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Flavone inhibits mutagen formation during heating in a glycine/creatine/glucose model system

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2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MelQx) and 2-amino-3,7,8-trimethylimidazo[4,5-*f*] quinoxaline (7,8-DiMelQx) were formed in a glycine/creatine/glucose model system. Addition of flavone into this model mixture gave a dose-related inhibition of mutagen formation. Furthermore, the major mutagenic fractions of basic extracts from the heated model mixture, with or without flavone, were purified and analysed by high-performance liquid chromatography. The 10-μmol flavone was present during heating in the model mixture and the mutagenic activities of MelQx and 7,8-DiMelQx were lowered by 31·3 and 27·8%, respectively. The inhibitory effects of flavone in the heated model system are possibly attributable to the reduction of the formation of Maillard reaction products. The findings may be a useful means for reducing the formation of mutagens of the 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) type during food processing.

INTRODUCTION

Specific heterocyclic amines are a potent class of food-borne mutagens produced by frying or broiling of meat or fish (Overvik *et al.*, 1984; Knize *et al.*, 1987) and refluxing of appropriate precursor extracts in a liquid-refluxing model system (Jagerstad *et al.*, 1983, 1984, 1986; Grivas *et al.*, 1985; Nes, 1987; Felton *et al.*, 1986). They have been demonstrated to be multipotential carcinogens in rodents (Sugimura, 1985). It is believed that many cases of (particularly) stomach and bowel cancers are caused by factors in the diet (Weisburger, 1986; Overvik & Gustafsson, 1990). Therefore, it is important to identify dietary factors responsible and thus to eliminate the formation of cooked-food mutagens during cooking processes.

Flavone is a naturally occurring flavonoid that is widely distributed in plants, particularly in edible and medicinal plants (Brown, 1980). Previous results showed that flavone exhibited a higher inhibitory effect on the mutagenicity of 2-amino-3-methylimidazo[4,5-*f*]-quinoline (IQ) and 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]-indole (Trp-P-2) by directly reducing the hepatic-microsomal activation level (Lee & Tsai, 1991). In the present studies, flavone was found to be an inhibitor of the formation of IQ-type mutagens inglycine/creatine/glucose refluxing model systems.

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MATERIALS AND METHODS

Chemicals

Flavone was purchased from Tokyo Kaisei Kogyo Co. (Tokyo, Japan). Glycine, creatine, glucose, glucose-6-phosphate, Amberlite XAD-2 and nicotinamide adenine dinucleotide phosphate (NADP) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and organic solvents were purchased from E. Merck Co. (Darmstadt, Germany). 2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MelQx) and 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline (7,8-DiMelQx) were kindly supplied by Dr Grivas of the Swedish University of Agriculture Sciences (Uppsala, Sweden).

Glycine/creatine/glucose refluxing model system

The methods described by Jagerstad *et al.* (1984) were used for the preparation of the model system and isolation of the mutagenic compounds. Glucose (17·5 mmol), glycine (35·0 mmol) and creatine (35·0 mmol), with or without flavone, were added to a medium (105 ml) of diethylene glycol containing 14% distilled water. After refluxing for 2 h, the boiled mixture was diluted with distilled water (400 ml) and subjected to Amberlite XAD-2 column chromatography (1·5 × 15 cm) to remove diethylene glycol. The column was washed with 500 ml of distilled water, and then the mutagenic fraction was eluted with 200 ml of methanol. The methanol fraction was evaporated to dryness. The residue was redissolved in 30 ml of 0·1 M hydrochloric acid and extracted three times with dichloromethane. The remaining aqueous fraction was then adjusted to pH 12·0 with 6·0 M sodium hydroxide and extracted three times with dichloromethane. The basic fraction thus obtained was redissolved in methanol for high-performance liquid chromatography (HPLC) purification and analysis.

HPLC purification and analysis

Partial purification by HPLC was carried out by using a semi-preparative μBondapack column (10-μm particle, 10 × 250 mm, Millipore Co., Milford, MA, USA) with a Waters 600 E controlled system and a model U6K injector coupled to a Model 441 UV detector (Millipore Co.). The HPLC separation conditions were performed as described previously (Lee & Tsai, 1991). The mobile phase was methanol: 10 mM hydrogen phosphate–sodium hydroxide (55 : 45, v/v, pH 7·2) at a flow rate of 1·5 ml/min. The elutants were collected by the fraction collector and 0·1 ml of each fraction was evaporated to dryness and re-dissolved in dimethylsulphoxide (DMSO) for mutagenicity testing. The active fractions were pooled and further analysed by HPLC analysis.

Active fraction (100 μg) was injected into a Lichrospher 100 RP-18 column (5-μm particle, 4·0 × 250 mm, E. Merck Co., Darmstadt, Germany) with the mobile phase, acetonitrile/water/diethylamine (18 : 82 : 01, v/v/v) at a flow rate of 1 ml/min (Lee & Tsai, 1991). The effluents were monitored at 254 nm and were also collected for mutagenicity testing as described above. The mutagenic compounds were determined by comparing the retention times of MelQx and 7,8-DiMelQx as standard compounds.

Mutagenicity assay

The samples were tested with *Salmonella typhimurium* TA98 strain for mutagenicity by the *Salmonella*/microsomal test described by Maron and Ames (1983). The bacterial strain was kindly supplied from Dr B. N. Ames (University of California, Berkeley, CA, USA). The liver homogenate supernatant (S9) was prepared from the liver of male Sprague-Dawley rats (180–200 g), which were treated with Aroclor 1254, as also described by Maron and Ames (1983).

Table 1. Effects of flavone on the mutagenic activity of basic extracts of heated glycine/creatine/glucose model mixture with *S. typhimurium* TA98 in the presence of S9 mix

Flavone (μmol)	Specific mutagenic activity (revertant/25 μg)	Extract amount (mg)	Total mutagenic activity (revertant × 10^5)	PI[a] (%)
0	1 730	53·6	37·1	0·0
10	1 235	65·7	32·5	12·4
20	1 529	49·7	30·4	18·1
40	1 336	29·6	15·8	57·4

[a] PI = (total mutagenic activity in the presence of flavone/total mutagenic activity in the absence of flavone) × 100.

RESULTS AND DISCUSSION

Table 1 shows that the mutagenic activity of basic extracts of the heated glycine/creatine/glucose model mixture was decreased by the addition of flavone with a dose-related response relationship. The mutagenic fractions of extracts of the heated model mixtures with or without flavone from semi-preparative HPLC were further analysed by HPLC and the mutagenicity of the effluents was determined using the strain of *S. typhimurium* TA98. The distribution of mutagenic activity on an analytical HPLC is shown in Fig. 1 and Table 2. The mutagenic fractions, 10–12 and 15–17, correspond to the peaks of authentic MelQx and 7,8-DiMelQx. The mutagenic activity of MelQx and 7,8-DiMelQx in extracts of the heated model mixture with added flavone exhibited 31·3 and 27·8% PI value, respectively. These results suggest that flavone could be an inhibitor of the formation of IQ-type mutagens in the glycine/glucose/creatine model system.

In previous studies, L-tryptophan has been shown to

Table 2. The distribution of mutagenic activity of heated glycine/creatine/glucose mixture, with or without flavone (10 μmol), using HPLC analysis

Mutagens	Glycine/creatine/glucose[a]	Glycine/creatine/glucose/flavone[a]	PI (%)[b]
Unknown	92	5	94·5
MelQx	1 423	1044	31·3
7,8-DiMelQx	615	444	27·8

[a] The revertants were calculated from the results of the mutagenicity test in the HPLC analysis (Fig. 1).

[b] PI = (the total revertants of mutagenic fractions in heated model mixture with flavone/the total revertants of mutagenic fractions in heated mixture without flavone) × 100.

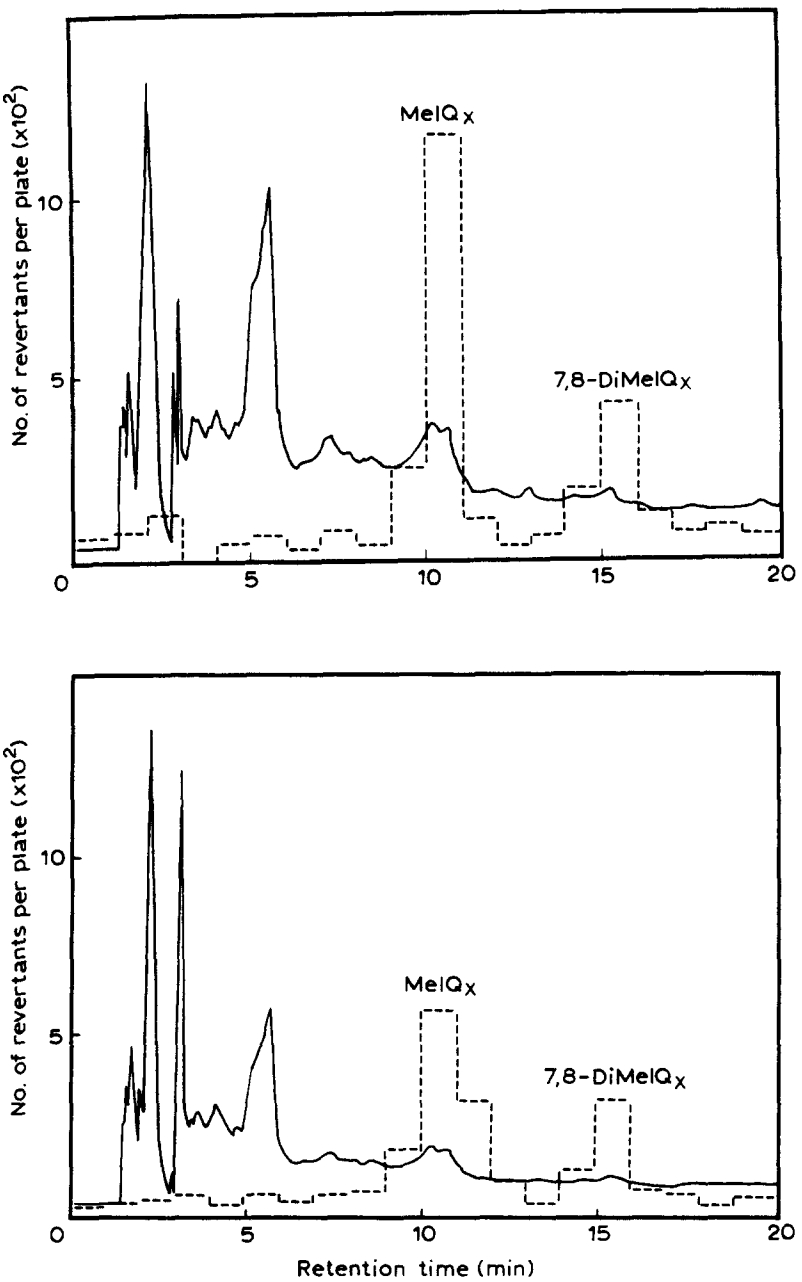

Fig. 1. Mutagenicity induced in TA98 and analytical HPLC profiles of the active fractions of heated model mixture extracts on a Lichrospher column. (----, no. of revertants; —, absorbance).

decrease mutagenic activity in both models of heated glycine/creatine/glucose mixtures and fried or boiled meat (Negishi *et al.*, 1984; Jones & Weisburger, 1988). Beef was fried with soy protein concentrate and wheat gluten, and chlorogenic acid and butylated hydroxyanisole (BHA) were also demonstrated to have reducing effects on mutagenicity (Wong *et al.*, 1982). Fukuhara *et al.* (1981) found that the mutagenic activity and the contents of 2-amino-9*H*-pyrido[2,3-*b*]indole in pyrolysates of albumin were decreased by the addition of tannic acid, quercetin, rutin, catechin, *n*-propyl gallate and BHA. This indicates that natural or synthetic antioxidants might retard the Maillard reaction in heated model mixtures. Some Maillard reaction intermediate compounds have been found to involve mutagen production (Jagerstad *et al.*, 1983) and are also proposed as precursors of IQ-type mutagens (Jagerstad *et al.*, 1983, 1986). Therefore, the inhibitory effects of flavone in the heated model system is possibly mediated through reducing the formation of Maillard reaction products.

In the home, meat is frequently cooked with various plant foods, such as potato and carrot, which contain various flavonoids, including flavone (Brown, 1980; Wollenweber & Dietz, 1981). The cooking conditions may eliminate the mutagen formation in pan-drippings and gravy, which are considered to be a significant additional source of mutagens or carcinogens in foods (Overvik *et al.*, 1987). The present findings may offer a useful means of reducing the formation of cooked-food carcinogens and of minimizing the hazard to human health.

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