The health implications of these findings and aspects of flavonoid structural requirements for mutagenic activity are discussed.*

Quercetin (5,7,3′,4′-tetrahydroxyflavone) is one of the most common phenolic compounds in vascular plants (*1*). It occurs in conjugated or free form in many plant products used for food, including many fruits, vegetables, and tea (*2*). The chemical and biological activities of many flavonoids, including quercetin, have been the subject of extensive study for many years (*3*). There is also considerable interest in the flavonoid derivatives as possible nonnutritive sweeteners (*4*).

Considering the widespread occurrence of quercetin in foods, relatively little work has been done on the toxicity of this compound. Ambrose *et al.* (*5*) found a low toxicity for quercetin in short-term and long-term (410 days) studies with rabbits and rats. Subsequent studies by Busby *et al.* (*6*) in which quercetin was implanted in bladders of mice indicated that quercetin might have tumorigenic activity. We have examined the mutagenic activity of quercetin and some related compounds using *Salmonella typhimurium* mutants to detect mutagenicity as described by Ames *et al.* (*7*).

Four *S. typhimurium* strains were used in this study: TA1535 and TA100 which carry the allele *hisG46* that is reverted by base-pair substitutions, and TA1538 and TA98 which carry the *hisD3052* allele that is reverted by frameshift mutations. Strains TA100 and TA98 carry the plasmid R factor pKM101, but TA1535 and TA1538 do not. Mutagenicity of substances is determined by incubating them with the bacterial strains and noting the number of histidine-independent (revertant) clones. In a parallel series of experiments the test substances and bacteria are incubated with a liver microsome preparation. An increased number of revertants under these conditions indicates a metabolic enhancement of mutagenic activity.

The flavonoids were obtained from commercial sources and derivatives were prepared synthetically by conventional procedures. All compounds were obtained in crystalline, analytically pure form and were used as solutions in dimethylsulfoxide in the mutagenicity tests.

Naringin, rutin, neohesperetin, hesperetin, quercetin, dihydroquercetin, quercetin pentaacetate, permethylquercetin, and two quercetin metabolites, *m*-hydroxyphenylacetic acid and *m,p*-dihydroxyphenylacetic acid, were assayed against TA98 and TA100 by the spot (100 μg per disk) and plate (50 μg, 250 μg, 1.0 mg, and 2.5 mg per plate) incorporation methods (*7*). Mutagenic activities of quercetin and quercetin pentaacetate were observed against both strains. Quercetin pentaacetate required metabolic activation for mutagenicity, whereas quercetin did not. The other compounds tested were not active against these strains. Corresponding experiments in which quercetin, dihydroquercetin, quercetin pentaacetate, permethylquercetin, *m*-hydroxyphenylacetic acid, and *m,p*-dihydroxyphenylacetic acid were assayed against TA1538 and TA1535 revealed mutagenic activity of quercetin against TA1538. Other compounds in this series were not active against TA1538 and no compound showed mutagenic activity against TA1535.

More detailed analysis of quercetin and quercetin pentaacetate revealed (Fig. 1) obvious dose-related responses for TA98 and TA100 and a less pronounced but definite relationship for quercetin when tested against TA1538.

Quercetin exhibits frameshift mutagenic activity by inducing reversions of the frameshift mutation in strains TA98 and TA1538. Like most frameshift mutagens, quercetin has a planar, polycyclic structure. Presumably, it acts by intercalating in the DNA base stack and inducing mispairing in a string of nucleotides. When such a mispaired DNA sequence is replicated, there is a greatly increased probability of adding or deleting a base pair in the nascent sequence and thus generating a frameshift mutation (*8*).

Molecular planarity appears to be a requirement for mutagenic activity in the compounds tested since the nonplanar flavonoids with a reduced pyrone ring, hesperetin, naringin, and most notably, dihydroquercetin, exhibit no measurable activity. However, since the planar compound, permethylquercetin, does not exhibit mutagenic activity, a requirement for free phenolic groups is also suggested. Indeed, whereas the planar com-

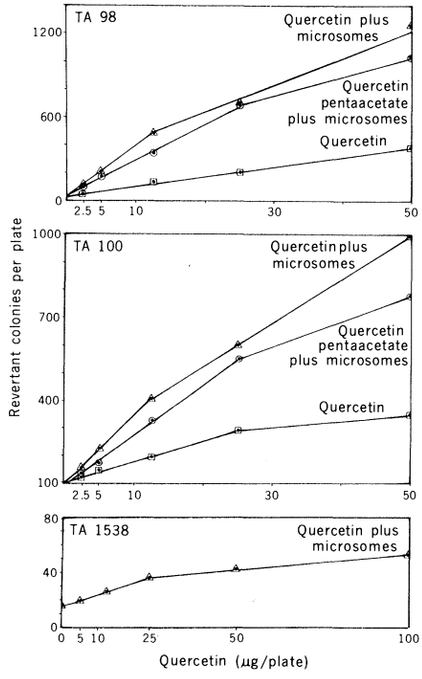

Fig. 1. The number of revertant colonies of the indicated strains as a function of the dose of mutagen. Each point is the result of a count of a separate plate. Spontaneous reversions are included with each count. The procedure was that described in (*7*), and 100 μl of liver preparation previously induced with phenobarbital was added to the appropriate plates.

pound, quercetin pentaacetate, is nonmutagenic in the absence of liver microsomes, activity nearly equivalent to quercetin is observed when the compound is metabolically activated, presumably with deacetylation occurring as an initial step. The inactivity of permethylquercetin may be due to the inability of the liver preparation to demethylate the compound.

Quercetin is clearly mutagenic in the absence of liver-mediated metabolism. However, the activity is approximately tripled in the presence of liver microsomes. The hydroxyphenylacetic acids tested, which are known metabolites of quercetin in mammals, were not active. Thus, the proximate mutagen is an intermediary metabolite of unknown nature.

Mutagenic activity of quercetin in bacteria is not proof of genetic hazard to higher organisms. Furthermore, quercetin is from one to three orders of magnitude less active against bacterial mutants than the highly potent mutagens aflatoxin B_1 and 2-aminofluorene. Also, the processes of mammalian absorption, adsorption, tissue distribution, and metabolism, which may have drastic effects on the biological activity of many compounds, are not duplicated in a single nonmammalian mutagen assay. However, evidence that most chemical carcinogens are mutagens in various bioassays (*9*) is increasing. Thus, the carcinogenic potential of a mutagen such as quercetin, which occurs widely in foods and is produced in mammals as a result of the metabolism of various food components, such as rutin in buckwheat (*10*), must not be overlooked.

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Quantitation of Cytoplasmic Tubulin by Radioimmunoassay

Abstract. *A radioimmunoassay has been developed for the quantitation of cytoplasmic tubulin. It measures tubulin between 20 and 1500 nanograms and does so independently of decay in colchicine-binding activity. In addition, the state of tubulin as subunit or polymer does not alter the measurement.*

Cytoplasmic microtubules are involved in a variety of cellular activities and differentiative events (*1*). Recently, major advances have been made in understanding the biochemistry of microtubule protein (*2, 3*), but the cellular control of microtubule assembly and disassembly in vivo remains to be elucidated. Ultimate understanding of these regulatory events requires the capability to measure the amount of tubulin, the microtubule subunit protein, present in the cells at various times. However, the only currently available methods for accurately determining the content of cytoplasmic tubulin are the time-decay colchicine-binding assay and the densitometric quantitation of material separated by electrophoresis in polyacrylamide gel systems (*4*), both of which have drawbacks and limitations. Recent reports (*5, 6*) that antibodies to tubulin can be used to localize microtubules in tissue cultured cells suggested to us that such antibodies could form the basis for a radioimmunoassay (RIA) as an alternative method for quantitating cytoplasmic tubulin.

Tubulin used in the RIA was prepared from lamb brain by two cycles of polymerization (*7*) followed by chromatography on a phosphocellulose column (*8*). When prepared by this procedure, tubulin is approximately 99 percent pure, as judged by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (*9*), and is free of microtubule-associated proteins (*8, 10*). Tubulin-specific antiserum was prepared by immunizing rabbits with highly purified tubulin that had been cross-linked with glutaraldehyde (*5*). The globulin fraction of this antiserum produced a faint precipitin line against tubulin in an Ouchterlony double-diffusion assay.

Purified tubulin was labeled with ^{125}I by a lactoperoxidase-catalyzed reaction (*11*), and free ^{125}I was removed by dialysis. Test tubes were coated with 50 μl of 1 mg of rabbit serum albumin (RSA) per milliliter in borate-buffered saline, *p*H 8.0. Radioactively labeled tubulin was added in a volume of 10 μl containing 0.5 ng of total tubulin protein ($\sim 10^5$ count/min). Ten microliters of the antiserum against tubulin was added and the tubes were agitated vigorously. This reaction mixture was incubated at 37°C for 4 hours with occasional agitation, and tubulin-antitubulin complexes were precipitated by adding enough of goat antiserum against rabbit immunoglobulin G (IgG) to precipitate all IgG in 10 μl of the rabbit antiserum. Precipitates and supernatants were separated by centrifugation and counted. Maximum specific binding for different preparations of labeled tubulin ranged from 45 to 70 percent.

Inhibition of a standard indirect RIA is widely used to quantitate numerous proteins, peptides, hormones, and small organic molecules. Unlabeled homologous antigen added to this type of assay competes with the labeled antigen for the limited number of antibody molecules available.

Unlabeled lamb brain tubulin to be used as inhibitor was prepared as described above. It was stored at 4°C in 0.05*M* phosphate buffer (*p*H 7.5) with 0.01 percent sodium azide. For quantitative inhibition studies, tubes were coated with RSA as above and 10 μl of unlabeled tubulin inhibitor was added. Next, 10 μl of rabbit antiserum against tubulin was added and the tubes were incubated at 37°C for 4 hours with occasional agitation; then 10 μl of labeled tubulin was added and the incubation was continued for an additional 4 hours at 37°C. Finally, goat antiserum against rabbit IgG was added and the incubation was continued for 2 hours at 37°C and overnight at 4°C. The precipitates were collected by centrifugation and washed twice with 0.5 ml of borate-buffered saline. Supernatants and precipitates from each tube were counted in a gamma-well scintillation counter. Counts in each precipitate were corrected for (i) background radioactivity, (ii) nonspecific binding (serum obtained prior to inoculation from the same rabbit that synthesized antibody against tubulin), and