Effect of Glucose–Lysine Maillard Reaction Products on Bacterial and Mammalian Cell Mutagenesis

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A glucose–lysine (gluc–lys) browning reaction mixture and its fractionated components were examined for mutagenic (*Salmonella typhimurium* strains TA-100 and TA-98) and clastogenic (chromosome aberration) activities, both in the presence and in the absence of a liver S-9 mix. Mutagenic activity was observed with the gluc–lys Maillard reaction products (MRP) in both TA-98 and TA-100 test strains without S-9 pretreatment but was reduced in TA-100 and eliminated totally in TA-98 with added S-9. Considerable variation in active dose ratios for both intact and nonvolatile neutral and basic fractions of MRP and melanoidin fractions was found with different short-term tests used to estimate genotoxicity. Clastogenic responses were observed in both MRP reactant intermediates and high molecular weight melanoidins without S-9 and were reduced in all fractions with S-9 pretreatment.

INTRODUCTION

The application of heat to food for the purpose of cooking or thermal processing enhances the reaction of various amino acids and proteins with reducing sugars to form nonenzymatic Maillard reaction products (MRP) (Hodge, 1953; Powrie *et al.*, 1986). The reaction products impart desirable aromas and brown color to the food, for enhancing palatability. Considerable research has been directed toward the mutagenic properties of MRP in both yeast (*Saccharomyces cerevisiae*; Rosin *et al.*, 1982) and bacterial assays (Bjeldanes and Chew, 1979; Shinohara *et al.*, 1980; Powrie *et al.*, 1981; Wei *et al.*, 1981; Cuzzoni *et al.*, 1988, 1989) and mammalian cells *in vitro* (Stich *et al.*, 1980; Powrie *et al.*, 1981; Lynch *et al.*, 1983; Vagnarelli *et al.*, 1991). With the Ames *Salmonella typhimurium* TA-98 and TA-100 strains, mutagenic responses occurred in heated model systems consisting of various types of amino acids and glucose, fructose, or ribose (Powrie *et al.*, 1981; Cuzzoni *et al.*, 1988). Other studies have reported antimutagenic activity of MRP (Chan *et al.*, 1982). Despite the present knowledge concerning the complex series of reactions between precursor amines and reducing sugars in model systems, there is little information on the relative mutagenic activity of different chemical fractions that comprise both low (carbonyl compounds) and high (melanoidins) molecular weight fractions.

In the present study, MRP in a heated lysine–glucose model system were fractionated by sequential ethyl ether and ethanol extractions into various molecular weight compounds. The fractions were examined for mutagenic and clastogenic activities using a battery of *in vitro* tests for the purpose of further assessing the genotoxic potential of MRP.

MATERIALS AND METHODS

Glucose–Lysine Browning Model System Preparation. An aqueous solution of 0.8 M D-glucose and 0.8 M L-lysine (reagent grade) was prepared in distilled water. The reaction solution was adjusted to pH 9.0 and heated at 121 °C for 1.0 h in an autoclave. An aliquot of the resulting dark brown reaction mixture was retained and the remainder extracted sequentially with ethyl ether under acidic (pH 2), neutral (pH 7.0), and basic (pH 11) conditions by liquid–liquid partition procedures. These fractions represented low molecular weight nonvolatile components. The residual aqueous soluion was re-extracted with absolute ethanol to a final proportion of 90% by volume. The ethanol suspension was centrifuged to yield supernatant and precipitate fractions. All fractions were brought to pH 7.0 before being tested for mutagenicity or clastogenic activity.

***In Vitro* Mutagenicity Assays.** Bacterial mutagenicity assays were performed using *S. typhimurium* TA-98 and TA-100 histidine-dependent mutant tester strains, both with and without microsomal S-9 pretreatment as described by Powrie *et al.* (1981). The microsomal activation mixture was prepared from liver tissue supernatant collected from male Wistar rats that were pretreated with Aroclor 1254. The spontaneous mutation frequencies with and without S-9 treatment were 39 ± 3.0 and 20 ± 4.0 revertants/plate, respectively, in the TA-98 strain, and the positive control using 2-nitroflurene (10 μg/plate) was 763 ± 28.9 revertants/plate. With the tester strain TA-100, the spontaneous mutation rates were 97 ± 9 revertants/plate without S-9 pretreatment and 103 ± 7 revertants/plate with S-9. The positive control using sodium azide (3 μg/plate) yielded 1084 ± 118 revertants/plate. Mutagenic activity tested with S-9 microsomal mixture was confirmed with benzo[*a*]pyrene (10 μg/plate = 594 ± 18.5 revertants/plate). The mutagenic activity recorded in both strains was corrected for spontaneous mutation. A dose response curve for each fraction tested was based on the minimal toxicity for the reaction mixture to the *Salmonella* tester strain.

Chromosomal Aberration Assay. Clastogenic activity from the gluc–lys reaction product and its fractions was tested using Chinese hamster ovary (CHO) cells, both with and without the Aroclor 1254 pretreatment mixture. The culture medium consisted of Eagle's minimal essential media (MEM) supplemented with 10% fetal calf serum, 1 mg/mL sodium bicarbonate, and an antibiotic mixture (streptomysin sulfate 29.6 μg/mL; penicillin 125 μg/L; kanamycin 100 μg/mL; fungizone 2.5 μg/mL). The concentrations of various reaction product fractions in the test assays were chosen on the basis of preliminary studies that identified both toxic and active dose concentrations. Chromosomal aberrations were detected by employing approximately 140 000 CHO cells to a 22-mm coverslip, positioned in a 3.5-cm Petri dish (Falcon plastics) containing MEM with 10% fetal calf serum at 37 °C for 2–3 days (Powrie *et al.*, 1981). Experiments were initiated after cells became 40–60% confluent. Colchicine (0.1%) in 2.5% MEM was added to the incubated samples at 16 h postexposure to Maillard reaction fractions to estimate the

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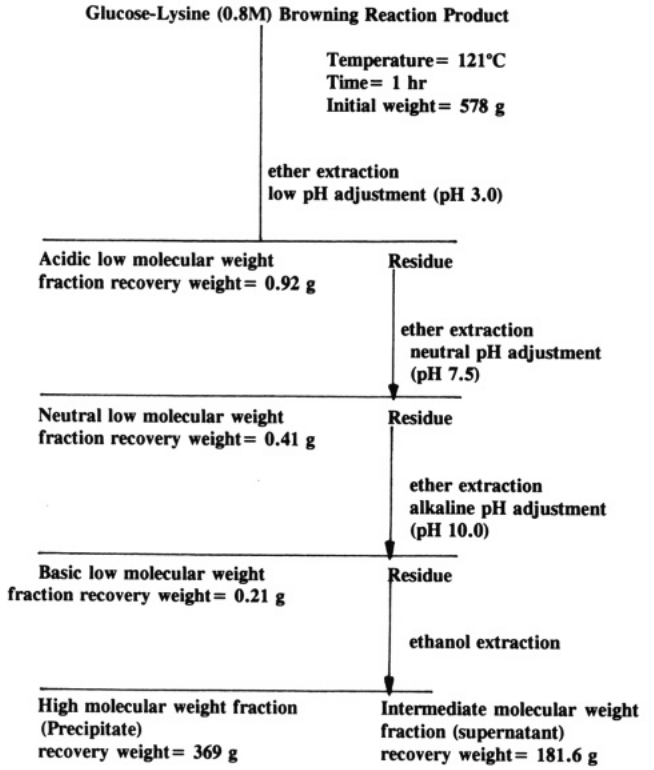

Figure 1. Flow chart of browning reaction product fractions.

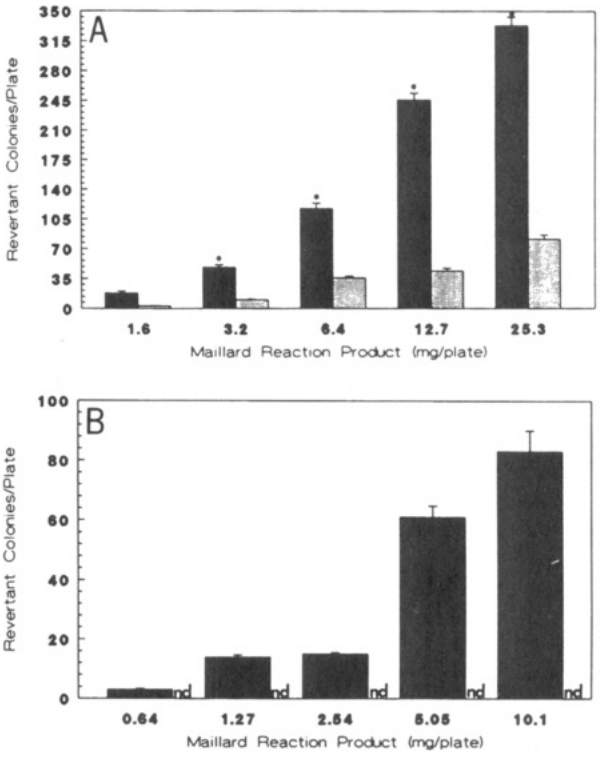

Figure 2. Mutagenicity response of intact gluc–lys MRP. [Values are means ± SD (n = 4). * = $P \leq 0.05$.] (A) *S. typhimurium* TA-100 strain. (Black bar) –S9 liver mix (spontaneous mutagenic activity = 97 ± 9 revertants/plate); (shaded bar) +S9 liver mix (spontaneous mutagenic activity = 103 ± 7 revertants/plate). (B) *S. typhimurium* TA-98 strain. (Black bar) –S9 liver mix (spontaneous mutagenic activity = 20 ± 4 revertants/plate); (shaded bar) +S9 liver mix (spontaneous mutagenic activity = 39 ± 3 revertants/plate). nd indicates that no detectable mutagenic activity above background was found.

chromosomal aberration frequency. Cells were fixed with ethanol–acetic acid (3:1) on glass slides and stained with 2% orcein in 50% acetic acid and water. For each test fraction examined, 200 metaphase plates were analyzed for chromosomal exchanges and chromatid breaks according to the method described by Powrie *et al.* (1981).

All data are expressed as mean ± SD. All samples were assayed in duplicate. Different mutagenic responses observed from MRP fractions with and without S-9 mix, within the same exposure dose, were analyzed by Student's t-test ($P \leq 0.05$).

RESULTS AND DISCUSSION

The gluc–lys model Maillard reaction system used in this study was chosen because of extensive information reported previously concerning the kinetics of this reaction (Pomeranz *et al.*, 1962; Powrie *et al.*, 1981). The fractionation procedure and subsequent recoveries of various intermediates isolated from the gluc–lys browning reaction model are presented in Figure 1. The collective recovery for all fractions was 96% (w/w) of the starting material. The majority of the MRP was recovered in the ethanol dispersion, with about 64% as the precipitate and 32% in the ethanol supernatant. MRP intermediates in acidic (0.16%), neutral (0.07%), and basic (0.04%) fractions were recovered in the ether extract fractions. These results indicate that the time, temperature, and molar ratio concentration of reactants used herein favored the high molecular weight melanoidin pigments, representing the final stages of Maillard browning reactions.

A dose response of mutagenic activity was observed with the unfractionated MRP system in both base pair substitute (TA-100; Figure 2A) and frame shift (TA-98; Figure 2B) mutant histidine auxotrophs when incubated in the absence of S-9 mix. With the Ames test, *S. typhimurium* strains TA-100, TA-102, and TA-104 detect mutagens causing base pair substitution, whereas strain TA-98 is used to measure the frame shift mutation (Maron and Ames, 1983). Mutagenic activity of the unfractionated MRP system incubated with S-9 was significantly ($P \leq 0.05$) reduced with the TA-100 test strain and absent with the TA-98 strain. Spingarn and Garvie (1979) reported a greater mutagenic response in sugar (rhamnose)–ammonia model systems following S-9 pretreatment in *Salmonella* frame shift organisms. Both bacterial strains were less responsive without metabolic activation. In contrast, Aeschbacher *et al.* (1981) observed greater mutagenic responses with a gluc–arg model browning reaction using TA-100 strain, compared to the TA-98 strain. The relative response between bacterial strains was reversed when fractions were pretreated with S-9 mix. In the present study, although the TA-98 strain provided greater sensitivity in detecting mutagens, the mutagenic response to MRP fractions was consistently greater over the nonlethal dose range using the TA-100 test strain (Figures 2–7). This result is evidence to conclude that different *Salmonella* strains and metabolic activation patterns occur when the mutagenic activity of different browning model systems is tested. The different mutagenic indices noted between tester strains in this and other studies likely involve not only the presence of different mutagenic reactants derived from various sugar–amino acid mixtures but also the relative sensitivity to toxic properties of Maillard reaction intermediates known to interfere with the Ames test (Shibamoto, 1980) and the chromosome aberration test (Vagnarelli *et al.*, 1991). The latter may ultimately mask the true mutagenic activity. To overcome this limitation and accurately estimate the relative genotoxic contribution of specific fractions derived from the model browning reaction, a maximum active dose weight applied to the assay and correspnding dose ratio, computed for both the *Salmonella* mutagenicity assay and the mammalian chromosomal aberration test, was used

Table I. Summary of Relative Active Doses from Model Glucose–Lysine Maillard Reaction Products Used with *in Vitro* Mutagenicity Testing

browning reaction fraction	*Salmonella* test: TA-98 active dose wt[a] (mg)	TA-98 dose ratio[b]	TA-100 active dose wt (mg)	TA-100 dose ratio	chromosome aberration test, chinese hamster ovary: active dose wt (mg)	chromosome aberration test, chinese hamster ovary: dose ratio
initial BRP						
ether extracts of low molecular wt fractions	10	1.73×10^{-5}	25	4.3×10^{-5}	2.17	0.38×10^{-5}
acid fraction	0.05	5.4×10^{-5}	0.05	5.4×10^{-5}	0.105	11×10^{-5}
neutral fraction	0.10	24.6×10^{-5}	0.20	49×10^{-5}	0.254	62×10^{-5}
basic fraction	0.05	5.4×10^{-5}	0.15	70×10^{-5}	0.127	59×10^{-5}
ethanol extracts of residue						
high molecular wt ppt (melanoidins)	15	4.1×10^{-5}	100	27×10^{-5}	3.75	1×10^{-5}
intermediate molecular wt (supernatant)	50	27.5×10^{-5}	100	55×10^{-5}	30	16×10^{-5}

[a] Active dose weight is the amount applied to the assay system. [b] Active dose ratio = active dose weight/original quantity recovered.

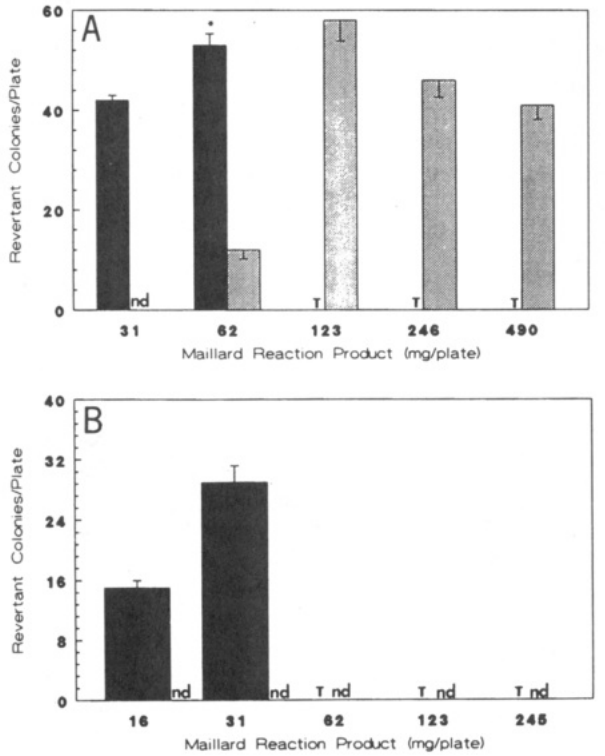

Figure 3. Mutagenicity response of MRP acidic fraction. [Values are means ± SD (n = 4). * = $P \leq 0.05$.] (A) *S. typhimurium* TA-100 strain (Black bar) –S9 liver mix (spontaneous mutagenic activity = 97 ± 9 revertants/plate); (shaded bar) +S9 liver mix (spontaneous mutagenic activity = 103 ± 7 revertants/plate). T indicates toxic activity; nd indicates that no detectable mutagenic activity above background was found. (B) *S. typhimurium* TA-98 strain. (Black bar) –S9 liver mix (spontaneous mutagenic activity = 20 ± 4 revertants/plate); (shaded bar) +S9 liver mix (spontaneous mutagenic activity = 39 ± 3 revertants/plate). T indicates toxic activity.

(Table I). The maximum MRP dose ratio was defined as the nontoxic dose applied to the assay, expressed as a proportion of the original quantity of reactant material recovered. In the specific cases of both the high and low melanoidin fractions, smaller active doses eliciting a genotoxic response were obtained in the CHO test as compared to both bacterial strains in the Ames test. This result confirms the potential for different genotoxic responses to be derived from specific compounds in various microbial and mammalian bioassays and the importance of employing more than one short-term test for predicting mutagenicity.

The acidic MRP fraction without S-9 treatment possessed low mutagenicity scores with both the TA-100 (Figure 3A) and TA-98 (Figure 3B) strains at low dose ranges (30–60 μg/plate). The loss of mutagenic activity, attributed to the toxicity of the acidic fraction, was observed at higher dose applications. Preincubation of the acidic fraction with the S-9 mix resulted in detoxification of constituents that were toxic to the TA-100 strain, as evidenced by the maximal mutagenicity observed at 123 μg/plate. In the mammalian chromosomal bioassay, the acidic fraction contributed to the greatest percentage of numerical aberrations when incubated in the absence of S-9 mix. The chromosomal aberrations were characterized by relatively large numbers of chromosomal breaks and exchanges (Table II). Structural aberrations were reduced when the acidic fraction was preincubated with microsomal enzymes. The relative small amount of mutagenic and genotoxic activity observed in the acidic fraction may be attributed to glyoxylic acid, which is produced from reactive aldehyde groups of the dicarbonyl intermediate, glyoxal. Other workers have similarly reported mutagenic activity from glyoxylic acid in the TA-100 tester strain (Yamaguchi and Nakagawa, 1983).

The neutral MRP fraction exhibited similar relative mutagenic activity, in both the *S. typhimurium* TA-100 and TA-98 strains without and with S-9 pretreatment. One exception was the significantly ($P \leq 0.05$) greater mutagenicity observed with S-9 pretreatment with the TA-100 strain at the second highest dose (111 mg/plate) of MRP (Figure 4A). This response was not observed in the TA-98 strain (Figure 4B). A toxic reaction to both strains of bacteria was noted at the highest dosages without S-9 pretreatment. Pretreatment of the largest dose of neutral MRP fractions with S-9 mix resulted in the highest mutagenic response. These results support previous studies that have identified various dicarbonyl compounds generated from aldehyde and ketone oxidation, such as maltol, glyoxal, and diacetyl, to yield a mutagenic dose response in TA-100 but not TA-98 strains (Bjeldanes and Chew, 1979). Methylglyoxal, reported to be the only mutagenic component in roasted coffee beans containing large amounts of MRP (Fujita *et al.*, 1985), could also have contributed to this activity. Alternatively, furan compounds derived from sugar dehydration reaction in the Amadori pathway represent additional sources of genotoxic compounds in the neutral fraction. Shinohara *et al.* (1986) found that furans incubated with S-9 mix had greater mutagenicity when tested with the TA-100 strain as compared to the TA-98 strain. According to Cuzzoni *et al.* (1989), the mutagenicity of Maillard reaction mixtures was associated with an increase in furfural content. Moreover, *in vitro* clastogenic activity of furfural and furfuryl alcohol has been observed with CHO cells (Stich *et al.*, 1980) and human lymphocytes (Gomez-Arroyo and Souza, 1985). The formation of 5-methylfurfural and

Table II. Effect of Heated Glucose–Lysine Solution and Nonvolatile Fraction Products on Chromosome Aberrations in Chinese Hamster Ovary Cells[a]

	number of structural aberrations					
	% numerical aberrations (% metaphases)		chromosome breaks (per cell)		chromosome exchanges (per cell)	
	−S9	+S9	−S9	+S9	−S9	+S9
gluc–lys BRP, no extraction						
2.89 mg/mL	31.4 (0.28)	1.8 (0.01)	0.94 (0.01)	0.1 (0.01)	0.28 (0.01)	0.01
2.17 mg/mL	25.5 (0.19)	0	0.82 (0.01)	0	0.19 (0.01)	0
gluc–lys BRP, extraction						
2.89 mg/mL	70.0 (0.31)	1.8 (0.02)	1.2 (0.1)	0	0.27 (0.02)	0.02
acidic fraction						
105 μg/mL	67.3 (0.29)	2.7 (0.04)	2.25 (0.05)	0.04	0.29 (0.04)	0
neutral fraction						
254 μg/mL	*[b]	33.6 (0.09)	*	0.80 (0.01)	*	0.05
31.7 μg/mL	38.2 (0.12)	0	0.89 (0.01)	0	0.12 (0.02)	0
basic fraction						
127.5 μg/mL	*	13.6 (0.02)	*	0.19 (0.01)	*	0.02
21.3 μg/mL	26.9 (0.07)	0	0.58 (0.02)	0	0.07 (0.01)	0
melanoidin						
3.75 mg/mL	36.4 (0.28)	2.7 (0.02)	1.14 (0.05)	0.01	0.28 (0.06)	0.02
ethanol supernatant						
30.0 mg/mL	31.8 (0.19)	11.8 (0.04)	1.09 (0.03)	0.15 (0.05)	0.19 (0.05)	0.04

[a] Values represent mean of duplicated chromosomal assays using Chineses hamster ovary cells. Average number of chromosome aberrations for untreated controls was zero. Values in parentheses are standard deviations. +S9, with S9 microsomal mixture; −S9, without S9 microsomal mixture. [b] An asterisk indicates toxic activity.

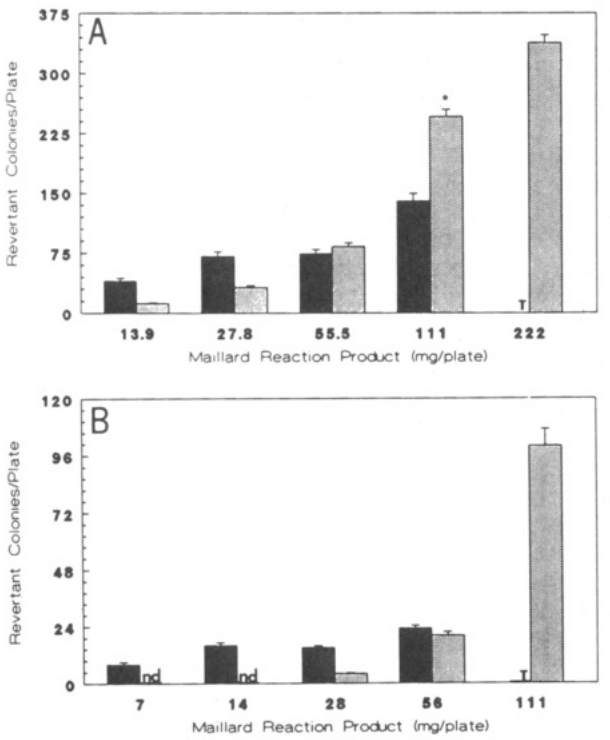

Figure 4. Mutagenicity response of MRP neutral fraction. (A) *S. typhimurium* TA-100 strain. (Black bar) −S9 liver mix (spontaneous mutagenic activity = 97 ± 9 revertants/plate); (shaded bar) +S9 liver mix (spontaneous mutagenic activity = 103 ± 7 revertants/plate). T indicates toxic activity. (B) *S. typhimurium* TA-98 strain. (Black bar) −S9 liver mix (spontaneous mutagenic activity = 20 ± 4 revertants/plate); (shaded bar) +S9 liver mix (spontaneous mutagenic activity = 39 ± 3 revertants/plate). T indicates toxic activity; nd indicates that no detectable mutagenic activity above background was found.

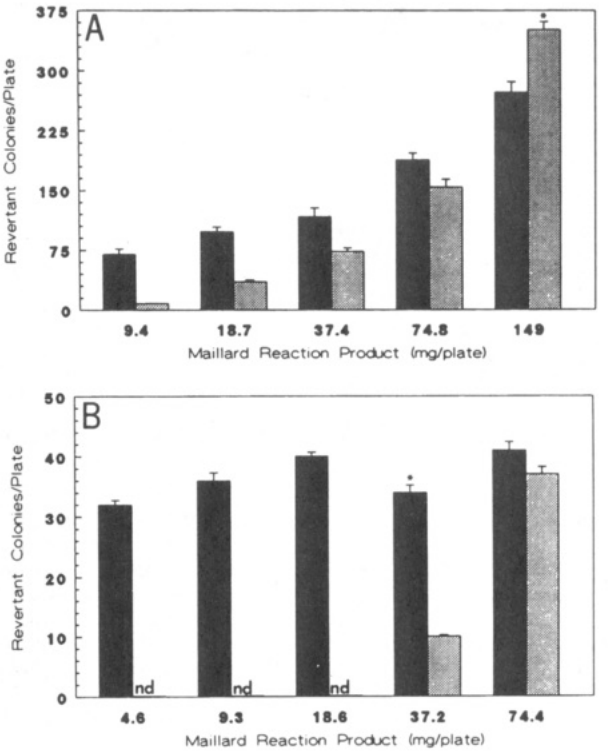

Figure 5. Mutagenicity response of MRP basic fraction. [Values are means ± SD (n = 4). * = $P \leq 0.05$.] (A) *S. typhimurium* TA-100 strain. (Black bar) −S9 liver mix (spontaneous mutagenic activity = 97 ± 9 revertants/plate); (shaded bar) +S9 liver mix (spontaneous mutagenic activity = 103 ± 7 revertants/plate). (B) *S. typhimurium* TA-98 strain. (Black bar) −S9 liver mix (spontaneous mutagenic activity = 97 ± 9 revertants/plate); (shaded bar) +S9 liver mix (spontaneous mutagenic activity = 103 ± 7 revertants/plate). nd indicates that no detectable mutagenic activity above background was found.

5-(hydroxymethyl)furfural by the reaction of hexose sugar or furfural with pentose sugar–amino acid mixtures occurs via the 1,2-eneaminol pathway from Amadori compounds. Triose reductones have also been reported to exhibit mutagenicity in the TA-100 tester strain (Omura *et al.*, 1978). The dicarbonyl breakdown products of the Strecker degradation reaction are produced in the 2,3-enediol pathway that yield a variety of fission products, including methylated reductones, aldehydes, and other neutral dicarbonyls (Powrie *et al.*, 1986).

Figure 5A shows the dose response revertants induced following exposure to the basic MRP fraction for *S. typhimurium* TA-100. A similar dose response was not obtained with the TA-98 strain tested under the same

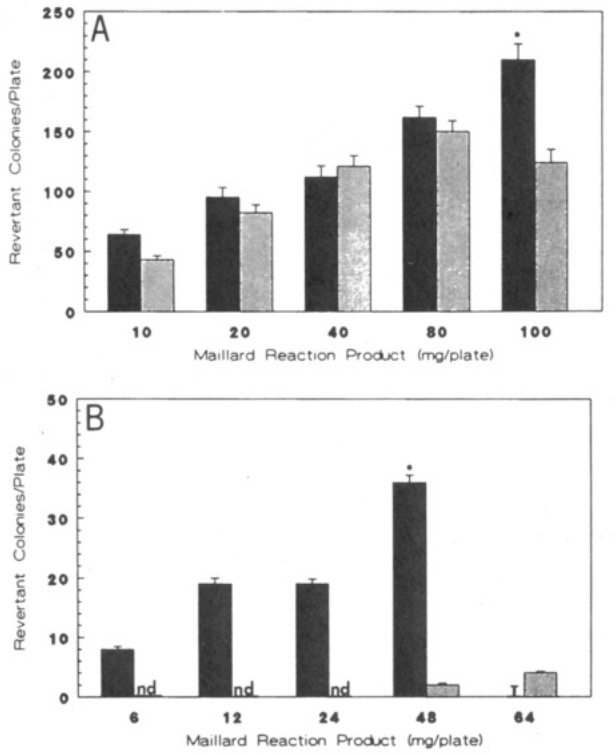

Figure 6. Mutagenicity response of MRP intermediate molecular weight fraction. [Values are means ± SD ($n = 4$). * = $P \leq 0.05$.] (A) *S. typhimurium* TA-100 strain. (black bar) –S9 liver mix (spontaneous mutagenic activity = 97 ± 0 revertants/plate); (shaded bar) +S9 liver mix (spontaneous mutagenic activity = 103 ± 7 revertants/plate). (B) *S. typhimurium* TA-98 strain. (Black bar) –S9 liver mix (spontaneous mutagenic activity = 20 ± 4 revertants/plate); (shaded bar) +S9 liver mix (spontaneous mutagenic activity = 39 ± 3 revertants/plate). T indicates toxic activity; nd indicates that no detectable mutagenic activity above background was found.

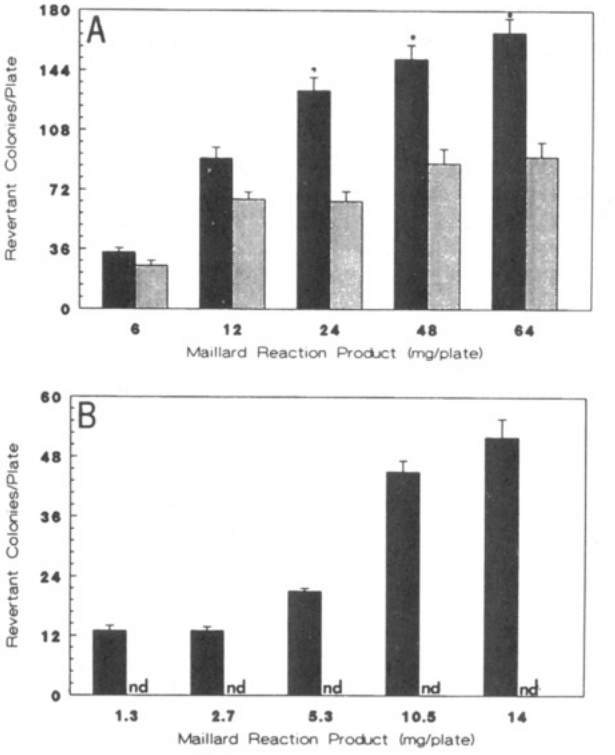

Figure 7. Mutagenicity response of MRP high molecular weight fraction. [Values are means ± SD ($n = 4$). * = $P \leq 0.05$.] (A) *S. typhimurium* TA-100 strain. (Black bar) –S9 liver mix (spontaneous mutagenic activity = 97 ± 9 revertants/plate); (shaded bar) +S9 liver mix (spontaneous mutagenic activity = 103 ± 7 revertants/plate). (B) *S. typhimurium* TA-98 strain. (Black bar) –S9 liver mix (spontaneous mutagenic activity = 20 ± 4 revertants/plate); (shaded bar) +S9 liver mix (spontaneous mutagenic activity = 39 ± 3 revertants/plate. nd indicates that no detectable activity above background was found.

conditions, both with or without the S-9 mixture (Figure 5B). Only the highest dose of basic MRP resulted in significantly ($P \leq 0.05$) greater mutagenicity following S-9 pretreatment with TA-100. The clastogenic activity of the basic fraction at high dosages was also observed only following incubation with the S-9 mixture. At the lower dose range, a higher number of chromosomal aberrations, predominantly in the form of chromosomal breaks, were detected without S-9 treatment. The number of chromosomal exchanges induced by this basic fraction was considerably lower than that observed with the neutral fraction at an equivalent dose. Of the nitrogen-containing heterocyclic MRP compounds, pyrazine and derivatives such as alkylpyrazines (2-methyl-, 2,5-dimethyl-, and 2,6-dimethylpyrazine) have given negative responses with the Ames TA-98 and TA-100 tester strains (Stich *et al.*, 1980; Shibamoto, 1983) but contribute significantly to the clastogenic activity of CHO cells incubated with heated sugar–amino acid reactions (Stich *et al.*, 1980). Spingarn and Garvie (1979) noted that the heating of arabinose–ammonia model systems resulted in the formation of pyrazines and mutagenic activity in MRP systems as assessed by the TA-98 tester strain with S-9 mix. Former studies have also observed strong mutagenicity from pyrolysis products yielding amino–carboline compounds, but this mutagenic activity occurred only after metabolic activation (Matsumoto *et al.*, 1978). Amino reductones or eneaminol compounds are also derived from the initial reaction between the hydroxyl group from triose reductones with the free amino group of the amino acid. Significant mutagenic activity derived from amino reductones from a gluc–lys browning reaction has been reported with the *in vivo* silkworm oocyte model (Omura *et al.*, 1983).

Melanoidins present in both the ethanol supernatant (Figure 6) and the precipitate (Figure 7) gave similar mutagenic dose responses in individual assays with TA-98 and TA-100 strains. This result was expected since mutagenic activity of MRP has often been correlated with the extent of browning (Shinohara *et al.*, 1980; Cuzzoni *et al.*, 1988), which in turn reflects the degree of polymerization of melanoidin molecules (Feather and Nelson, 1984). The number of revertants detected above background was greater in treatments without S-9 mix than counterparts exposed to S-9 in both TA-98 and TA-100 strains. Following S-9 pretreatment, a significant ($P \leq 0.05$) reduction in the mutagenic dose response from melanoidins in the ethanol supernatant fraction was obtained only at the highest dose. A significant ($P \leq 0.05$) reduction in mutagenic activity observed with TA-100 was also observed over a wider dose range with the ethanol-insoluble (high molecular weight) melanoidins preincubated with S-9 mix (Figure 7). Pretreatment of ethanol-insoluble MRP fractions with S-9 completely eliminated the mutagenic response with the TA-98 bacterial strain. A similar finding was obtained when melanoidin fractions were tested for chromosomal aberration (Table II). The frequencies of chromosomal aberrations scored in CHO cells were similar in both melanoidin fractions without S-9 mix, albeit the active dose for the high molecular pigments was a magnitude smaller, indicating greater toxicity to the text organism. A substantial reduction in

clastogenic activity, in the form of both chromosomal breaks and exchanges, was observed when melanoidin pigments were incubated with microsomal enzymes.

CONCLUSION

In this study, we have evaluated the effect of MRP fractions on bacterial and mammalian cell mutagenesis *in vitro*. Low molecular weight MRP intermediates, in both the neutral and basic fractions and high molecular weight melanoidins, gave positive responses in a base shift mutation bacterial assay and a clastogenic response in mammalian chromosomal aberration test. The induction of mutagenicity as well as chromosomal abberations by individual MRP fractions was reduced dramatically with prior exposure to S-9.

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LITERATURE CITED

Aeschbacher, H. U.; Chappuis, C.; Manganel, M.; Aeschbach, R. Investigation of Maillard products in bacterial mutagenicity test systems. *Prog. Food Nutr. Sci.* **1981**, *5*, 279–293.

Bjeldanes, L. F.; Chew, H. Mutagenicity of 1,2-dicarbonyl compounds: Maltol, kojic acid, diacetyl and related substances. *Mutat. Res.* **1979**, *67*, 367–371.

Chan, R. I. M.; Stich, H. F.; Rosin, M. P.; Powrie, W. D. Antimutagenic activity of browning reaction products. *Cancer Lett.* **1982**, *15*, 27–33.

Cuzzoni, M. T.; Stoppini, G.; Gazzani, G.; Mazza, P. Influence of water activity and reaction temperature of ribose-lysine and glucose-lysine Maillard systems on mutagenicity, absorbance and content of furfurals. *Food Chem. Toxicol.* **1988**, *26*, 815–822.

Cuzzoni, M. T.; Stoppini, G.; Gazzani, G.; Mazza, P. Effect of storage on mutagenicity, absorbance and content of furfural in the ribose-lysine Maillard system. *Food Chem. Toxicol.* **1989**, *27*, 209–213.

Feather, M. S.; Nelson, D. Maillard polymers derived from D-glucose, D-fructose, 5-(hydroxymethyl)-2-furaldehyde and glycine and methionine *J. Agric. Food Chem.* **1984**, *32*, 1428–1432.

Fujita, Y.; Wakabayashi, K.; Nago, M.; Sugimura, T. Characteristics of major mutagenicity of coffee. *Mutat. Res.* **1985**, *142*, 145–148.

Gomez-Arroyo, S.; Souza, V. *in vitro* and occupational induction of sister-chromatid exchange in human lymphocytes with furfuryl alcohol and furfural. *Mutat. Res.* **1985**, *156*, 233–238.

Hodge, J. E. Dehydrated foods: chemistry of browning reactions in model reactions in model system. *J. Agric. Food Chem.* **1953**, *1*, 928–943.

Lynch, S. C.; Gwenwedel, D. W.; Russel, G. F. Mutagenic activity of a nitrosated early Maillard product: DNA synthesis (DNA repair) induced in HeLa S,3 carcinoma cells by nitrosated 1-(N-L-tryptophan)-1-deoxy-D-fructose. *Food Chem. Toxicol.* **1983**, *21*, 551–556.

Maron, D. M.; Ames, B. N. Revised methods for the salmonella mutagenicity test. *Mutat. Res.* **1983**, *113*, 173–215.

Matsumoto, T.; Yoshida, D.; Mizudaki, S.; Okamoto, H. Mutagenic activity of pyrolysates of peptides and proteins. *Mutat. Res.* **1978**, *56*, 281–288.

Omura, H.; Shinohara, K.; Maida, H.; Nonaka, M.; Murakami, H. Mutagenic action of triose reductone and ascorbic acid of Salmonella typhimurium TA-100 strain. *J. Nutr. Sci. Vitaminol.* **1978**, *24*, 185–194.

Omura, H.; Johan, N.; Shinohara, K.; Murakami, H. Formation of mutagens by the Maillard Reaction. In *The Maillard Reaction in Foods and Nutrition*; Waller, G. R., Feather, M. S., Eds.; ACS Symposium Series 215; American Chemical Society: Washington, DC, 1983; pp 537–563.

Pomeranz, Y.; Johnson, J. A.; Shallenberger, J. A. Effects of various sugars on browning. *J. Food Sci.* **1962**, *27*, 350–362.

Powrie, W. D.; Wu, C. H.; Rosin, M. P.; Stich, H. F. Clastogenic and mutagenic activities of Maillard reaction model systems. *J. Food Sci.* **1981**, *46*, 1433–1438.

Powrie, W. D.; Wu, C. H.; Molund, V. P. Browning reaction systems as sources of mutagens and antimutagens. *Environ. Health Perspect.* **1986**, *67*, 47–54.

Rosin, M. P.; Stich, H. F.; Powrie, W. D.; Wu, C. H. Induction of mitotic gene conversion by browning reaction products and its modulation by naturally occurring agents. *Mutat. Res.* **1982**, *101*, 189–197.

Shibamoto, T. Mutagenicity of 1,5(or 7)-dimethyl-2,3,6,7-tetrahydro-1H,5H-biscyclopentapyrazine obtained from a cyclotene/NH3 browning model system. *J. Agric. Food Chem.* **1980**, *28*, 883–884.

Shinohara, K.; Wu, R. T.; Johan, N.; Tanaka, M.; Morinaga, N.; Murakami, H.; Omura, H. Mutagenicity of the browning mixtures by amino-carbonyl reaction on Salmonella typhimurium TA-100. *Agric. Biol. Chem.* **1980**, *44*, 671–672.

Shinohara, K.; Kim, E. H.; Omura, H. Furans as the mutagens formed by amino-carbonyl reactions. In *Amino-Carbonyl Reactions in Food and Biochemical Systems*; Fujimaki, M., Namiki, M., Kato, H., Eds.; Developments in Food Science 13; Kodansha: Tokyo, 1986; pp 353–362.

Spingarn, N. E.; Garvie, C. T. Formation of mutagens in sugar-ammonia model systems. *J. Agric. Food Chem.* **1979**, *27*, 1318–1321.

Stich, H. F.; Stich, W.; Rosin, M. P.; Powrie, W. D. Clastogenic activity of pyrazine derivatives: A comparative study with Salmonella typhimurium, Saccharomyces cerevisiae and Chinese hamster ovary cells. *Food Cosmet. Toxicol.* **1980**, *18*, 581–584.

Vagnarelli, P.; De Sario, A.; Cuzzoni, M. T.; Mazza, P.; De Carli, L. Cytotoxicity and clastogenic activity of ribosome-lysine browning model system. *J. Agric. Food Chem.* **1991**, *39*, 2237–2239.

Wei, C. I.; Kitamura, K.; Shibamoto, T. Mutagenicity of Maillard browing products obtained from a starch-glycine model system. *Food Cosmet. Toxicol.* **1981**, *19*, 749–751.

Yamaguchi, T.; Nakagawa, K. Mutagenicity of and formation of oxygen radicals by trioses and glyoxal derivative. *Agric. Biol. Chem.* **1983**, *47*, 2461–2465.

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