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Bacterial antimutagenesis by hydroxycinnamic acids from plant cell walls

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Abstract

We have determined the abilities of (*E*)-ferulic acid, (*E*)-*p*-coumaric acid and (*E*,*E*)-5-5-dehydrodiferulic acid to protect against different types of mutation in a simple bacterial model. These antimutagenic properties were compared with those of the related compound curcumin, and also with those of an extract containing hydroxycinnamic acids obtained by the saponification of the cell walls of wheat coleoptiles. Three known mutagens, bleomycin, hydrogen peroxide and 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) were used to chemically induce reversion mutation, while the known antimutagen Trolox was used as a positive control. Both the pure hydroxycinnamic acids and the extract from the cell walls showed antimutagenic properties. It is known that hydroxycinnamic acids ester-linked to plant cell walls can be released in the human colon by the action of microbial esterases. Providing the current data extrapolate to mammalian cells, they suggest that antimutagenic properties of hydroxycinnamic acids released from plant cell walls could play a role in dietary fibre protection against cancer.

Keywords: 5-5-Dehydrodiferulic acid; Antimutagen; Curcumin; Dietary fibre; Ferulic acid; Hydroxycinnamic acid; *p*-Coumaric acid; *Salmonella*

1. Introduction

The health promoting properties of dietary fibre have long been attributed to its polysaccharide composition [1]. It is certainly true that more than 95% of the dietary fibre in Western diets is made up of plant cell walls, and these are predominantly composed of polysaccharides [2]. However, plant cell walls contain other components that may confer on them health promoting properties. In particular, the unlignified primary cell walls of grasses and cereals and of other families of commelinoid monocotyledons, and of families in the dicotyledon order Caryophyllales, contain the hydroxycinnamic acid ferulic acid, together with small amounts of *p*-coumaric acid and 5-5-dehydrodiferulic acid ester-linked to cell wall polysaccharides [3–8] (Fig. 1). Dehydrodiferulic acids coupled in other ways, including 8-*O*-4, 8-5, and 8-8 and 4-*O*-5, have also

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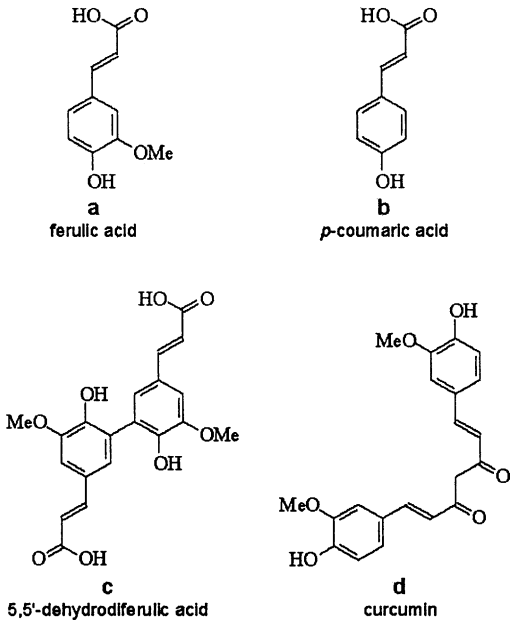

Fig. 1. Structures of hydroxycinnamic acids and of curcumin: (a) ferulic acid; (b) *p*-coumaric acid; (c) 5-5-dehydrodiferulic acid; (d) curcumin.

been found in the cell walls of grasses and cereals [9–11]. All of the hydroxycinnamic acids occur mostly as their (*E*)- or *trans*-forms. Other than grasses and cereals, many of the plants with hydroxycinnamic acids in their cell walls are food plants and include spinach, beetroot and pineapple.

In the colon, these hydroxycinnamic acids, which are ester-linked to cell-wall polysaccharides, can be released from the walls by the action of bacterial esterases [12]. These acids are then either absorbed or rapidly converted to other compounds by colonic bacteria. Kroon et al. [12] postulated that in humans, 95% of the total feruloyl groups are released into the colon, thus making them bioavailable. It is, therefore, of considerable interest to understand whether they could play any significant role in protection against cancer, possibly through acting as antimutagens, as do certain other plant phenolics [13].

Other laboratories have described the in vitro free radical scavenging properties of ferulic acid and its dehydrodimers [14,15]. However, although there have been a few studies on the antimutagenic properties of ferulic acid [16–18], there have been no comparable reports on the effects of dehydrodimers of ferulic acid. In the present study, we tested the hypothesis that the hydroxycinnamic acids that occur in plant cell walls can act as antimutagens, and that this might be a mechanism by which they could reduce cancer risk. We determined the abilities of (*E*)-ferulic acid, (*E*)-*p*-coumaric acid and (*E*,*E*)-5-5-dehydrodiferulic acid to protect against different types of mutation in a simple bacterial model. We also tested an extract containing hydroxycinnamic acids obtained by the saponification of the cell walls of wheat coleoptiles. The cell walls of wheat coleoptiles are mostly primary and have previously been shown to contain ferulic acid and dehydrodiferulic acids [19]. Microbial hydroxycinnamoyl esterases, which are responsible for releasing ester-linked hydroxycinnamic acids from plant cell-wall polysaccharides in the colon, are not available commercially. Thus, we have saponified the isolated cell walls with 1 M NaOH and, after acidification, extracted the hydroxycinnamic acids from this with diethyl ether and tested it in the assay system.

The Ames mutagenicity test has been used as a simple primary screen for antimutagenesis [20]. Three known mutagens, bleomycin, hydrogen peroxide and 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), were used to chemically induce reversion mutation, and a known antimutagen, Trolox, was used as a positive control. We have also compared these data with comparable findings for the related compound, curcumin (Fig. 1).

2. Materials and methods

2.1. Chemicals

(*E*)-Ferulic and *p*-coumaric acids were obtained from Sigma Chemical Co., St. Louis, MO, USA. The (*E*,*E*)-5-5-dehydrodiferulic acid was synthesized as described previously (except that labeled substrates were not used) [21]. Bleomycin (15000 IU) was from Bristol-Myers Squibb, Pharmaceutical Pty. Limited, Victoria, Australia. 4-Nitroquinoline-*N*-oxide (NQNO, 98%), 4-nitro-*O*-phenylenediamine (4NOPD, 98%), curcumin, and Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) were from Aldrich Chemical Company, Milwaukee, WI, USA. 2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ) was

from Toronto Research Chemicals Inc., Downsview, Ontario, Canada. S9 Aroclor 1254 rat liver LS9 (Moltox) was from Molecular Toxicology Incorporated, Boone, NC, USA.

2.2. Plant material

Grains of wheat (*Triticum aestivum* cv. Kotuku) were kindly provided by Dr. W.B. Griffen, Crop Research Institute, Christchurch. Grains (25 g) were soaked in water for 2 h at room temperature, placed on wet Whatman no. 1 chromatography paper and incubated at 25 °C in the dark, except for a 2 h exposure daily to red light to prevent mesocotyl elongation. Coleoptiles were harvested after 5 days.

2.3. Isolation of coleoptile cell walls

Cell walls were isolated using the technique described by Smith and Harris [22]. All procedures were carried out at 4 °C. Coleoptiles were homogenized in Mops-KOH buffer (20 mM, pH 6.8) using a Polytron blender (Kinematica, Kriens-Luzern, Switzerland) followed by a Tenbroeck ground-glass homogenizer. The homogenates were centrifuged (450 × *g*, 5 min) and the pellets washed by centrifugation, four times with buffer followed by four times with water. The cell walls were filtered onto nylon mesh (pore size 11 μm, Nybolt), washed with water until the filtrate was clear, dried by solvent exchange by successively washing with ethanol, methanol and *n*-pentane, and stored under vacuum over silica gel. Histochemical examination of the isolated cell walls using iodine in a potassium iodide solution [23] showed that starch granules were absent.

2.4. Treatment of coleoptile cell walls with NaOH

This was done in two ways: (a) to quantify using UV spectrophotometry the amount of (*E*)-ferulic acid equivalents extracted; and (b) to obtain an extract for antimutagenicity testing. (a) Cell walls (15 mg) were shaken with 1 M NaOH (1 ml) for 20 h at 25 °C under argon. The suspension was filtered (glass microfibre filter, type CF/C, Whatman, Maidstone, UK) and the residue washed with 1 M NaOH (five washings of 2 ml). The combined filtrate and washings were diluted to 25 ml with 1 M NaOH and the UV absorption spectrum (210–430 nm) of this solution was measured. The (*E*)-ferulic acid equivalents released from the cell walls were calculated by comparing the absorbance at λ_{max} 333 nm with the extinction coefficient of sodium (*E*)-ferulate at 333 nm (determined using reference solutions of (*E*)-ferulic acid in 1 M NaOH) [4,24]. (b) Cell walls (340 mg) were shaken with 1 M NaOH (20 ml) and filtered as described in (a). The combined filtrates and washings were adjusted to pH 1.5 with 6 M HCl, saturated with NaCl, and the hydroxycinnamic acids extracted by shaking with diethyl ether (4 × 20 ml). The combined extracts were evaporated in a stream of N_2, and dried under vacuum over silica gel [25]. The antimutagenicity of the cell-wall extract dissolved in 50% aqueous ethanol was determined as described below.

2.5. Bacterial strains for Salmonella antimutagenicity assays

Salmonella typhimurium strains TA98 and TA102 were kindly supplied by B.N. Ames (Biochemistry Dept, University of California, Berkeley, CA, USA). We have determined previously that using aliquots of frozen stock is necessary for reproducibility of experiments and essential for quantitative comparisons. Therefore, the bacteria were initially grown to stationary phase in bacterial complete medium, and frozen (in 10% aqueous DMSO) in 1 ml aliquots at −80 °C.

The original stock cultures were tested for genetic markers, including sensitivity or resistance to UV radiation (uvrB) and sensitivity to crystal violet (deep rough character, **rfa**). Strains were routinely characterised for spontaneous reversion characteristics and reversion rates in response to 4-nitro-*o*-phenylene diamine (TA98) or bleomycin (TA102).

2.6. Bacterial antimutagenicity assay (standard protocol)

For each experiment, a frozen vial of bacteria was used to inoculate 20 ml L-broth which was then incubated in a 37°C shaking water bath for 3 h. The absorbance at 600 nm of the suspension (blanked with L-broth) was measured and the cultures used for all the experiments had absorbances of 0.080–0.100.

Aliqots of the bacterial culture (100 μl), a known mutagen (50 μl at optimal concentration), either 500 μl of S9 mix or 500 μl of phosphate buffer, and the test antimutagen (50 μl) were all added directly to 2 ml of the histidine/biotin supplemented soft agar for plating. The known mutagens and test antimutagens were dissolved in 50% aqueous ethanol before testing. Initially, the following mutagen concentrations were tested: hydrogen peroxide 10,000–80,000 ng per plate, bleomycin 20–20,000 ng per plate and IQ 5–100 ng per plate. A positive control containing NQNO (49,000 ng per plate) was included in duplicate. When the agar solidified, the plates were inverted and incubated at 37 °C in the dark for 72 h.

Numbers of revertant colonies were scored using an automated programme (Countermat, IUL Instruments, Barcelona, Spain) or a colony counter to count the colonies manually, as appropriate. Average numbers of revertant colonies and standard errors were calculated and plotted. All experiments were done in duplicate or triplicate. The dose-range used was estimated from preliminary experiments which estimated toxicity, and the final doses selected to be non-toxic. Toxicity was also checked by visual examination of the background lawn in each experiment. We have accepted an antimutagenicity result as positive if there is a dose–response effect seen, and if the data at a given dose of an antimutagen is significantly lower than seen in experiments without an antimutagen.

2.7. Bacterial antimutagenicity assay (pre-incubation protocol)

The 1100 μl of S9 mix, 220 μl of the bacterial culture and 110 μl of the test chemical at different concentrations were mixed in test tubes. As a positive control, 1100 μl of S9 mix, 220 μl of the bacterial culture and 108,000 ng of the known mutagen 4NOPD were mixed. As a negative control, 1100 μl of S9 mix, 220 μl of the bacterial culture and 110 μl of sterile distilled water were mixed and incubated in a 37 °C waterbath, shaking at 120 rpm for 15 min. From each mixture, duplicate aliquots (650 μl) were mixed with 2 ml of soft agar supplemented with histidine and biotin, plated, incubated and counted as above.

3. Results

3.1. Mutagenicity of bleomycin, hydrogen peroxide and IQ

Dose–response curves were generated using standard methodology with strain TA102 (bleomycin, peroxide) or TA98 (IQ) (Fig. 2). From these data, optimum amounts per plate of mutagens against which to test the putative antimutagens were chosen as follows: bleomycin 500 ng, hydrogen peroxide 34,000 ng, IQ 50 ng (standard protocol) and 25 ng (pre-incubation protocol).

3.2. Antimutagenic effects of ferulic and p-coumaric acids

Fig. 3 illustrates the dose–response of ferulic and *p*-coumaric acids when tested as antimutagens in the presence of a set concentration of each of the three mutagens. Neither test chemical was mutagenic when tested alone in TA98 and TA102 (data not presented). Both showed an ability to reduce the mutagenesis of all three mutagens. For hydrogen peroxide, both ferulic and *p*-coumaric acids require about 1000 μg per plate to reduce the response, whereas for bleomycin, the responses are all greatly reduced until a concentration of 500 μg per plate is reached. In the standard protocol with IQ, there is no reduction up to 1000 μg per plate and possibly higher, and no reduction by *p*-coumaric acid until a concentration over 600 μg per plate is reached. The antimutagenic effect was more strongly marked when a pre-incubation protocol was being used (Fig. 3).

Antimutagenesis dose–response curves of Trolox against the mutagenesis of each of these three chemicals are included for comparative purposes.

3.3. Antimutagenic effects of curcumin

Curcumin was antimutagenic in the presence of hydrogen peroxide (Fig. 4), leading to a plateau dose–response after 1 μg per plate. However, when tested against either bleomycin or IQ, curcumin showed co-mutagenic effects. In the case of bleomycin, curcumin was co-mutagenic at lower concentrations, although antimutagenic at higher concentrations. Curcumin appeared co-mutagenic towards IQ-induced

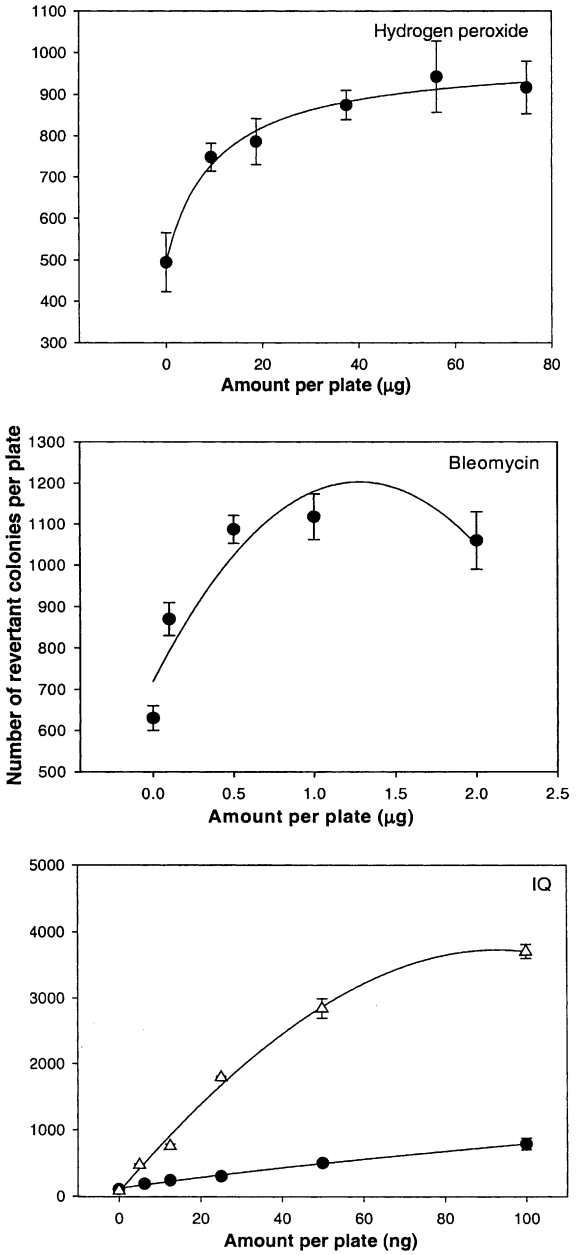

Fig. 2. Dose–response of three different mutagens in *Salmonella typhimurium*. Different amounts per plate of hydrogen peroxide or bleomycin were plated with strain TA102, or of IQ with strain TA98, using standard methodologies (closed symbols) or the pre-incubation protocol (open symbols). Each data point represents mean ± S.E. from two experiments.

mutation when tested using the pre-incubation protocol (Fig. 4).

3.4. Antimutagenic effects of 5-5-dehydrodiferulic acid

The 5-5-dehydrodiferulic acid was antimutagenic at concentrations higher than 50 μg per plate (Fig. 5). Ferulic acid has been included as a positive control in each of these experiments. When tested against hydrogen peroxide, the 5-5-dehydrodiferulic acid was effective at a considerably lower concentration than necessary for ferulic acid, but unlike ferulic acid, the response appeared to reach a plateau after around 50 μg per plate (Fig. 5). Although ferulic acid itself showed only a weak antimutagenic effect towards bleomycin, the dimer reduced mutations further and at lower concentrations (Fig. 5). Effects towards IQ were only tested using the pre-incubation protocol, and the data suggested that the dimer was antimutagenic at a lower concentration, but did not reduce mutation to the same level as ferulic acid itself (Fig. 5).

3.5. Antimutagenic effects of the cell-wall extract from wheat coleoptiles

The antimutagenic activity of the cell-wall extract was compared with that of ferulic acid in reducing mutations induced by either the oxidizing mutagen bleomycin, or the alkylating dietary mutagen, IQ. This comparison was made by using (*E*)-ferulic acid equivalents for the extracts calculated on the basis that the coleoptile cell walls yielded 16.6 mg equivalents/g cell wall. The cell-wall extract reduced mutations to a lower level at an equivalent dose to ferulic acid (Fig. 6). Against IQ a greater reduction of the IQ response was seen but only in the dose window between 1 and 10 μg per plate.

4. Discussion

We believe that this is the first report of antimutagenic effects of an extract containing hydroxycinnamic acids obtained by the saponification of primary cell walls of a cereal. It is also the first report of the antimutagenic effects of 5-5-dehydrodiferulic acid. Comparison of the cell-wall fraction antimutagenicity data with

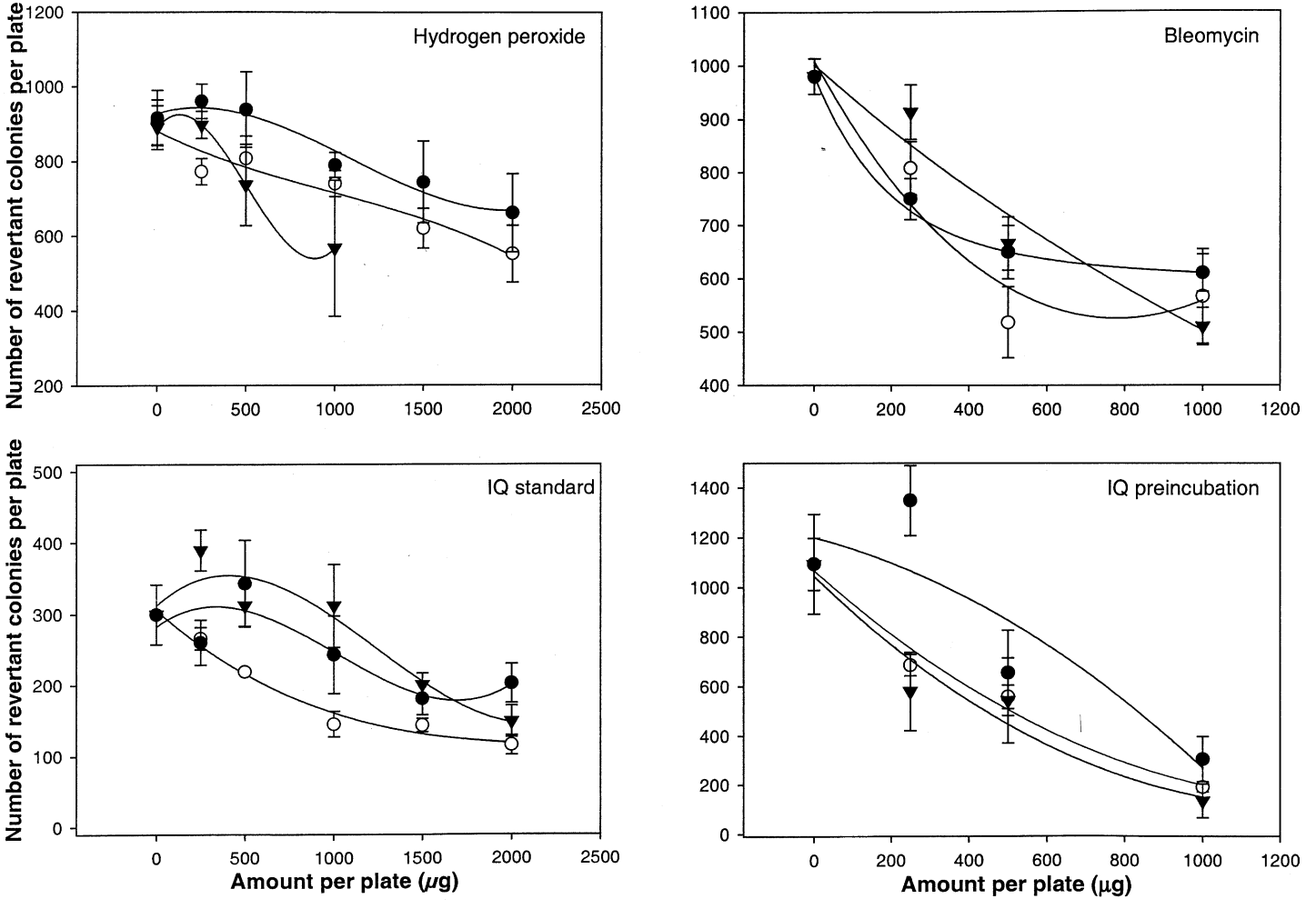

Fig. 3. Effect of ferulic acid, *p*-coumaric acid and of Trolox on mutations induced by three different mutagens in *Salmonella typhimurium*. Different amounts per plate of the compounds were plated with strain TA102 in the presence of 34,000 ng per plate of hydrogen peroxide, or 500 ng per plate of bleomycin, or with strain TA98 in the presence of 25 ng per plate IQ, using standard methodologies, or 50 ng per plate of IQ using the pre-incubation protocol. Each data point represents mean ± S.E. from two experiments. Closed circles represent *p*-coumaric acid, open circles are ferulic acid, and triangles are Trolox.

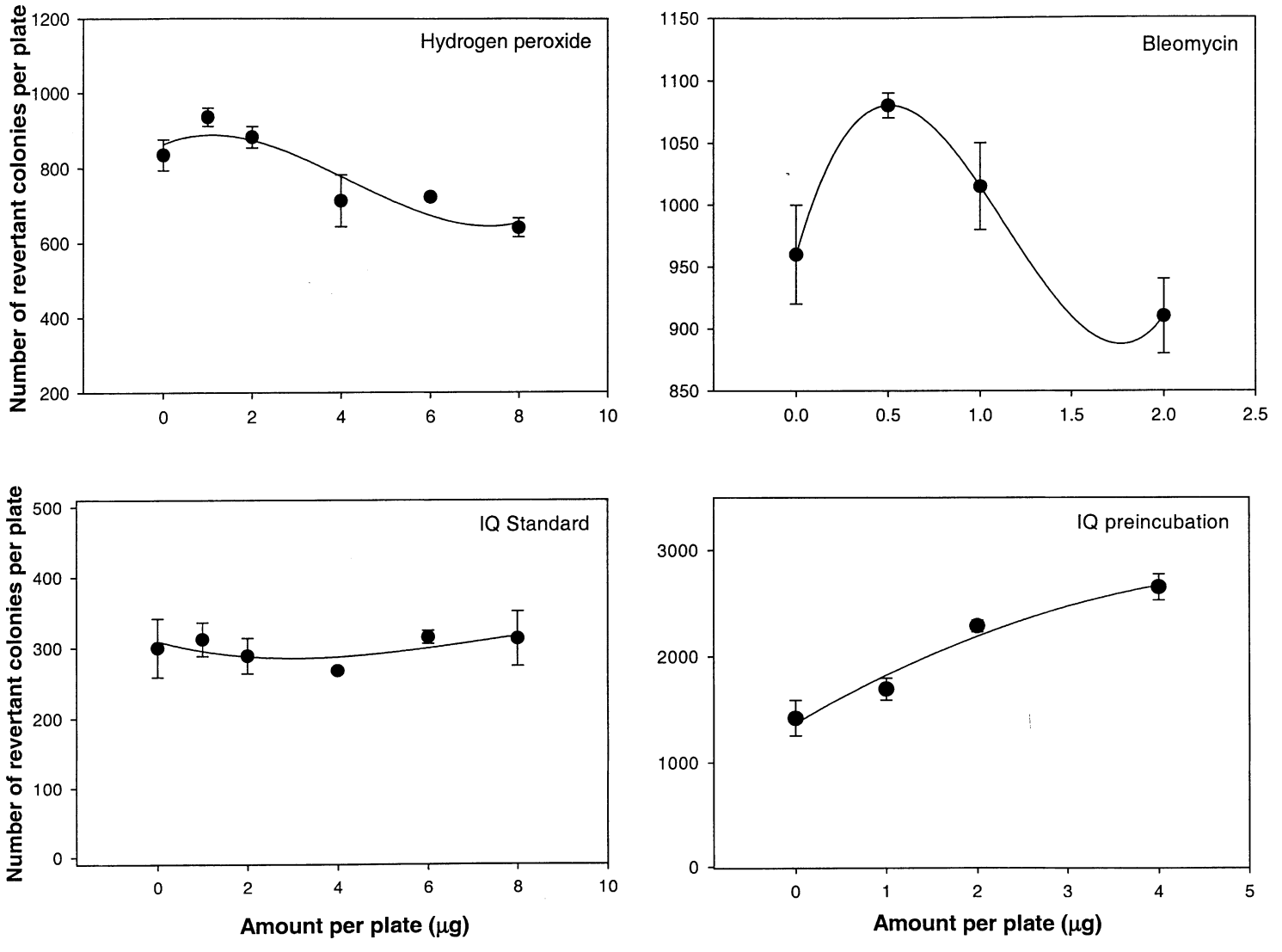

Fig. 4. Effect of curcumin on mutations induced by three different mutagens in *Salmonella typhimurium*. Different amounts of the compounds were plated with strain TA102 in the presence of 34,000 ng per plate of hydrogen peroxide, or 500 ng per plate of bleomycin, or with strain TA98 in the presence of 25 ng per plate of IQ, using standard methodologies, or 50 ng per plate of IQ using the pre-incubation protocol. Each data point represents mean ± S.E. from two experiments, each done in triplicate.

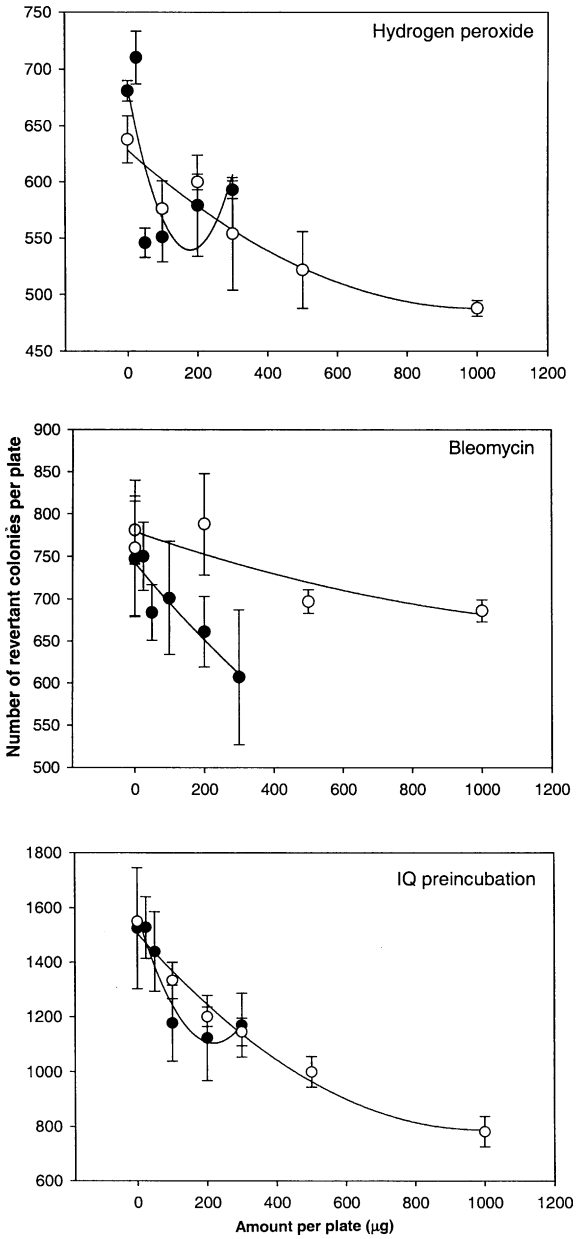

Fig. 5. Effect of 5-5-dehydroferulic acid on mutations induced by three different mutagens in *Salmonella typhimurium*. Different amounts per plate of the compound were plated with strain TA102 in the presence of 34,000 ng per plate of hydrogen peroxide, or 500 ng per plate of bleomycin, or with strain TA98 in the presence of 25 ng per plate of IQ using the pre-incubation protocol. Each data point represents mean ± S.E. from two experiments, each done in triplicate. Closed circles represent 5-5-dehydroferulic acid; open circles represent ferulic acid.

the effects of the individual hydroxycinnamic acids is consistent with the explanation that virtually all the antimutagenicity of this fraction is due to the hydroxycinnamic acids present.

The cell walls isolated from wheat coleoptiles contained more phenolic constituents measured as (*E*)-ferulic acid equivalents than did mesophyll cell walls isolated from perennial ryegrass (*Lolium perenne*) leaves [4], although the cell walls from both sources were mainly primary walls. The mesophyll cell walls yielded 10.1 mg (*E*)-ferulic acid equivalents/g cell walls [4], whereas the wheat coleoptiles cell walls in the present study yielded 16.6 mg/g cell walls. The hydroxycinnamic acids of primary cell walls of Poaceae species extracted by this method will include mostly (*E*)-ferulic acid, but will also include smaller amounts of (*Z*)-ferulic acid, (*E*)-*p*-coumaric acid, and dehydrodimers of ferulic acid [7,9,19].

Bleomycin binds to guanosine–cytosine-rich portions of DNA. In the presence of iron (Fe(II)) and molecular oxygen, this complex can lead to highly reactive free radicals and Fe(III) [26,27]. The free radical product of bleomycin is able to induce double strand breaks in DNA through oxidation of the deoxyribose sugar backbone, thereby generating chromosomal aberrations [26,27]. Hydrogen peroxide is able to generate hydroxyl radicals both extracellularly and intracellularly. The radicals are capable of inducing chromosomal aberrations through oxidative base damage as well as through direct strand breakage in the DNA [28]. As the hydroxycinnamic acids are known antioxidants [14,15], the simplest explanation of their inhibitory effect on the mutagenicity of bleomycin and hydrogen peroxide is that they reduce the concentration of mutagenic oxidation products by free radical scavenging.

Free radical scavenging effects are unlikely to explain the antimutagenicity of these compounds towards IQ. This mutagen requires metabolic activation and probably exerts its effects by covalently binding to the DNA, distorting its normal base bonding. The mutation site in TA98 contains alternating C–G sequences which are the bases preferred by IQ for bonding to cause frame-shift mutation.

Hydroxycinnamic acids have several hydrogen atoms available for abstraction and can remain stable through delocalization of electrons across the conjugated ring and sidechains. It is also possible

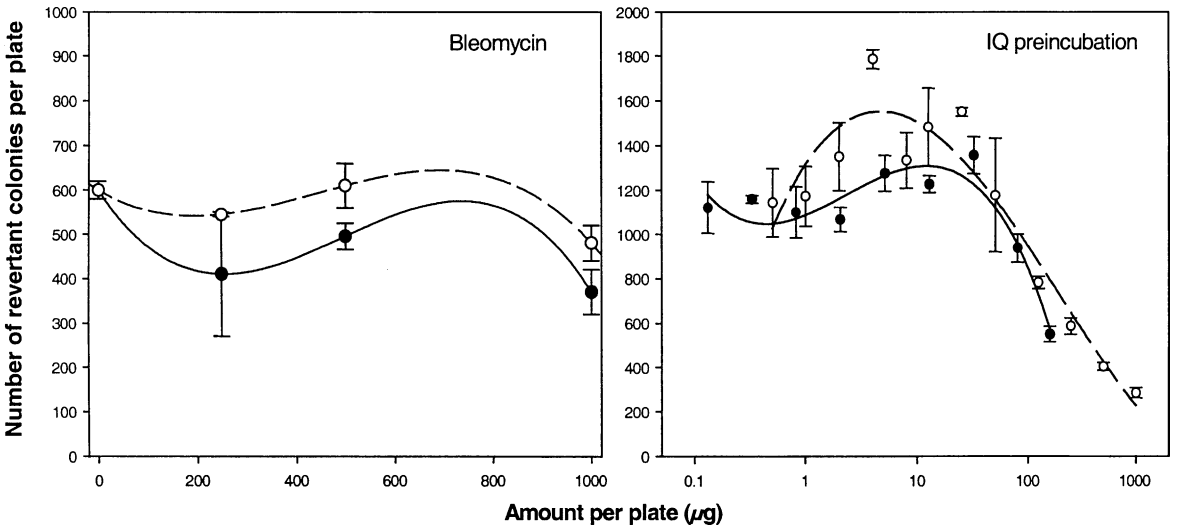

Fig. 6. Effect of cell-wall extracts as compared with ferulic acid on mutations induced by two different mutagens in *Salmonella typhimurium*. Different amounts per plate of the compounds were plated with strain TA102 in the presence of 500 ng per plate of bleomycin, or with strain TA98 in the presence of 25 ng per plate of IQ using the pre-incubation protocol. Each data point represents mean ± S.E. from two experiments, each done in triplicate. Closed circles represent the cell-wall extract acid; open circles represent ferulic acid.

that these hydrogen atoms may form hydrogen bonds with the nitrogen atoms of IQ, reducing its affinity to the DNA. This bulky complexed molecule may not be able to enter the groove and bind to the DNA as well. Thus, it could decrease the bioavailability and exposure of IQ to DNA.

In these same studies, curcumin showed both antimutagenic and co-mutagenic effects. Even though curcumin shows some structural similarity to the hydroxycinnamic acids, it did not show the consistency found for these compounds in protecting against mutations induced by reactive oxygen species and chemicals. Ahsan et al. [29] showed that in the presence of Cu(II), curcumin caused strand cleavage in DNA through generation of reactive oxygen species and that curcumin has three binding sites for Cu(II) to mediate this adverse effect. Therefore, it is likely that at different concentrations, curcumin may be able to act as either an antioxidant or as a pro-oxidant. This may explain the protective and adverse effects found in these studies. We note that although curcumin shows protection against cancer in many studies, it has also been shown to be a co-carcinogen in other studies and at other tumour sites [30]. Although it has been described as an anticarcinogen, it seems likely that it could also potentiate the development of some types of cancer.

These studies extend the possible range of biological effects of plant cell-wall phenolic compounds. Although there are obvious limitations in extrapolating bacterial antimutagenicity data to mammalian cells, such an approach provides a useful prescreen that reveals some, but not all, possible mammalian properties, as well as providing possible mechanistic explanations [20]. Given that cell-wall hydroxycinnamic acids are likely to be released in the human colon, their antimutagenic properties could have significance for dietary fibre protection against cancer. These results suggest that an increased intake of food plants with cell walls rich in ferulic acid and its dehydrodimers may be beneficial in reducing dietary carcinogen-induced mutation as well as reducing the spontaneous mutation and other mutations associated with reactive oxygen species in the body.

References

[1] H.N. Englyst, J.H. Cummings, Improved method for measurement of dietary fibre as the non-starch polysaccharides (NSPs) in plant foods, J. Assoc. Off. Anal. Chem. 71 (1988) 808–814.

[2] P.J. Harris, L.R. Ferguson, Dietary fibre: its composition and role in protection against colorectal cancer, Mut. Res. 290 (1993) 97–110.

[3] P.J. Harris, R.D. Hartley, Detection of bound ferulic acid in cell walls of the Gramineae by ultraviolet fluorescence microscopy, Nature 259 (1976) 508–510.
[4] P.J. Harris, R.D. Hartley, K.H. Lowry, Phenolic constituents of mesophyll and non-mesophyll cell walls from leaf laminae of *Lolium perenne*, J. Sci. Food Agric. 31 (1980) 959–962.
[5] R.D. Hartley, P.J. Harris, Phenolic constituents of the cell walls of dicotyledons, Biochem. Syst. Ecol. 9 (1981) 189–203.
[6] P.J. Harris, M.R. Kelderman, M.F. Kendon, R.J. McKenzie, Monosaccharide composition of unlignified cell walls of monocotyledons in relation to the occurrence of wall-bound ferulic acid, Biochem. Syst. Ecol. 25 (1997) 167–179.
[7] P.J. Harris, Compositions of monocotyledon cell walls: implications for biosystematics, in: K.L. Wilson D.A. Morrison (Eds.), Monocots: Systematics and Evolutions, CSIRO, Melbourne, 2000, pp. 114–126.
[8] B.G. Smith, P.J. Harris, Ferulic acid is esterified to glucurono-arabinoxylans in pineapple cell walls, Phytochemistry 56 (2001) 513–519.
[9] J. Ralph, S. Quideau, J.H. Grabber, R.D. Hatfield, Identification and synthesis of new ferulic acid dehydrodimers present in grass cell walls, J. Chem. Soc., Perkin Trans. 1 (1994) 3485–3498.
[10] J. Ralph, R.D. Hatfield, J.H. Grabber, H.G. Jung, S. Quideau, R.F. Helm, Cell wall cross-linking in grasses by ferulates and diferulates, in: N.G. Lewis, S. Sarkanen (Eds.), Lignin and Lignan Biosynthesis, American Chemical Society, Washington, DC, 1998, pp. 209–236.
[11] M. Bunzel, J. Ralph, J. Marita, H. Steinhart, Identification of 4-*O*-5′-coupled diferulic acid from insoluble cereal fiber, J. Agric. Food Chem. 48 (2000) 3166–3169.
[12] P.A. Kroon, C.B. Faulds, P. Ryden, J.S. Robertson, G. Williamson, Release of covalently bound ferulic acid from fiber in the human colon, J. Agric. Food Chem. 45 (1997) 661–667.
[13] L.R. Ferguson, Role of plant polyphenols in genomic stability, Mut. Res. 475 (2001) 89–112.
[14] E. Graf, Antioxidant potential of ferulic acid, Free Rad. Biol. Med. 13 (1992) 435–448.
[15] M.T. Garcia-Conesa, G.W. Plumb, K.W. Waldron, J. Ralph, G. Williamson, Ferulic acid dehydrodimers from wheat bran: isolation, purification and antioxidant properties of 8-*O*-4-diferulic acid, Redox Rep. 3 (1997) 319–323.
[16] A.W. Wood, M.T. Huang, R.L. Chang, H.L. Newmark, R.E. Lehr, H. Yagi, J.M. Sayer, D.M. Jerina, A.H. Conney, Inhibition of the mutagenicity of bay-region diol epoxides of polycyclic aromatic hydrocarbons by naturally occuring plant phenols: exceptional activity of ellagic acid, Proc. Natl. Acad. Sci. U.S.A. 79 (1982) 5513–5517.
[17] A.J. Alldrick, J. Flynn, I.R. Rowland, Effects of plant-derived flavonoids and polyphenolic acids on the activity of mutagens from cooked food, Mut. Res. 163 (1986) 225–232.
[18] J. Yamada, Y. Tomita, Antimutagenic activity of caffeic acid and related compounds, Biosci. Biotechnol. Biochem. 60 (1996) 328–329.
[19] K. Wakabayashi, T. Hoson, S. Kamisaka, Osmotic stress suppresses cell wall stiffening and the increase in cell wall-bound ferulic and diferulic acids in wheat coleoptiles, Plant Physiol. 113 (1997) 967–973.
[20] L.R. Ferguson, Antimutagens as cancer chemopreventive agents in the diet, Mut. Res. 307 (1994) 395–410.
[21] S. Quideau, J. Ralph, Lignin–ferulate cross-links in grasses. Part 4. Incorporation of 5-5-coupled diferulate into lignin, J. Chem. Soc., Perkin Trans. 1 (1997) 2351–2358.
[22] B.G. Smith, P.J. Harris, Polysaccharide composition of unlignified cell walls of pineapple (*Ananas comosus* (L.) Merr.) fruit, Plant Physiol. 107 (1995) 1399–1409.
[23] W.A. Jensen, Botanical Histochemistry: Principles and Practice, Freeman and Co., San Francisco, 1962.
[24] R.D. Hartley, E.C. Jones, Phenolic components and degradability of cell walls of grass and legume species, Phytochemistry 16 (1977) 1531–1534.
[25] S.M. Carnachan, P.J. Harris, Ferulic acid is bound to the primary cell walls of all gymnosperm families, Biochem. Syst. Ecol. 28 (2000) 865–879.
[26] R.T. Dorr, Bleomycin pharmacology: mechanism of action and resistance, and clinical pharmacokinetics, Sem. Oncol. 19 (2 (Suppl 5)) (1992) 3–8.
[27] L.F. Povirk, M.J.F. Austin, Genotoxicity of bleomycin, Mut. Res. 257 (1991) 127–143.
[28] A.R. Collins, V.L. Dobson, M. Dusinska, G. Kennedy, R. Stetina, The comet assay: what can it really tell us? Mut. Res. 375 (1997) 183–193.
[29] H. Ahsan, N. Parveen, N.U. Khan, S.M. Hadi, Prooxidant, antioxidant and cleavage activities on DNA of curcumin and its derivatives demethoxycurcumin and bisdemethoxy-curcumin, Chem. Biol. Interact. 121 (1999) 161–175.
[30] N. Frank, J. Knauft, F. Amelung, J. Nair, H. Wesch, H. Bartsch, No prevention of liver and kidney tumors in long-Evans cinnamon rats by dietary curcumin but inhibition at other sites and of metastases, Mut. Res. 523–524 (2003) 127–136.